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Note

Liquid chromatographic separation of racemates on acetylated or carbamoylated β-cyclodextrin-bonded stationary phases

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Cyclodextrins (CDs) and their chemically modified derivatives have been the subject of numerous investigations and have been used for various purposes. It is well known that the chemical modification of CDs brings about changes in the depth of the CD cavity, in the hydrogen-bonding ability and in various other physical properties. In previous papers, we reported selectivity changes in solute retention after acetylation[1–3] or carbamoylation[4] of unmodified CD-bonded stationary phases in high-performance liquid chromatography (HPLC).

Recently, there has been considerable interest in separating racemates with CD-bonded stationary phases. The unmodified β-CD phases have been especially widely employed for this purpose[5–12]. It is of great interest to investigate optical resolution with chemically modified CD stationary phases, because their selectivity change may be reflected in the separation of racemates. To our knowledge, only the separation of optical isomers of norgestrel on an acetylated β-CD stationary phase has been reported briefly[6].

In this paper, we describe the preliminary HPLC separation of some racemates on acetylated or carbamoylated β-CD stationary phases.

EXPERIMENTAL

Preparation of β-CD stationary phases

Sodium salt of unmodified β-CD was coupled to 3-glycidoxypropyl silica (Gp-silica) in N,N-dimethylformamide at 130°C for 4 h[13]. The stationary phase thus obtained is denoted CD-Gp-silica after end-capping of the silanols with 1,1,1,3,3,3-hexamethyldisilazane in hexane. The amount of β-CD immobilized was evaluated to be 54.2 μmol/g by elemental analysis.

CD-Gp-silica (2.0 g) was treated with acetic anhydride (8 ml) in dry pyridine (30

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ml) at 45°C for 6 h. The acetylated stationary phase is denoted Ac-CD-Gp-silica.

CD-Gp-silica (2.5 g) was carbamoylated in pyridine (100 ml) at 30°C by adding methyl isocyanate (1 g) three times at intervals of 24 h. Similarly, CD-Gp-silica (2.5 g) was treated with phenyl isocyanate (2.4 g) in pyridine (80 ml) at 70°C for 10 h. The resulting methyl- or phenylcarbamoylated β-CD stationary phase is denoted Me-CD-Gp-silica or Ph-CD-Gp-silica, respectively.

Materials and chromatography

Silica gel (5 μm) and β-CD were obtained from Wako (Osaka, Japan) and other chemicals of analytical-reagent grade from Wako, Tokyo Kasei (Tokyo, Japan) or Sigma (St. Louis, MO, U.S.A.).

The HPLC system used consisted of a Shimadzu (Kyoto, Japan) LC-6A pump and a Waters Assoc. (Milford, MA, U.S.A.) 440 UV detector operating at 254 nm. Each stationary phase was packed into a stainless-steel column (15 cm × 4 mm I.D.) by the balanced-density slurry method. The flow-rate of the eluent [methanol–1% triethylammonium acetate (TEAA) mixtures] was 1.0 ml/min.

RESULTS AND DISCUSSION

In this study, the extent of separation between the two peaks of a racemate is represented by

$$R' = \frac{H - H'}{H} \cdot 100$$

where H and H' are the height of the first eluted peak and that of the valley between the two peaks, respectively, for a clear separation. In this definition, the greater the value of R', the better is the resolution, and $R' = 100$ represents a complete separation of the two peaks.

Table I gives the R' values for eight pairs of dansylamino acid enantiomers together with their retention times (t_R) and separation factors (α) on both CD-Gp-silica and Ac-CD-Gp-silica in methanol–TEAA at pH 5.0. A decrease in retention was found for these solutes on CD-Gp-silica with increasing proportion of methanol in the eluent. A similar decrease in the retention also occurred as the pH of TEAA increased; the solute retention in methanol–TEAA (50:50) at pH 6.0 was about half with that at pH 5.0. Considering the separations of the enantiomers and the total analysis times, the optimum eluent was methanol–TEAA (50:50) at pH 5.0 for CD-Gp-silica, as indicated in Table I.

In chiral recognition and separation in chromatographic processes using unmodified CD, the interaction of the guest (solute) with the 2- or 3-hydroxy groups at the mouth of the CD cavity is considered to play an important role, in addition to a tight fit between the guest and the CD host[10]. Therefore, it is interesting to compare the separation before and after the chemical modification of these hydroxyl groups. In this work, acetylation and methyl- and phenylcarbamoylation were investigated, because each modified CD stationary phase could be obtained by treating the same unmodified phase and probably contained the same amount of CD. It is apparent from Table I that the acetylation of CD-Gp-silica considerably enhances the separation of

TABLE I

SEPARATION OF DANSYLAMINO ACID ENANTIOMERS ON β-CD STATIONARY PHASES BEFORE AND AFTER ACETYLATION IN METHANOL–TEAA (50:50) AT pH 5.0.

Dansylamino acid	*Isomer*	*CD-Gp-silica*			*Ac-CD-Gp-silica*			*Ac-CD-Gp-silica*[a]		
		$t_R(min)$	α	R'	$t_R(min)$	α	R'	$t_R(min)$	α	R'
α-Amino-*n*-butyric acid	L	8.70	1.08	22.4	17.25	1.15	94.4	9.36	1.17	98.7
	D	9.30			19.92			10.71		
Leucine	L	11.70	1.25	93.5	21.30	1.18	100	11.10	1.20	98.2
	D	14.10			24.74			13.05		
Methionine	L	9.75	1.09	49.7	17.85	1.12	80.2	10.50	1.15	88.3
	D	10.56			19.75			11.70		
Norvaline	L	9.06	1.09	28.1	16.20	1.13	90.0	9.24	1.15	94.8
	D	9.69			18.15			10.44		
Phenylalanine	L	16.95	1.14	72.9	31.95	1.14	91.7	15.00	1.15	95.6
	D	18.96			36.90			17.01		
Serine	L	7.95	1.09	34.7	14.40	1.17	94.8	10.23	1.19	100
	D	8.49			16.50			11.91		
Threonine	L	8.16	1.13	81.4	16.68	1.26	100	10.26	1.29	100
	D	9.12			20.58			12.84		
Valine	L	9.54	1.13	64.1	20.40	1.20	98.8	10.50	1.23	100
	D	10.50			24.30			12.54		

[a] Eluted with methanol–TEAA (70:30) at pH 5.0.

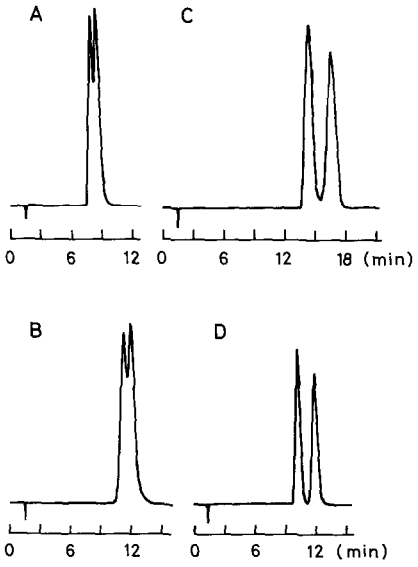

Fig. 1. Enantiomeric separation of dansyl-D,L-serine on CD-Gp-silica in methanol–TEAA at pH 5.0, (A) (50:50) and (B) (40:60), and on Ac-CD-Gp-silica in methanol–TEAA at pH 5.0, (C) (50:50) and (D) (70:30).

TABLE II

SEPARATION OF ENANTIOMERS ON β-CD STATIONARY PHASES BEFORE AND AFTER CHEMICAL MODIFICATION

The methanol content and pH of TEAA in the eluent are given in parentheses.

Solute	*CD-Gp-silica*			*Ac-CD-Gp-silica*			*Me-CD-Gp-silica*			*Ph-CD-Gp-silica*		
	$t_R(min)$	α	R'	$t_R(min)$	α	R'	$t_R(min)$	α	R'	$t_R(min)$	α	R'
Alanine-β-naphthylamide	(30%, pH 5.1)			(10%, pH 4.5)			(20%, pH 4.95)			(30%, pH 5.1)		
L	3.66	1.36	93.1	9.60	1.24	98.0	5.85	1.12	36.0	9.15	1.27	50.5
D	4.41			11.55			6.45			11.34		
α-Aminoethylbenzene				(0%, pH 5.1)			(0%, pH 4.95)					
L				2.30	1.22	84.3	2.26	1.09	15.8			
D				2.57			2.42					
α-Hydroxyethylferrocene	(30%, pH 5.1)			(30%, pH 5.0)								
	27.30[a]	1.09	59.5	65.40[a]	1.10	63.0						
	29.70			71.70								
Mandelic acid methyl ester				(0%, pH 5.0)								
R				15.06	1.06	18.1						
S				15.90								

[a] Retention time of the first-eluted enantiomer.

the enantiomers for each dansylamino acid eluted with methanol–TEAA (50:50). Each solute, however, exhibited a relatively long retention time on Ac-CD-Gp-silica in this instance. Consequently, the methanol content in the eluent was changed from 50 to 70%, in order to reduce the retention [nearly comparable to that on CD-Gp-silica eluted with methanol-TEAA (50:50)]. This change resulted in a further improvement in separation, except for dansylleucine.

Fig. 1 shows typical liquid chromatograms for the separation of dansyl-D,L-serine. Fig. 1A and C represent the separation of the enantiomers on CD-Gp-silica and Ac-CD-Gp-silica, respectively, with methanol–TEAA (50:50) at pH 5.0. Apparently, the separation of the enantiomers is much better after the acetylation. The methanol concentration was adjusted to give the same retention time (*ca.* 12 min) for both CD stationary phases. As the result, the R' value decreased from 34.7 (Fig. 1A) to 25.9 (Fig. 1B) on CD-Gp-silica, whereas it increased from 94.8 (Fig. 1C) to 100 (Fig. 1D) on Ac-CD-Gp-silica. The enantiomers can be separated completely on the acetylated CD stationary phase with methanol–TEAA (70:30) at pH 5.0. Hence acetylation of CD-Gp-silica enhanced the chiral recognition for the dansylamino acid enantiomers, whereas both methyl- and phenylcarbamoylation removed it.

An attempt was also made to separate several other solutes, and some of the results are given in Table II. Although the eluent conditions were not identical, acetylation improved the enantiomeric separation for these solutes. In contrast, methyl- and phenylcarbamoylation resulted in a substantial decrease in the enantioselectivity of the modified CD stationary phases.

Unmodified CD-Gp-silica has many terminal hydroxyl groups produced by the deactivation of the unreacted 3-glycidoxypropyl groups with CD on Gp-silica (*ca.* 600 μmol/g). These hydroxyl groups are probably acetylated or carbamoylated in addition to those of the immobilized CD moiety. Moreover, the spacer arm linking CD to silica gel in CD-Gp-silica contains one hydroxyl group formed on coupling CD. It is also possible for this hydroxyl group to be acetylated or carbamoylated. In this context, it is reasonably accepted that the chemical modification of some and/or all of these hydroxyl groups affects the interaction between the solute and the stationary phase (the CD moiety and/or the non-CD-containing moiety) and that this brings about the considerable change in the enantioselectivity after the modification. Further work is needed for a more convincing explanation.

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