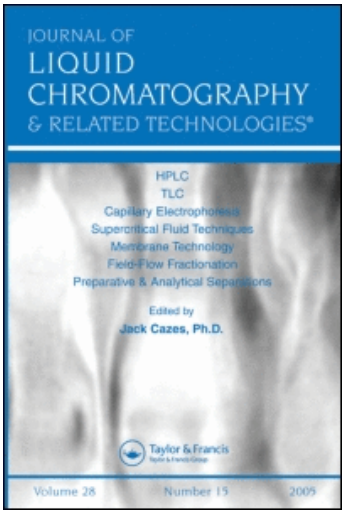

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Evaluation of 3,5-Dimethylphenyl Carbamoylated α-, β-, and γ-Cyclodextrins as Chiral Stationary Phases for HPLC

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EVALUATION OF 3,5-DIMETHYLPHENYL CARBAMOYLATED α-, β-, AND γ-CYCLODEXTRINS AS CHIRAL STATIONARY PHASES FOR HPLC

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ABSTRACT

Optical resolving power of chiral stationary phases (CSPs) containing 3,5-dimethylphenyl carbamoylated β-cyclodextrin chemically bonded to silica was compared with those of two commercial stationary phases containing carbamoylated β-cyclodextrin (β-CD), Cyclobond I SN and Cyclobond I DMP. For most of examined 14 racemates, higher selectivities were obtained on our CSPs than on the commercial CSPs. This may be ascribed to the higher degree of substitution of carbamate groups on β-CD and to the opposite orientation of the immobilized β-CD on our materials. The present CSPs were evaluated under reverse, normal and supercritical fluid chromatographic conditions. The highest selectivities were usually obtained under normal phase chromatographic conditions.

The influence of cyclodextrins, α-, β- and γ-CD, on the enantioselectivity was compared. Each CSP containing different cyclodextrin showed the highest enantioselectivity for different racemates.

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By using silica particles with different average pore size (60, 100 and 300 Å), the influences of the amount and surface concentration of immobilized carbamoylated β-CD on the enantioselectivity were studied for similar amounts of immobilized β-CD. Higher enantioselectivity was observed for the material with high surface concentration.

INTRODUCTION

Preparation of chiral stationary phases (CSPs) for high-performance liquid chromatgraphy (HPLC) is of great interest since it is well known that enantiomers can have quite different pharmacological and toxicological effects in human body. A number of CPSs are now commercially available [1] and their number is steadily increasing since one single CSP can not separate all enantiomers. Some of the more successful CSPs contain 3,5-dimethylphenylcarbamate functionalized polysaccharides, such as cellulose and amylose, on which 343 (80%) of 483 racemic compounds have been resolved [2].

Recently, we evaluated the optical resolving powers of 3,5-dimethylphenylcarbamates of α, β and γ-cyclodextrins as CSPs for HPLC. We have also recently reported on the preparation and chromatographic evaluation of the CSPs containing 3,5-dimethylphenyl carbamoylated β-cyclodextrin (β-CD) chemically bonded to silica [3]. In this paper, two commercial CSPs, containing carbamate functionalized β-CD, are compared to some of our previously prepared carbamoylated β-CD materials. The enantioselectivity of some new CSPs, containing carbamoylated α-, β- or γ–CD, is also presented together with results on the effect of average pore diameter of the silica particles and surface concentration of immobilized functionalized cyclodextrin on the enantioselectivity .

EXPERIMENTAL

Chemicals

The racemic solutes were obtained from different sources. β-Cyclodextrin (β-CD), α-CD and γ-CD, guaranteed reagent grade, were purchased from Nacalai Tesque. The spacers 4,4'-diphenylmethane diisocyanate (A), hexamethylene diisocyanate (B) and suberoyl chloride

(C) were all of guaranteed reagent grade and obtained from Tokyo Kasei. 3,5-Dimethylphenyl isocyanate was synthesized from 3,5-dimethylaniline and phosgene by a conventional method. A silanizing reagent 3-aminopropyltriethoxysilane (99%) was obtained from Janssen Chimica. Develosil, totally porous spherical silica gels with mean particle sizes of 5 or 10 μm and with mean pore diameters of 60, 100 and 300 Å were purchased from Nomura Chemical. Daisogel, a totally porous spherical silica gel with a mean particle size of 5 μm and with a mean pore diameter of 120 Å was kindly supplied by Daiso . The Cyclobond I DMP and Cyclobond I SN columns (250 mm x 4.6 (id) mm) were obtained from Advanced Separation Technologies Inc. (Astec). All solvents used in the preparation of the CSPs were of at least analytical grade and carefully dried before use. Solvents used in the chromatographic experiments were of HPLC grade.

Preparation of chiral stationary phases

A complete description of the preparation methods 1, 2 and 3 of the CSPs with β-CD was previously reported [4]. In method 1, β-CD was first allowed to react with 3,5-dimethylphenyl isocyanate (85-95% of hydoxy groups of β-CD) and then remaining secondary hydroxy groups at position C_2 and C_3 were chemically bonded to aminopropyl silica with a spacer. In methord 2, the primary hydroxy groups of β-CD was first immobirized to aminopropyl silica with a spacer and then remaining hydroxy groups of β-CD were reacted with an excess of 3,5-dimethylphenyl isocyanate. In method 3, a small amounts of a spacer was first reacted with the primary hydroxy groups of β-CD followed by carbamoylation of the remaining hydroxy groups with an excess of 3,5-dimethylphenyl isocyanate. Other CSPs used here were prepared in the same way.

Preparation of amino functionalized silica gel

The preparation of this silica gel has been reported previously [4].

Apparatus and chromatography

The specific surface area of amino functionalized silica gel was measured by a Micromeritic Flowsorber, Flowsorb II 2300 .

TABLE I
Characterization Data on β-CD Containing CSPs [a]

	IA2	IC2	IIC	IIE
Method[b]	1	1	2	3
Spacer	A	C	C	E
nr spacer/β-CD[c]	1	1-2	1	2
Weight % carbamoylated β-CD[d]	14	17	6-8	6

a) Particle size 5 μm, surface area 120 m^2/g.
b) See Experimental section.
c) Molar ratio of spacer to β-CD used.
d) Based on gravimetric analysis of silica bonded carbamoylated β-CD.

The carbamoylated β-CD bonded to silica gels was packed into 250x4.6 i.d. mm stainless steel columns by conventional high-pressure slurry-packing procedures. The chromatographic experiments were performed on a Jasco BIP-I HPLC-pump, a Jasco 875 UV-detector (254 nm), a Jasco DIP-181C polarimeter (Hg lamp, no filter, 5x0.3 cm i.d.) and a Jasco RC-228 recorder at room temperature. About 1-5 μl of a solution of a racemate was injected in the chromatographic system (20 μl loop) with a Rheodyne 7125 injector. The dead time (t_0) was determined with 1,3,5-tri-*tert*-butylbenzene. Most columns exhibited a theoretical plate number of about 3000 per column with benzene as solute. SFC experiment was performed on a Jasco Super-200, System-2 SFC instrument.

RESULTS AND DISCUSSION

All chiral stationary phases presented in this paper, except for one of the commercial CSPs, contain 3,5-dimethylphenyl isocyanate functionalized cyclodextrin (carbamoylated CD). Our CSPs (Tables I and II) are classified according to the orientation of immobilized CD, to the used spacer and in some cases to the used cyclodextrin (α or γ if not β-CD is used). CSPs, where the cyclodextrin molecule is immobilized to the silica surface via a secondary hydroxy group of CD, are labeled with

TABLE II
Characterization Data on α-, β- or γ-CD Containing CSPs

	Material I Bα	Material I B2	Material I Bγ
Cyclodextrin	α-CD	β-CD	γ-CD
Particle size (μm)	5	5	5
Average pore diameter (Å)	120	100	120
Surface area (m^2/g) a)	290	280	290
wt% of carbamoylated CD	13	17	8
Surface conc. (μmol/m^2)	0.16	0.17	0.07

a) Surface area of amino functionalized silica particles.

roman numeral I, while CSPs, where the CD molecule is immobilized to the silica surface via a primary hydroxy group, are labeled with roman numeral II (Fig. 1). The spacers presented in Fig. 2 are labeled with A, B, C or E. Structures of the racemic solutes are presented in Fig. 3.

Comparison between five carbamoylated β-CD CSPs

The enantioselectivity of two commercial, carbamoylated β-CD materials was compared with our materials. The two commercial materials were Cyclobond I DMP and Cyclobond I SN containing 3,5-dimethylphenyl carbamoylated β-CD (DMP) and (S)-naphthylethyl carbamoylated β-CD (SN), respectively. The two materials were probably prepared by immobilization of β-CD molecule through reaction between non-nitrogen containing spacers on the silica surface and primary hydroxy groups (pos C_6) on β-CD (Fig. 1). The immobilized β-CD is functionalized by the derivatization agent to different degrees of substitution. Cyclobond I SN probably has a degree of substitution of about 7 carbamate groups per β-CD [5]. No value of the degree of substitution has been reported for the Cyclobond I DMP material but a similar material containing 2,6-dimethylphenyl carbamoylated β-CD was reported to have a degree of substitution of about 10 [5]. The enantioselectivity of the two commercial materials was quite different to each other (Table III). Only 2 of the 13 used racemates were separated on Cyclobond I SN while 7 of 13 racemates were separated on Cyclobond I DMP under normal phase chromatographic conditions.

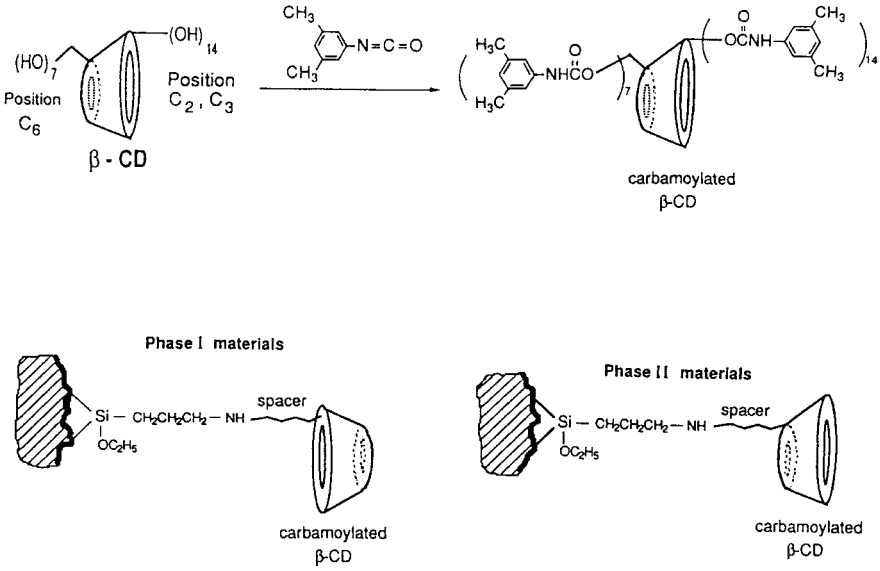

Fig. 1 Schematic model of β-cyclodextrin and phase I and phase II chiral stationary phases.

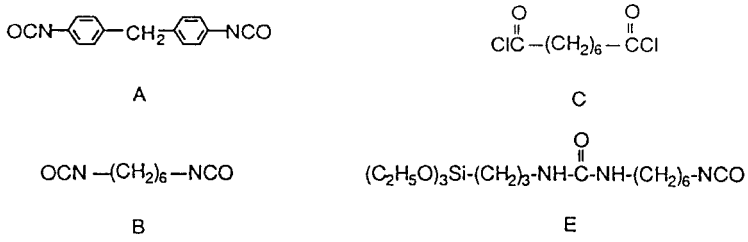

Fig. 2 Di-functional spacers used for immobilization of carbamoylated cyclodextrins to silica particles.

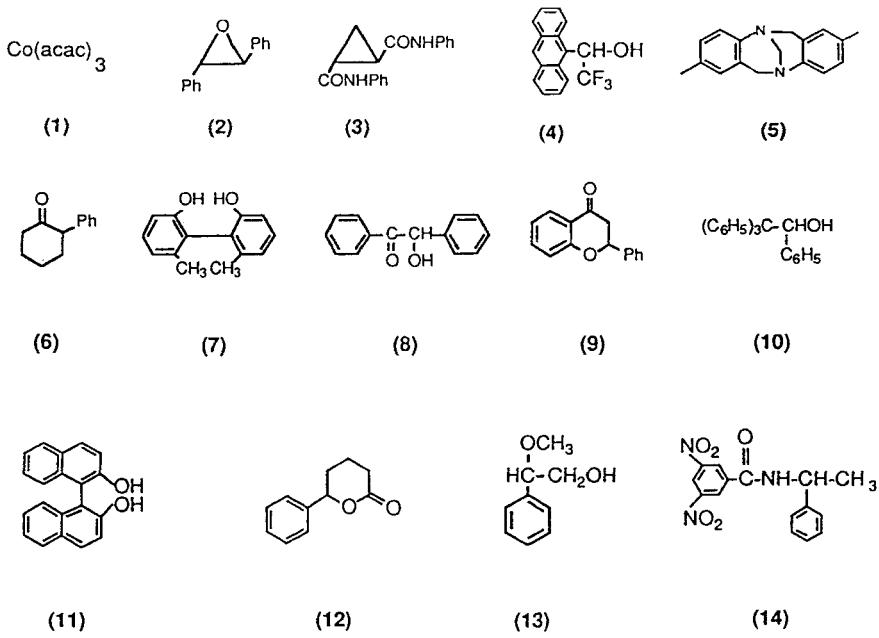

Fig. 3 Structures of racemic solutes .

Cyclobond I DMP was compared with our CSPs (Table III). Although only 14 racemates were chosen for comparison, the results of evaluation with these racemates seem to be analogously extended to about 500 racemates which we have used so far [2]. Similar enantioselectivity of material II C and Cyclobond I DMP suggests that Cyclobond I DMP may be prepared in a similar way to our method and that the degree of substitution is comparable with material II C, *i.e.*, about 11. Material II E was prepared by immobilization of completely carbamoylated β-CD to silica particles by preparation method 3 [4]. The functionalized β-CD molecule is bonded to the silica surface through position C_6 on the β-CD molecule. The increased enantioselectivity of material II E, compared with Cyclobond I DMP and material II C, may be due to the higher degree of substitution of carbamate groups on β-CD. The material with higher enantioselectivity, compared to Cyclobond I DMP, is material I C2. This

TABLE III
Normal Phase HPLC Separations of a Variety of Racemic Solutes (Fig. 3) on Different CSPs[a)]

Solute	Cyclobond I SN		Cyclobond I DMP		Material II C		Material II E		Material I C2	
nr.	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	1.48(+)	~1	2.07(+)	1.14	3.82(+)	~1	0.82	1.0	1.60(-)	~1
2	0.34(+)	~1	0.40(+)	~1	0.29(+)	~1	0.15(+)	~1	0.85(-)	~1
3	1.86(+)	~1	2.04(+)	1.14	0.86(+)	1.23	0.38(+)	1.43	3.17(+)	1.42
4	2.34	1.0	1.88	1.0	1.59	1.0	0.46	1.0	4.4	1.0
5	1.0	1.0	1.33	1.0	0.55(-)	~1	0.25(-)	~1	1.10(-)	1.09
6	0.79(+)	~1	0.98(-)	~1	0.74(-)	~1	0.56	1.0	1.42(-)	1.09
7	2.17(+)	1.05	2.00(+)	1.05	1.54	1.0	0.50(-)	~1	5.39(-)	1.05
8	2.24(+)	~1	2.46(+)	1.07	1.50(+)	1.07	0.50	1.0	3.96(+)	1.03
9	1.0	1.0	1.19(+)	1.12	0.75(+)	1.13	0.34(+)	1.82	2.19(+)	1.69
10	1.0	1.0	0.98	1.0	0.66	1.0	0.31	1.0	1.79(-)	1.09
11	9.14	1.0	5.22(+)	1.04	3.15(+)	~1	0.69(+)	1.09	12.7(+)	1.02
12	3.0(+)	~1	4.37(+)	~1	2.78(+)	~1	0.79(-)	~1	6.79(-)	1.13
14 b)	3.62(-)	1.78	3.69(-)	1.22	4.35(-)	1.12	1.4(-)	1.23	9.95(-)	1.35

a) k'_1=capacity factor and optical rotation of the first eluted enantiomer. Separation factor (α)=(k'_2/k'_1). Mobile phase=n-hexane/2-propanol 90/10 (v/v). Flow rate=1.0 ml/min.
b) Mobile phase=n-hexane/2-propanol 70/30 (v/v).

material is prepared by immobilization of completely carbamoylated β-CD according to the preparation method 1 [4]. The functionalized β-CD is bonded to the silica surface through position C_2 or position C_3, *i.e.*, facing its larger opening of the truncated cone towards the silica surface (Fig. 1). The high enantioselectivity of material I C2 over II C indicates that the orientation of the functionalized β-CD molecule affects the enantioselectivity. The structure of spacer also influences the enantioselectivity as observed for materials IIC and IIE. Material I C2 appears to be a superior CSP to commercial I DMP. This is attributed to higher degree of substitution of carbamate groups on β-CD and the opposite orientation of the immobilized β-CD molecule. As the plate number is comparable for all the CSPs in Table III, the increased selectivity of material I C2 enhances this column efficiency.

Interestingly, Cyclobond I DMP separates racemate nr 1 which can not be separated on any of our materials. This can perhaps be explained by the different spacer and/or the silica surface functionality since these factors have an effect on the enantioselectivity [4].

Comparison between CSPs containing α-, β- or γ-carbamoylated cyclodextrins

Three chiral stationary phases were prepared by the same method as the preparation method 1 but by using carbamoylated α-, β- or γ-CD as the chiral selector. The completely 3,5-dimethylphenyl carbamoylated cyclodextrins were immobilized to silica particles with isocyanate spacer A by reaction between the position C_2 or position C_3 of CD and amino groups on the silica surface; thus the somewhat larger opening on the truncated cone of CD was immobilized towards the silica surface (Fig. 1). Even if different amounts of carbamoylated CD were immobilized on the prepared materials (Table II), the results in Table IV clearly show that the enantioselectivity of prepared materials indeed depends on the α-, β- or γ-CD. Racemates nr 7 and 10 were only separated on the α-CD functionalized material, racemates nr 6 and 12 were only separated on the β-CD functionalized material while racemate nr 8 was only separated

TABLE IV

Normal Phase HPLC Separations of a Variety of Racemic Solutes (Fig. 3) on α-, β-, or γ-CD Containing CSPs[a)]

	Material I Bα		Material I B2		Material I Bγ	
Solute	α-CD		β-CD		γ-CD	
nr.	k'_1	α	k'_1	α	k'_1	α
1	5.77(+)	~1	4.82(+)	~1	3.62(+)	~1
2	0.47(+)	1.27	0.63(+)	1.16	0.28(+)	~1
3	1.81(+)	1.14	2.94(+)	1.45	1.19(+)	1.21
4	2.22	1.0	2.76	1.0	1.62	1.0
5	1.06	1.0	1.33(-)	~1	0.75(-)	~1
6	1.13(-)	~1	1.68(-)	1.05	0.69(+)	~1
7	2.35(+)	1.08	4.13(+)	~1	2.03(-)	~1
8	2.68(-)	~1	1.65	1.0	1.62(+)	1.08
9	1.29	1.0	2.22(+)	1.27	0.66(+)	1.09
10	1.19(+)	1.11	1.45	1.0	0.75	1.0
11	5.10(+)	1.07	11.8(+)	1.07	4.19(+)	1.04
12	4.16(+)	~1	7.30(-)	1.03	2.50(+)	~!
13	1.42(-)	~1	2.51(+)	~1	1.00(+)	~1
14 [b)]	12.0(-)	1.14	12.4(-)	1.27	3.00(-)	2.19

a) Chromatographic conditions : see Table III.
b) Mobile phase=n-hexane/2-propanol 70/30 (v/v).

on the γ-CD functionalized material. Furthermore, compared to all other 3,5-dimethylphenyl carbamoylated β-CD materials presented in this and the previous papers [4], the highest selectivity was obtained for racemate nr 2 on the α-CD functionalized material and for racemates nr 8 and 14 on the γ-CD functionalized material. It is, however, difficult to explain why the enantioselectivity is different between the α-, β- or γ-CD functionalized materials (Table IV). Under normal phase chromatographic conditions, it is not believed that the separation mechanism is due to inclusion complexation of solutes in the rather hydrophobic cavity of the truncated cone.

The influence of the cyclodextrin on the enantioselectivity is confirmed by the results reported on 3,5-dimethylphenyl carbamoylated α-, β- and γ-cyclodextrins adsorbed to silica particles [3]. The cyclodextrins were

completely functionalized with 3,5-dimethylphenyl isocyanate prior to adsorption to 3-aminopropyl functionalized silica particles. The separation factors (α) for racemates nr 1 and 5 increase while the separation factor (α) for racemate nr 4 decreases as the cyclodextrin is changed from α- to β-CD, and β- to γ-CD [3]. The same observation with an increasing or decreasing change on the separation factor (α), with a change of cyclodextrin, was also obtained for racemates nr 2 and 14 on the present cyclodextrin materials (Table IV). The separation factors for racemates nr 1, 2, 4, 5 and 6 were higher on the adsorbed carbamoylated α-, β- or γ-CD materials [3] compared to the present materials. Interestingly, the adsorbed materials show quiet different enantioselectivity, *e.g.*, racemates nr 1, 2 and 4 are not separated at all on the present materials while they are separated fairly good on the adsorbed materials. The average pore size of silica particle for the adsorbed CSPs is 400 nm and the amount of adsorbed carbamoylated CD was about 20 weight% which results in a concentration of adsorbed carbamoylated CD of 5.8 μmol/m^2. This corresponds to about 30 times higher surface concentration of CD as to the CSPs presented in this and a recently reported paper [4]. The pore of the adsorbed materials is thus more or less coated with a multilayer of carbamoylated CD. Other possibilities of interaction for the enantiomers, due to the very high surface concentration of CD, is probably responsible for the different enantioselectivity for the adsorbed materials.

Separation of racemates using different chromatographic conditions

The ability to separate racemates under other phase conditions, was briefly studied using material I C2. The results presented in Table V show that even under reverse phase conditions and under supercritical fluid chromatographic conditions, some racemates are separated.

Some of the enantioselectivities of CSPs were compared under reverse phase conditions using water/acetonitril 1/1 (v/v) as mobile phase. The two rather similar materials Cyclobond I DMP and material II E showed lower selectivities than the two I C2 and I A2 materials (Table VI). This is probably due to the higher degree of substitution of carbamate

TABLE V. HPLC Separations of a Variety of Racemic Solutes (Fig. 3) on Material I C2 Using Different Mobile Phases[a)]

Solute	Hex/IPA 90/10		Hex/THF 90/10		SFC		H_2O/ACN 50/50	
nr.	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	1.60(-)	~1	15.9	1.0	6.48	1.0	0.80	1.0
2	0.85(-)	~1	0.87(+)	1.11	1.91	1.05	5.07(+)	1.03
3	3.17(+)	1.42			3.96	1.0	2.73(+)	1.12
4	4.4	1.0	3.06	1.0	7.16	1.0	5.1	1.0
5	1.10(-)	1.09	1.97(+)	1.06	9.58	1.0	1.57	1.0
6	1.42(-)	1.09	2.48(-)	~1	1.85	1.0	7.5	1.0
7	5.39(-)	1.05	2.64	1.0	6.88	1.0	1.57	1.0
8	3.96(+)	1.03	2.03	1.0	3.29	1.0	1.73(+)	~1
9	2.19(+)	1.69	1.26(-)	~1	2.78	1.16	4.23(-)	1.07
10	1.79(-)	1.09	3.57	1.0	8.39	1.0	11.0(-)	1.03
11	12.7(+)	1.02			0.78	1.0	4.13(+)	~1
12	6.79(-)	1.13			3.1	1.0	1.2	1.0
13			5.35(-)	~1	1.67	1.0	0.57(-)	~1
14	9.95(-)[b)]	1.35					7.13(-)	1.32

a) Flow rate=1.0 ml/min. SFC conditions: Flow rate (CO_2)= 3.0 ml/min. Flow rate (ethanol)= 0.2 ml/min. Column pressure=200 bar. Column temperature=60 °C.
b) Mobile phase=n-hexane/2-propanol 70/30 (v/v)

TABLE VI. Reverse Phase HPLC Separations of a Variety of Racemic Solutes (Fig. 3) on Different CSPs[a)]

Solute	Cyclobond I DMP		Material II E		Material I C2		Material I A2	
nr.	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	0.84(+)	~1	0.91	1.0	0.8	1.0	0.77	1.0
2	3.6(+)	~1	4.44(+)	~1	5.07(+)	1.03	4.0(+)	1.07
3	2.28(+)	1.05	1.88(+)	~1	2.73(+)	1.12	2.0(+)	1.20
4	3.48	1.0	2.88	1.0	5.1	1.0	3.63	1.0
5	2.24	1.0	0.22	1.0	1.57	1.0	2.23(-)	~1
6	19.4	1.0	2.4	1.0	7.5	1.0	7.3	1.0
7	1.24	1.0	1.69	1.0	1.57	1.0	1.27	1.0
8	1.48(+)	~1	1.38	1.0	1.73(+)	~1	1.4(+)	~1
9	2.98(-)	1.05	3.44(-)	~1	4.23(-)	1.07	3.73(-)	1.09
10	6.16(-)	~1	11.1(-)	~1	11.0(-)	1.03	8.73(-)	1.06
11	2.72(+)	~1	3.31(+)	~1	4.13(+)	~1	3.23	1.0
12	1.16	1.0	1.09	1.0	1.2	1.0	1.0	1.0
13	0.72	1.0	0.50(-)	~1	0.57(-)	~1	0.47(-)	~1
14	4.76(-)	1.21	4.44(-)	1.07	7.13(-)	1.32	6.20(-)	1.32

a) Mobile phase=water/acetonitrile 50/50 (v/v). Flow rate=1.0 ml/min.

groups and the opposite orientation of the immobilized β-CD on materials I C2 and I A2 compared to Cyclobond I DMP and material II E. It is difficult to determine if another separation mechanism is responsible for the selectivity under reverse phase conditions on the completely carbamoylated β-CD materials I C2 and I A2. With the racemates used in this paper, probably very similar separation mechanism is responsible for the separations independent of the chromatographic mode. The results recently reported on separations of racemates on Cyclobond I DMP show that this material, which is partly carbamoylated, works with different separation mechanism depending on the used chromatographic modes [6], *i.e.*, the material is described as a true bimodal CSP. Further work has to be done to investigate if also the complete carbamoylated materials, presented in this and the recent papers [4], have the ability to separate racemates with inclusion complexation mechanism under reverse phase conditions.

The influence of average pore diameter of the silica particles

The influences of pore diameter of the silica particles on the amount of immobilized β-CD and on the enantioselectivity were studied. The average pore diameter for the original silica particles on material I A(60), I A1(100), I A2(100) and I A(300) were 60, 100 and 300 Å, respectively. The results in Table VII show that very low amounts of functionalized β-CD were immobilized on material I A(60), perhaps due to steric restrictions of the carbamoylated β-CD molecules to enter the smallest pores in the amino functionalized silica particles. Rather low amount of functionalized β-CD was also immobilized on material I A(300). This is probably due to the limited available surface area of the silica particles. Although the surface concentration of carbamoylated β-CD was highest on material I A(300), about 0.17 $\mu mol/m^2$, the highest amount of immobilized carbamoylated β-CD was obtained on material I A2(100), about 11 weight% carbamoylated β-CD. A simple calculation on the degree of covering of the silica surface with carbamoylated β-CD results in that about 32 % of the surface on material I A2(100) is covered with the chiral selector while about 46 % of the surface on material I A(300) is

TABLE VII
Charactarization Data of CSPs Prepared by Using Silica Particles of Different Pore Size

	Material I			
	A(60)	A 1(100)	A 2(100)	A(300)
Particle size (μm)	5	5	5	10
Average pore diameter (Å)	60	100	100	300
Surface area (m^2/g)[a]	340	281	281	101
wt% carbamoylated β-CD	3	6	11	7
Surface conc (μmol/m^2)	0.02	0.05	0.10	0.17

a) Surface area of amino functionalized silica particles.

covered with the chiral selector. The calculations are based on an estimated surface area of one carbamoylated β-CD molecule with a shape of a circle of 3.1 nm^2 and assuming a close packing of circles.

The enantioselectivity of the materials was compared and the results are presented in Table VIII. The enantioselectivity of material I A(60) is lower than those of other materials. This is expected as this material contains the lowest amount as well as the lowest surface concentration of carbamoylated β-CD. Comparison of the enantioselectivity of material I A 1(100), I A 2(100) and I A(300) shows that the surface concentration affects the enantioselectivity of the materials . The separation factor (α) for racemate nr 3 and 9 increases while the separation factor for racemate nr 10 decreases as the surface concentration of carbamoylated β-CD increases. The increasing enantioselectivity can be associated with reduced nonspecific interactions between the enantiomers and the silica surface or with increased stereospecific interaction possibilities between the enantiomers and a number of closely orientated carbamoylated β-CD molecules. An increase of enantioselectivity with an increase of surface concentration of functionalized β-CD is also reported for similar CSPs prepared with two silicas having different pore sizes, 300 and 120 Å [7]. The CSP with the highest surface concentration of functionalized β-CD (about 0.2 μmol/m^2), prepared by using the silica particle with the pore size of 300 Å, almost always showed the higher enantioselectivity.

TABLE VIII

NORMAL PHASE HPLC SEPARATION ON CSPs PREPARED WITH SILICA PARTICLES OF DIFFERENT PORE SIZE

Solute	Material I A(60)		Material I A1(100)		Material I A2(100)		Material I A(300)	
nr.	k'_1	α	k'_1	α	k'_1	α	k'_1	α
3	1.10(+)	1.23	1.45(+)	1.25	2.31(+)	1.44	1.26(+)	1.60
5	1.97(-)	~1	0.99	1.0	0.99(-)	1.16	0.58(-)	~1
9	0.98(+)	1.19	1.42(+)	1.39	2.24(+)	1.98	1.25(+)	1.57
10	0.73	1.0	1.27(-)	1.07	1.95(-)	1.12	0.81	1.0

Separation data of a variety of racemic solutes (Fig 3). k'_1 = capacity factor and optical rotation of the first eluted enantiomer. Separation factor (α) = (k'_2/k'_1). Mobile phase = n-hexane/2-propanol, 90/10 (v/v). Flow rate = 1.0 mL/min.

CONCLUSION

Compared to the commercial Cyclobond I DMP material, higher enatioselectivity and an increase number of separable racemates were observed on material I C2, at least for the 14 racemates examined here. The enantioselectivity of the materials was increased with an increasing degree of substitution of carbamate groups on cyclodextrin and an increasing surface concentration of cyclodextrin, if the cyclodextrin was immobilized to the silica particles at position C_2 or C_3. The enantioselectivity of the materials was also affected by the type of α-, β- or γ-CD. Separation of racemates was also performed under reverse phase and SFC chromatographic conditions but better selectivities were observed under normal phase chromatographic conditions.

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