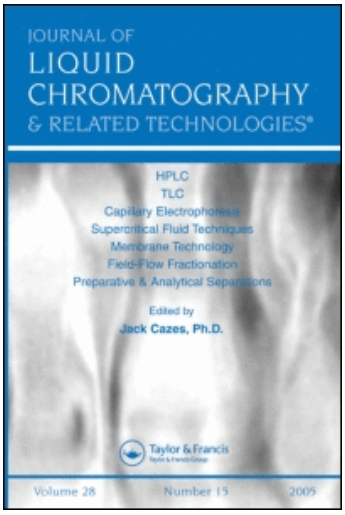

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Comparison of the Enantioselectivity of Phenethyl- and Naphthylethyl-Carbamate Substituted Cyclodextrin Bonded Phases

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COMPARISON OF THE ENANTIOSELECTIVITY OF PHENETHYL- AND NAPHTHYLETHYL-CARBAMATE SUBSTITUTED CYCLODEXTRIN BONDED PHASES

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ABSTRACT

(R)-, (S)- and racemic-phenethylcarbamate substituted-β-cyclodextrin bonded stationary phases were prepared and evaluated for the high performance liquid chromatographic separation of enantiomers. The results were compared to corresponding separations on an (S)-(-)-1-(1-naphthyl)ethyl-carbamate substituted β-cyclodextrin bonded phase. Successful separations were obtained in the reversed-phase and normal phase modes as well as with mobile phases of intermediate polarity. Different mechanisms of separation seem to be operative for various classes of compounds, depending upon the polarity of the mobile phase. The columns act as a novel cyclodextrin phase in the reversed phase mode, π-complex-hydrogen bonding chiral stationary phase in the normal phase mode, and

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cellulosic-like-phase with polar organic solvents such as methanol. With different chromatographic modes, it resolves completely different sets of enantiomers thereby serving as several columns in one.

INTRODUCTION

Prior to 1990 enantiomeric separations reported on cyclodextrin bonded phases occurred in the reversed phase mode. The chiral recognition mechanisms is this mode is thought to be dependent on inclusion complex formation between the hydrophobic moiety of the analyte and the relatively nonpolar interior of the cyclodextrin cavity. The utility of native cyclodextrin bonded phases and derivatized cyclodextrin bonded phases in the reversed phase mode for chiral separations has been well documented (1-7). Recently several derivatized bonded phases (*e.g.*, acetyl and toluoyl esters as well as naphtylethyl and 2,6-dimethylphenylcarbamates) have been developed for normal phase mode (8).

It has been demonstrated that the new derivatized cyclodextrin (CD) phases exhibit unique selectivities towards a wide variety of compounds and their chromatographic behavior is somewhat analogous to the derivatized cellulosic phases. It is most likely that under normal phase conditions the hydrophobic CD cavity is occupied by apolar solvent. Thus, no inclusion complexation is thought to take place. Chiral recognition comes from stereoselective hydrogen bonding between donor and acceptor sites of the analyte with the residual secondary hydroxyl groups and other polar moieties at the mouth of the cyclodextrin cavity, as well as π-π interactions with the aromatic substituents.

The naphthylethylcarbamate (NEC) functionalized β-CD stationary phase, due to the high stability and unique selectivity, has been proved to be the most widely applicable of all the various derivatives. Previous work has

demonstrated the applicability of NEC-β-CD phases for many separation problems (8-12). The NEC phases, which were originally developed for normal-phase separations, can be used in different modes of operation. In each mode the NEC-β-CD column provided a very unique enantioselectivity and resolved quite different set of enantiomers.

The present study examines the new (R)-, (S)- and (S,R) phenethylcarbamate substituted β-cyclodextrin bonded stationary phases. The comparison of retention characteristics in different modes for these new phases and (S)-naphthylethyl carbamate β-CD provides insight into the chiral recognition mechanism.

Figure 1 illustrates the structures (generated by computer) of the native phenethylcarbamate and the naphthylethylcarbamate derivatized-β-cyclodextrin.

EXPERIMENTAL

Materials and Methods: (S,R), (R)- and (S)- phenethylisocyanate and (S)-1-(1-naphthyl) isocyanate as well as pyridine were obtained from Aldrich Chemical Company (Milwaukee, WI). All chiral analytes were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). The structures of the analytes are given in the tables. The solvents were obtained from EM Science, Gibbstown, NJ (Omnisolv:Grade) and used without further purification. The water used for the triethylamine acetate buffer solution was distilled and filtered through a Barnstead (Dubuque, IA) no. D8922 filter.

The solutes were derivatized by placing a few mg of the solute in a vial, diluting with acetone and adding dinitrobenzoyl chloride. The solution was heated approximately 15 minutes.

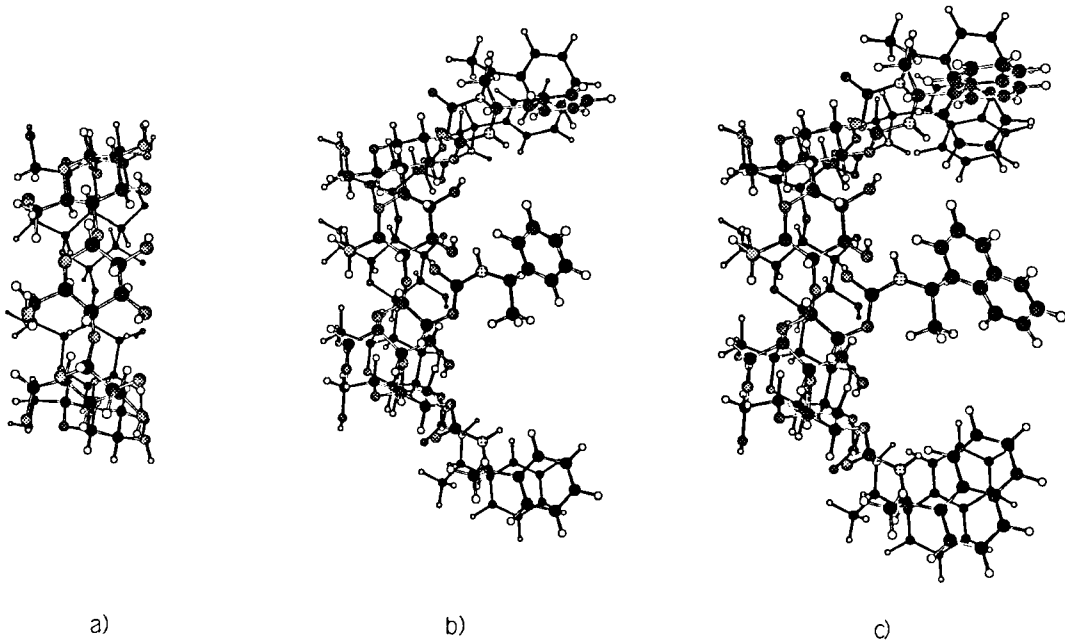


Figure 1. A side view computer generated structures of (a) native underivatized β-cyclodextrin molecule, (b) phenethylcarbamate derivatized-β-cyclodextrin molecule, and (c) naphthylethylcarbamate-β-cyclodextrin molecule.

The chromatographic experiments were performed on a Shimadzu (Columbia, MD) LC-6A Chromatograph interfaced with a CR3A Chromatopac Data System. Detection was accomplished using a Shimadzu SPD-6A variable wavelength detector. Additional evidence for an enantiomeric separation was obtained by injecting the compound again and varying the detection wavelength.

Bonded sorbents: The four phases used in the study were prepared using identical procedures. Briefly, Cyclobond I packing material (obtained from Advanced Separation Technologies, Inc., Whippany, NJ) was dried and placed in 3 necked round bottom flask with pyridine. Water was removed (as an azeotrope into a Dean-Stark trap). The appropriate isocyanate derivatizing reagent was added and the mixture was refluxed for 4 h. The derivatized bonded phase was collected on a fritted glass filter and washed with approximately 100 mL of pyridine followed by 200 mL of methanol and air-dried. The bonded sorbents were submitted for carbon analysis (Galbraith Labs, Knoxville, TN). The results are listed in Table I. The sorbents were all packed into 250 x 4.6 mm i.d. stainless steel columns.

RESULTS AND DISCUSSION

The surface concentration of cyclodextrin and the degree of substitution on the β-CD were calculated according to Reference 8. The data are listed in Table I. Although the derivatization reaction with the appropriate phenethylisocyanate and (S)-1-(1-naphthyl)ethyl isocyanate derivatizing reagent was carried out under the same experimental conditions the differences in the overall % C loading and the degree of substitution were obtained for the derivatized bonded β-CD phases investigated. The (S)-naphthylethyl carbamate-β-CD (S-NEC-β-CD) had the highest % C loading as well as the highest degree of substitution. As can be seen from the results

Table I

List of Bonded Sorbents

Column designation	Substituent configuration	% C_{spacer} [1]	%C_{CD} [2]	%C_{TOT} [3]	Degree of substitution
R-PEC*	*R*	2.60	4.87	6.33	3.3
S-PEC*	*S*	2.60	4.87	6.88	4.6
(S,R)-PEC*	S,R	2.60	4.87	6.35	3.4
S-NEC**	*S*	2.60	4.87	8.89	6.6

[1] %C due to the spacer used to link the cyclodextrin to the silica substrate.
[2] %C due to the spacer linkage plus the CD.
[3] %C of the derivatized bonded phase, including spacer, CD and the carbamate linked substituent group.
* Phenethyl carbamate-β-cyclodextrin column
** Naphthylethylcarbamateβ-cyclodextrin column

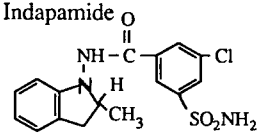
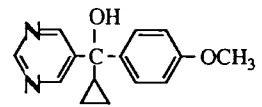
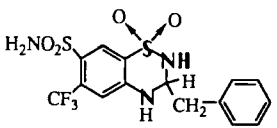
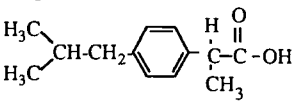
presented in Table I, the coverage of (S)-phenethylcarbamate-β-CD (S-PEC) sorbent is significantly higher when compared to the (R)-phenethylcarbamate-β-CD (R-PEC) bonded phase. This suggests that the S-form of phenethylisocyanate exhibits higher affinity or reaction rate to the chiral β-CD bonded phase which results in differences in the overall loading and the degree of substitution. Thus, it is possible that sorbent obtained in the reaction between the chiral β-CD bonded phase and the racemic mixture of phenethylisocyanate reagent is not homogeneous in respect to the degree of substitution of S- and R- PEC groups to the CD moiety. Therefore this sorbent will be referred as the (S,R)-PEC.

The chromatographic data obtained from this study have been divided into three categories, depending upon the polarity of the mobile phase used. In each mode, these new bonded phases were able to separate compounds that the native cyclodextrin phases could not (*e.g.*, 3,5-dinitrobenzoyl-DL-phenylglycine in a normal phase mode and indapamide in reversed phase mode). The unique enantioselectivity afforded by these new phases seems to be due, in part, to the incorporation of an additional stereogenic center onto the bonded ligand.

1. Reversed Phase Separations

Chromatographic data for the reversed-phase separations are given in Table II. Under reversed-phase conditions, the mechanism for enantioselectivity with derivatized cyclodextrin bonded phases is thought to involve not only inclusion complex formation but also additional interactions between the analyte and the cyclodextrin substituents. The role of the cyclodextrin substituent may be inferred by comparing the selectivities achieved on the (S) naphthylethyl and the (R)-, (S)- and (S,R)- phenethyl carbamate-substituted-β-cyclodextrin phases. In the cases of indapamide,

Table II Enantiomeric Separations with Derivatized-β-cyclodextrins using Polar Mobile Phases

Name & Strucuture	k'	α	Mobile Phase	Column
Indapamide 	17.34	1.17	20%MeCN/80%buffer[a]	*S*-NEC[1]
	4.52	1.06	20%MeCN/80%buffer[a]	*S*-PEC[2]
	3.82	1.07	20%MeCN/80%buffer[a]	*R*-PEC[3]
	4.32	1.06	20%MeCN/80%buffer[a]	(S,R)-PEC[4]
Ancymidol 	11.38	1.14	20%MeCN/80%buffer[a]	*S*-NEC[1]
	3.78	1.10	20%MeCN/80%buffer[a]	*S*-PEC[2]
	3.43	1.10	20%MeCN/80%buffer[a]	*R*-PEC[3]
	3.18	1.09	20%MeCN/80%buffer[a]	(S,R)-PEC[4]
Bendroflumethiazide 	15.03	1.11	30%MeCN/70%buffer[b]	*S*-NEC[1]
	3.93	1.05	30%MeCN/70%buffer[b]	*S*-PEC[2]
	2.15	1.00	30%MeCN/70%buffer[b]	*R*-PEC[3]
	3.44	1.00	30%MeCN/70%buffer[b]	(S,R)-PEC[4]
Ibuprofen 	4.07	1.14	40%MeCN/60%buffer[a]	*S*-NEC[1]
	5.30	1.11	40%MeCN/60%buffer[a]	*S*-PEC[2]
	2.29	1.09	40%MeCN/60%buffer[a]	*R*-PEC[3]
	4.70	1.13	40%MeCN/60%buffer[a]	(S,R)-PEC[4]

[1] (*S*)-1-(1-naphthyl)ethylcarbamate-β-cyclodextrin
[a] triethylamine acetate buffer pH=7.1
[3](*R*)-phenethylcarbamate-β-cyclodextrin
[4](S,R) phenethylcarbamate-β-cyclodextrin
[2](*S*)-phenethylcarbamate-β-cyclodextrin
[b] triethylamine acetate buffer pH=4.1

ancymidol and ibuprofen, the fact that the selectivities obtained on all three phenethyl phases are very similar suggests that the chirality of the cyclodextrin substituent does not contribute to the overall enantiorecognition. The cyclodextrin substituent seems to be more important for nonstereospecific π-π interactions. The higher retention obtained on the naphthylethyl column for all of these compounds (except, interestingly, ibuprofen) as well as selectivity, in these cases, may reflect the larger hydrophobic surface of the π-π interaction site of the naphthyl moiety. Furthermore, the (S)-phenethyl column, which had the highest loading of the phenethyl phases, exhibited a higher retention for these solutes than the lower loaded phenethyl phases.

In contrast to the results discussed above, the interaction afforded by the CD moiety does not appear to be enough to separate bendroflumethiazide. The (R)-phenethylcarbamate- and the racemic-phenethyl carbamate column yielded no resolution. Both the (S) phenethylcarbamate-β-cyclodextrin and (S)-(-)-1-(1-naphthyl)ethylcarbamate-β-cyclodextrin resolved bendroflumethiazide. Since the size of the (R)-phenethylcarbamate-β-cyclodextrin and the (S)-phenethylcarbamate-β-cyclodextrins are the same, the distinctions between these two columns are the degree of substitution and the configuration of the substituent. The (S)-phenethylcarbamate-substituent group and the (S)-(-)-1-(1-naphthyl)ethylcarbamate may be interacting in a synergistic fashion to the enantioselective interaction of the cyclodextrin moiety. Conversely, the (R)-phenethylcarbamate-group may have been interacting in an antagonistic manner to the cyclodextrin moiety. Therefore, in the separations where the (R)- and (S,R)- substituted cyclodextrins could not separate the compounds, the configuration of the substituent group appears to play an important role.

2. Intermediate Polarity Mobile Phases

The chromatographic data for separations in mobile phases of intermediate polarity are given in Table III. Figure 2 shows the chromatographic separation of 3,5-dinitrobenzoyl-derivatized-DL-phenylalanine on all four columns using a mobile phase of 99% methanol and 1% acetic acid.

Two types of compounds (i.e., 3,5-dinitrobenzoyl and dansylated amino acids) were investigated in this mode. In the case of the 3,5-dinitrobenzoyl derivatized compounds, the 3,5-dinitrophenyl ring is a π-acid. In almost all cases, the best separations of the 3,5-dinitro compounds were obtained on (S)-1-(1-naphthyl)ethylcarbamate derivatized cyclodextrin. With 3,5-dinitrobenzoyl-DL-phenylalanine and 3,5-dinitrobenzoyl-DL-alanine the naphthylethyl carbamate column was the only column that resolved the enantiomers. The naphthylethyl group is larger than the phenethyl group of the derivatized cyclodextrin. This size difference may account for the difference in the ability of enantioseparation. In the case of 3,5-dinitrobenzoyl-DL-phenylglycine the substituent played an important role as the change of the substituent configuration causes the change in elution order and the (S,R)- form of the phenethylcarbamate-β-cyclodextrin was unable to resolve the racemic mixture. The enantioresolution of 3,5-dinitrobenzoyl-DL-homophenylalanine is mainly due to the stereospecific interaction with the base CD-moiety. Consequently as in all cases investigated in this group the highest selectivity is observed on NEC-β-CD column. This seems to be the result of the large size of the chiral substituent and the high coverage or the degree of substitution on the CD moiety. However, the substituent doesn't influence the enantioselectivity, *i.e.*, the elution order is unchanged and the α-values obtained on all columns are very similar.

Table III. Enantiomeric Separations with Derivatized-β-Cyclodextrins using Mobile Phases of Intermediate Polarity

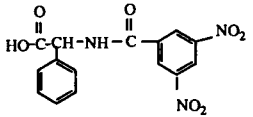
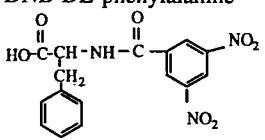
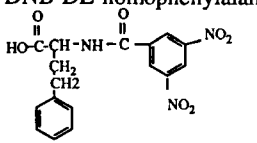
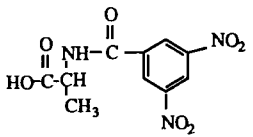
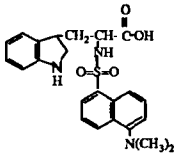
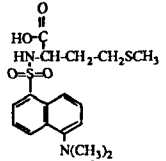
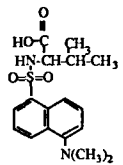
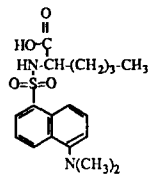
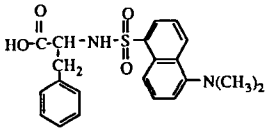
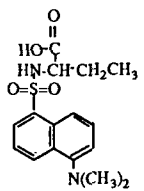
Name & Strucuture	k'	α	Mobile Phase	Column
DNB-DL-phenyglycine 	23.70	1.43	99%MeOH/1% HOAc	*S*-NEC[1]
	12.61	1.03	99%MeOH/1% HOAc	*S*-PEC[2]
	2.43	1.20	99%MeOH/1% HOAc	*R*-PEC[3]
	9.01	1.00	99%MeOH/1% HOAc	(S,R)-PEC[4]
DNB-DL-phenylalanine 	11.93	1.10	99%MeOH/1% HOAc	*S*-NEC[1]
	6.92	1.00	99%MeOH/1% HOAc	*S*-PEC[2]
	5.60	1.00	99%MeOH/1% HOAc	*R*-PEC[3]
	4.52	1.00	99%MeOH/1% HOAc	(S,R)-PEC[4]
DNB-DL-homophenylalanine 	11.72[D]	1.12	99%MeOH/1%HOAc	*S*-NEC[1]
	5.40[D]	1.14	99%MeOH/1%HOAc	*S*-PEC[2]
	2.67[D]	1.17	99%MeOH/1% HOAc	*R*-PEC[3]
	3.89[D]	1.14	99%MeOH/1% HOAc	(S,R)-PEC[4]
DNB-DL-alanine 	6.25	1.08	99%MeOH/1% HOAc	*S*-NEC[1]
	3.56	1.00	99%MeOH/1%HOAc	*S*-PEC[2]
	1.71	1.00	99%MeOH/1% HOAc	*R*-PEC[3]
	2.45	1.00	99%MeOH/1% HOAc	(S,R)-PEC[4]

table III (cont'd)

table III (cont'd)

Name & Strucuture	k'	α	Mobile Phase	Column
Dansyl-DL-tryptophan	2.39	1.03	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-NEC[1]
	3.88	1.00	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-PEC[2]
	4.80	1.06	49.5%MeCN/49.5%EtOH/1%HOAc	*R*-PEC[3]
	2.81	1.04	49.5%MeCN/49.5%EtOH/1%HOAc	S,R-PEC[4]
	2.90	1.06	49.5%MeCN/49.5%EtOH/1%HOAc	β-CD[5]
Dansyl-DL-methionine	3.33	1.03	49.5%MeCN/49.5%EtOH/1%HoAc	*S*-NEC[1]
	5.54	1.00	49.5%MeCN/49.5%EtOH/1%HoAc	*S*-PEC[2]
	5.22	1.04	49.5%MeCN/49.5%EtOH/1%HoAc	*R*-PEC[3]
	3.84	1.04	49.5%MeCN/49.5%EtOH/1%HoAc	S,R-PEC[4]
	3.69	1.09	49.5%MeCN/49.5%EtOH/1%HoAc	β-CD[5]
Dansyl-DL-valine	1.39	1.02	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-NEC[1]
	2.92	1.07	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-PEC[2]
	2.10	1.06	49.5%MeCN/49.5%EtOH/1%HOAc	*R*-PEC[3]
	1.76	1.08	49.5%MeCN/49.5%EtOH/1%HOAc	S,R-PEC[4]
	1.92	1.14	49.5%MeCN/49.5%EtOH/1%HOAc	β-CD[5]
Dansyl-DL-norleucine	1.58	1.00	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-NEC[1]
	3.37	1.00	49.5%MeCN/49.5%EtOH/1%HOAc	*S*-PEC[2]
	2.85	1.05	49.5%MeCN/49.5%EtOH/1%HOAc	*R*-PEC[3]
	2.05	1.03	49.5%MeCN/49.5%EtOH/1%HOAc	S,R-PEC[4]
	2.15	1.10	49.5%MeCN/49.5%EtOH/1%HOAc	β-CD[5]

Dansyl-DL-phenylalanine 	3.53[L]	1.12	49.5%MeCN/49.5%EtOH/1%HOAc *S*-NEC[1]
	5.46[L]	1.10	49.5%MeCN/49.5%EtOH/1%HOAc *S*-PEC[2]
	5.28[L]	1.13	49.5%MeCN/49.5%EtOH/1%HOAc *R*-PEC[3]
	3.99[L]	1.11	49.5%MeCN/49.5%EtOH/1%HOAc S,R-PEC[4]
	3.99[L]	1.12	49.5%MeCN/49.5%EtOH/1%HOAcβ-CD[5]
Dansyl-DL-norvaline 	1.61	1.00	49.5%MeCN/49.5%EtOH/1%HOAc *S*-NEC[1]
	3.94	1.00	49.5%MeCN/49.5%EtOH/1%HOAc *S*-PEC[2]
	2.90	1.05	49.5%MeCN/49.5%EtOH/1%HOAc *R*-PEC[3]
	2.11	1.04	49.5%MeCN/49.5%EtOH/1%HOAc*S,R*-PEC[4]
	2.19	1.09	49.5%MeCN/49.5%EtOH/1%HOAcβ-CD[5]

[1] (*S*)-1-(1-naphthyl)ethylcarbamate-β-cyclodextrin
[2](*S*)-phenethylcarbamate-β-cyclodextrin
[3](*R*)-phenethylcarbamate-β-cyclodextrin
[4](S,R) phenethylcarbamate-β-cyclodextrin
[5]native β-cyclodextrin

[L] L-form eluted first
[D]D-form eluted first
MeCN=acetonitrile
EtOH=ethanol
HOAc=acetic acid

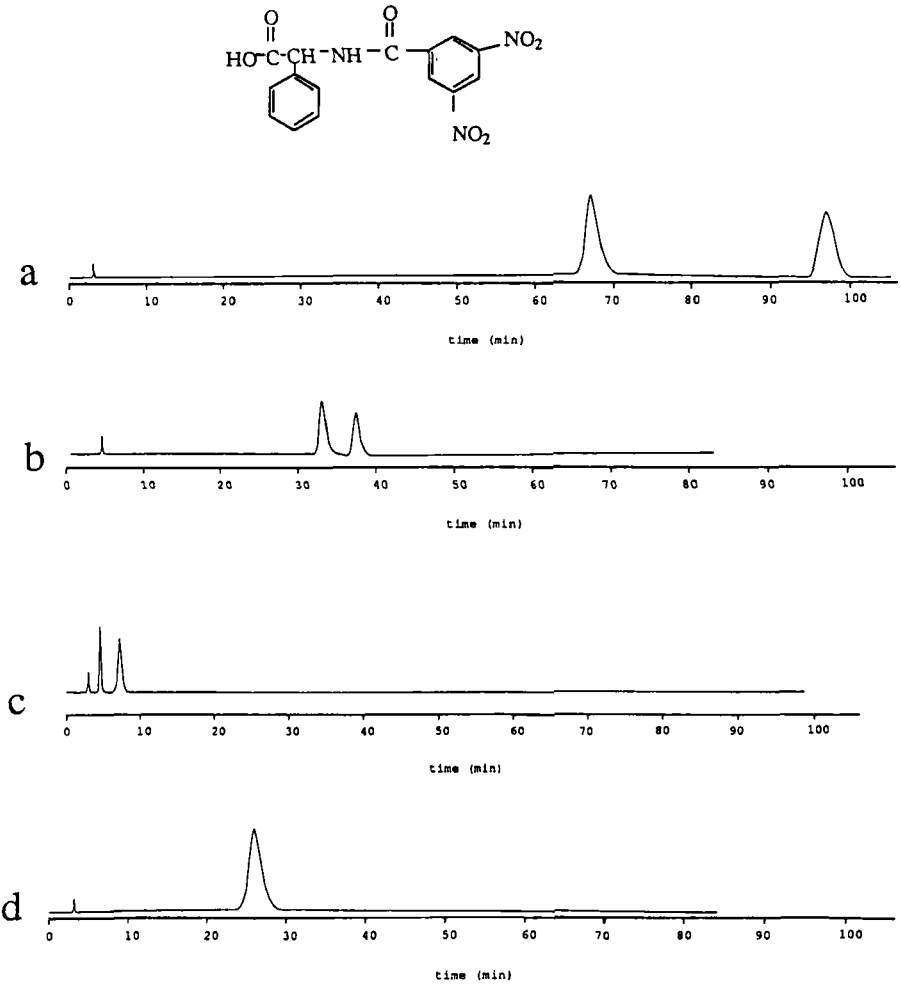
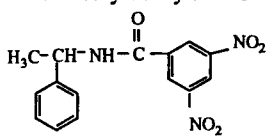
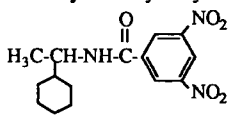
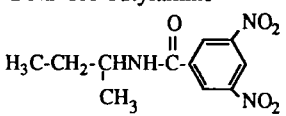


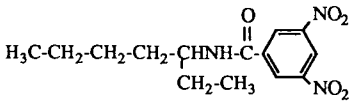
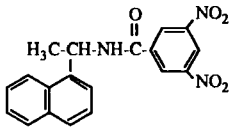
Figure 2. Dinitrobenzoyl-derivatized-DL-phenylglycine was separated on (a) S-naphthylethylcarbamate-derivatized-β-cyclodextrin, (b) S-phenethylcarbamate-derivatized-β-cyclodextrin, (c) R-phenethylcarbamate-derivatized-β-cyclodextrin and (d) R,S-phenethylcarbamate-derivatized-β-cyclodextrin which yielded no resolution. Mobile phase 99% methanol and 1% acetic acid at 1 ml/min.

In the case of the dansyl amino acids, the dansyl moiety is somewhat π-basic. Therefore, π-π interactions between the naphthyl moiety on the solute and the aromatic moieties (naphthyl or phenyl) of the bonded ligand are expected to play a lesser role in retention. The fact that the retention of most compounds on the naphthyl column, which has the highest degree of substitution, is less than that obtained on the phenethyl columns suggest that repulsive interactions may play a more important role in chiral recognition for these solutes. This conclusion is further supported by the fact that for almost all of the dansylated amino acids (except phenylalanine), the lowest selectivity was obtained on the naphthylethyl substituted column.

Separations are obtained for all of the dansylated amino acids on the native β-CD as well on the (S,R) phenethyl column. This indicates that the enantiorecognition is governed mainly by the cyclodextrin moiety. In case of dansyl-DL-phenylalanine the elution order and the enantioselectivity observed is the same for all derivatized CD stationary phases as well for the native β-CD column. However, it is apparent from the data collected in Table 3 that the derivatization significantly influences the enantioselectivity exhibited by the native β-CD. Substitution of the cyclodextrin's hydroxyl groups at by relatively large PEC and NEC groups reduces the number of available hydroxyls for stereoselective hydrogen bonding and also may reduce access of the solute to some parts of the cyclodextrin. This results in reduced selectivities. However, the reduced selectivities obtained on the (S)- and (S,R) phenethyl column relative to those obtained on the R column suggests that the S configuration of the substituent may contribute in a slightly antagonistic manner to the chiral selectivity of the cyclodextrin. This type of behavior has been reported previously on the naphthylethylcarbamate β-cyclodextrin phases (10)

Table IV. Enantiomeric Separations with Derivatized-β-Cyclodextrins using Apolar Mobile Phases

Name & Structure	k'	α	Mobile Phase	Column
DNB-α-methylbenzylamine 	8.52	2.15	70%HEX/30%IPA	*S*-NEC[1]
	1.73	1.28	70%HEX/30%IPA	*S*-PEC[2]
	0.93	1.05	70%HEX/30%IPA	*R*-PEC[3]
	1.69	1.00	70%HEX/30%IPA	S,R-PEC[4]
DNB-1-cyclohexylethylamine 	21.60[R]	1.11	90%HEX/10%IPA	*S*-NEC[1]
	5.73[R]	1.39	90%HEX/10%IPA	*S*-PEC[2]
	2.70[R]	1.25	90%HEX/10%IPA	*R*-PEC[3]
	5.28[R]	1.18	90%HEX/10%IPA	S,R-PEC[4]
DNB-sec-butylamine 	24.10	1.10	90%HEX/10%IPA	*S*-NEC[1]
	5.98	1.00	90%HEX/10%IPA	*S*-PEC[2]
	5.92	1.00	90%HEX/10%IPA	*R*-PEC[3]
	5.10	1.00	90%HEX/10%IPA	S,R-PEC[4]

DNB-3-aminoheptane	15.08	1.21	90%HEX/10%IPA	*S*-NEC[1]
	3.81	1.07	90%HEX/10%IPA	*S*-PEC[2]
	3.82	1.00	90%HEX/10%IPA	*R*-PEC[3]
	3.24	1.00	90%HEX/10%IPA	S,R-PEC[4]
DNB-1-(1-naphthyl)ethylamine	10.71[R]	4.28	70%HEX/30%IPA	*S*-NEC[1]
	2.05[R]	1.48	70%HEX/30%IPA	*S*-PEC[2]
	1.13[S]	1.56	70%HEX/30%IPA	*R*-PEC[3]
	2.16	1.00	70%HEX/30%IPA	(S,R)-PEC[4]

[1] (*S*)-1-(1-naphthyl)ethylcarbamate-β-cyclodextrin
[2](*S*)-phenethylcarbamate-β-cyclodextrin
[3](*R*)-phenethylcarbamate-β-cyclodextrin
[4](S,R)- phenethylcarbamate-β-cyclodextrin
[R] R-form eluted first
[S] S form eluted first

3. Normal Phase Separations.

The chromatographic data for the normal phase separations on the three phenethyl derivatized and the naphthylethyl derivatized-β-cyclodextrin columns are given in Table IV. All of the solutes analyzed in the normal phase mode incorporated a 3,5-dinitrobenzoyl moiety. In the normal phase mode, the cyclodextrin cavity is most likely occupied by the most nonpolar component of the mobile phase. It is likely that interaction occurs between the solute and bonded ligand outside of the cyclodextrin cavity. Therefore, the strong π-acid dinitrobenzoyl group of the derivatized solutes prefer to interact with the larger, stronger π-base-naphthyl moiety rather than the smaller phenethyl moiety. However, the fact that the degree of substitution was larger for the naphthylethyl phase than for any of the phenethyl phases also may account for the generally higher selectivities obtained on the naphthylethyl phases relative to those obtained on the phenethyl phases.

In most cases (e.g., 3,5-dinitrobenzoyl-α-methylbenzylamine, 3,5-dinitrobenzoyl-3-aminoheptane, and 3,5-dinitrobenzoyl-1-(1-naphthyl)ethylamine), the configuration of the substituent groups of the cyclodextrin seems to play a fairly important role in the enantioselectivity for these solutes in the normal phase mode. This is substantiated by the lack of resolution with the (S,R)-phenethyl substituted β-cyclodextrin phase and the reversal elution order found for enantioseparation of 3,5-dinitrobenzoyl-1-(1-naphthyl)ethylamine.

CONCLUSION

Carbamoylation of cyclodextrin yields columns with multimodal behavior and therefore offers a wider applicability to resolve enantiomeric compounds. In each different mode the mechanism of separation appears to be different. The enantiomeric solutes have the potential to interact with two

chiral moieties (i.e., the chiral cyclodextrin and the chiral substituent). With a combination of two chiral moieties, the resultant chiral selectivities can be result of one constituent dominating or a combination of the two working together. In hydro-organic solvents the carbamyolated β-CD phases behave similarly to native cyclodextrin bonded phases although the enantioselectivity is different than the native cyclodextrin bonded phase (9,11). In the reversed phase mode the cyclodextrin portion of the stationary phase contributes mainly to the enantioseparation. In hexane/isopropanol mobile phases the stationary phases act as a π-complex-hydrogen bonding or Pirkle-type column; in this mode the pedant NEC or PEC groups appear to control the enantioselectivity (9,10,13). In neat alcohol or acetonitrile solvents the stationary phases act like functionalized cellulosic stationary phases (8,14,15). Both the cyclodextrin moiety and chiral pendant can influence the enantioselectivity.

These derivatized cyclodextrin high performance liquid chromatographic phases have similar stability to native cyclodextrins in relationship to mobile phase durability. Each of these columns were used in reversed and normal phase modes without column damage. Furthermore, no problems concerning the integrity of the chiral selector were encountered in switching back from one mode to the other.

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