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Preparation and chromatographic evaluation of 3,5-dimethylphenyl carbamoylated β-cyclodextrin stationary phases for normal-phase high-performance liquid chromatographic separation of enantiomers

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ABSTRACT

Thirteen chiral stationary phases (CSPs) were prepared from β-cyclodextrin (β-CD) and their chiral recognition abilities were evaluated under normal-phase conditions by high-performance liquid chromatography. All materials were prepared by three different methods and contained 3,5-dimethylphenylcarbamate residues. The influence of different spacers, the amount of immobilized carbamoylated β-CD and the degree of substitution on β-CD on the enantioselectivity of the CSPs was considered. The results suggest that the enantioselectivity of the materials is favoured by high degrees of substitution of carbamate groups on β-CD and large amounts of chemically bonded carbamoylated β-CD. The highest selectivities were obtained on CSPs where complete carbamoylated β-CD was immobilized on the silica surface.

INTRODUCTION

β-Cyclodextrin (β-CD) is a cyclic natural oligomer containing seven glucose units connected via α-(1,4)-linkages. Schematically, the shape of β-CD can be presented as a truncated cone with seven primary hydroxy groups attached to the smaller opening of the cone while the remaining fourteen secondary hydroxy groups are located on the larger opening of the cone. Both unfunctionalized and functionalized β-CDs, chemically bonded to silica, have been reported to separate a variety of analytes by HPLC under reversed-phase conditions [1,2]. The separation mechanism has often been claimed to depend on the formation of inclusion complexes where the guest molecule enters the relative hydrophobic interior of the cone from the larger side of the opening of the cone. This has traditionally resulted in β-CD being chemically bonded to support particles via the primary hydroxy groups situated on the smaller opening of the cone.

Recently, the normal-phase separation of enantiomers on a chiral stationary phase (CSP)-containing partly naphtylethylcarbamate-substituted β-CD has been reported [3,4]. As the maximum degree of substitution per β-CD is about 7 [4], both carbamate groups and remaining unreacted hydroxy groups can interact with the enantiomeric analytes and thus contribute more or less to the separation mechanism. The separation mechanism on this CSP has been considered to act more like the Pirkle-type chiral stationary phases [3].

Enantiomeric separations on 3,5-dimethylphenylcarbamate-functionalized polysaccharides have been reported to be successful [5]. In fact, 343

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(80%) of 483 racemic compounds could be resolved on at least one of the CSPs containing tris (3,5-dimethylphenylcarbamate)-functionalized cellulose or amylose [6]. The complete substitution of hydroxy groups with 3,5-dimethylphenyl isocyanate on α, β- or γ-cyclodextrin has recently been reported [7]. The carbamoylated cyclodextrins were adsorbed on silica particles and chiral separations were obtained using the normal-phase chromatographic mode. The practical use of the prepared CSPs is limited, however, as highly non-polar solvents must be used as eluents.

In this paper we report the use of CSPs containing 3,5-dimethylphenylcarbamate-functionalized β-CD, chemically bonded to silica, thus avoiding the limited practical use of the above-mentioned adsorbed carbamoylated CD materials. Thirteen CSPs were prepared by using different preparation methods and different spacers. Enantioselectivity of the CSPs was chromatographically evaluated under normal-phase conditions with a variety of racemic solutes. The influence of the degree of substitution on the β-CD and the amount of immobilized carbamoylated β-CD on the enantioselectivity is discussed briefly.

EXPERIMENTAL

Chemicals

The racemic solutes were obtained from different sources. β-Cyclodextrin (β-CD) of guaranteed reagent grade was purchased from Nacalai Tesque (Kyoto, Japan). Triphenylmethyl chloride (trityl chloride) and the spacers 4,4′-diphenylmethane diisocyanate (A), hexamethylene diisocyanate (B) and suberoyl chloride (C) were of guaranteed reagent grade from Tokyo Kasei (Tokyo, Japan) and dodecanedioyl dichloride (D) (98%) was obtained from Aldrich (Milwaukee, WI, USA). 3,5-Dimethylphenyl isocyanate was synthesized from 3,5-dimethylaniline by a conventional method. The silylating reagent 3-aminopropyltriethoxysilane (99%) was obtained from Janssen Chimica (Geel, Belgium) and the silylating agent 3-isocyanatopropyltriethoxysilane (spacer F) from Lancaster Synthesis (Morecambe, UK). Develosil, a totally porous spherical silica gel with a mean particle size of 5 μm, a mean pore diameter of 100 Å and a specific surface area of 345 m^2/g, was purchased from Nomura Chemical (Nagoya, Japan). Daisogel, a totally porous spherical silica gel with a mean particle size of 5 μm, a mean pore diameter of 120 Å and a specific surface area of 309 m^2/g, was a gift from Daiso (Osaka, Japan). All solvents used in the preparation of the CSPs were of at least analytical-reagent grade and carefully dried before use. Solvents used in the chromatographic experiments were of HPLC grade.

Preparation of chiral stationary phases

Method 1. β-CD (0.5 g) was added to either pure pyridine (12 ml) or tetrahydrofuran (THF)–pyridine (10 + 2 ml), depending on whether isocyanate (pyridine) or acid chloride (THF–pyridine) functionalized spacers (A, B, C or D) were used in the derivatization procedure. About 90 mol% of the available hydroxy groups on the β-CD were reacted with 3,5-dimethylphenyl isocyanate (1.1–1.3 g) for 3 h at 90°C under a nitrogen atmosphere. The disappearance of the isocyanate groups, –N=C=O (2200–2300 cm^{-1}) and appearance of carbonyl groups (1700 cm^{-1}) was monitored by FT-IR spectrometry. A twofold excess of the spacer (A, B, C or D), based on moles of unreacted hydroxy groups on β-CD, was added to the reaction mixture and the reaction was continued for 3 h at 90°C. After evaporation fo the solvents under reduced pressure, the modified β-CD was washed with 3 × 10 ml of hexane and then dissolved in THF–pyridine (10 × 2 ml). The β-CD solution was injected on to previously vacuum-dried (180°C, 4 h) amino-functionalized silica gel (3.0 g) and reacted at 90°C overnight. The CSPs obtained were filtered and washed carefully with an excess of THF, N,N-dimethylacetamide (DMA), pyridine, methanol, water, THF, and hexane and finally dried under vacuum at 60°C for about 5 h. The carbon contents of phase I materials are approximately comparable to the immobilized carbamoylated β-CD contents determined by the mass difference of the dried particles before and after functionalization.

Method 2. To a suspension containing previously vacuum-dried amino-functionalized silica gel (3.0 g, 180°C, 4 h) and THF–pyridine (10 + 1 ml) was added 0.03 mmol of a spacer (A, B, C or D) per gram of silica. The amount of spacer added corresponds to the same amount of immobilized carbamoylated β-CD prepared by method 1 if every spacer only binds to one β-CD molecule. After

completion of reaction with the spacer (3 h, 90°C, nitrogen atmosphere), a small excess of β-CD (about 1.3 mol excess based on moles of spacer) was added to the reaction solution together with DMA–pyridine (10 + 1 ml) and reacted for 3 h at 90°C. A twofold excess of 3,5-dimethylphenyl isocyanate (based on moles of unreacted hydroxy groups on β-CD) was finally added to the reaction mixture and reacted overnight at 90°C. The CSPs obtained were filtered and washed as described in method 1. The amount of immobilized β-CD was measured as the mass difference of the dried particles before and after functionalization.

Method 3. Pure spacer F, or a previously prepared spacer solution of E (see below), was dropwise added to a pyridine solution (10 ml) of β-CD (0.5 g) and reacted at 90°C for 1 h under a nitrogen atmosphere. A twofold excess of 3,5-dimethylphenyl isocyanate (2.8 g) (based on moles of unreacted hydroxy groups) was then added to the reaction solution and reacted for about 4 h at 90°C. The reaction solution was finally added to previously vacuum-dried silica gel (3.0 g, 180°C, 4 h) and reacted overnight at 90°C. The CSPs obtained were filtered and washed as described in method 1. The materials obtained were end-capped with trimethylchlorosilane (2 ml) in benzene–pyridine (30 + 1 ml). The end-capped materials were filtered and washed as described in method 1. The amount of immobilized β-CD was measured as the mass difference of the dried particles before and after functionalization with β-CD, *i.e.*, before end-capping of the material.

The spacer solution of E was prepared the following way: a solution of spacer B in pyridine (3 ml) was prepared so that the final amount of prepared spacer E would correspond to two spacers per β-CD molecule (about 10 mol% of available hydroxy groups on β-CD). A small excess of 3-aminopropyltriethoxysilane (1.1 mol excess based on moles of added spacer B) was added dropwise to the solution for 5 min at 50°C and allowed to react for 1 h at 50°C.

Preparation of NMR samples

The completely carbamoylated β-CD sample was prepared by adding an excess of 3,5-dimethylphenyl isocyanate (2.5 g) to a pyridine solution (10 ml) of β-CD (0.5 g). After 4 h of reaction at 90°C, the carbamoylated β-CD was precipitated in methanol–water (4:1, v/v), filtered and vacuum dried at 110°C for 4 h. Elemental analysis data supported the formation of almost completely carbamoylated β-CD (found, C 65.45, H 6.11, N 6.80; calculated, C 65.66, H 6.18, N, 6.96%) [7]. The partly carbamoylated β-CD sample was prepared as described above with the difference that only 20 mol% of available hydroxy groups on β-CD were reacted with 3,5-dimethylphenyl isocyanate. The position C-2- and C-3-carbamoylated β-CD sample was prepared by adding an excess of trityl chloride (1.3 g) to a pyridine solution (14 ml) of β-CD (0.5 g) [8]. After reaction overnight at 90°C, an excess of 3,5-dimethylphenyl isocyanate (1.9 g) was added to the solution and allowed to react for 5 h at 90°C. The product was precipitated in methanol–water (4:1, v/v), filtered and vacuum dried at 80°C for 4 h. The trityl groups on position C-6 were removed by treatment with concentrated hydrochloric acid–methanol solution for 1 h at room temperature. The methanol-soluble products were precipitated in pure water, filtered and finally vacuum dried at 110°C overnight.

Preparation of amino-functionalized silica gel

Vacuum-dried silica gel (20 g, 180°C, 4 h) was suspended in a mixture of 250 ml of dry benzene and 3 ml of dry pyridine under a nitrogen atmosphere. 3-Aminopropyltriethoxysilane (5 ml) was added to the suspension and the reaction mixture was refluxed for about 15 h. The amino functionalized silica gel obtained was washed thoroughly with an excess of THF, methanol, acetone and hexane and finally dried under vacuum at 60°C for about 5 h. The amount of bonded 3-aminopropyltriethoxysilyl groups was determined by elemental analysis: for develosil, C 4–5, N 1.2%; for Daisogel, C 3.2, N 1.0%.

Treatment of amino-functionalized silica gel with isocyanate

3,5-Dimethylphenyl isocyanate (0.65 g) was added to a suspension of previously vacuum-dried amino-functionalized silica gel (3.0 g, 160°C, 2 h) in pyridine (10 ml). The reaction mixture was refluxed overnight and the partly isocyanate-treated silica gel obtained was filtered and washed as described for the preparation of amino-functionalized silica gel.

Apparatus and chromatography

The specific surface area of amino-functionalized silica gel was measured with a Micromeritics (Norcross, GA, USA) Flowsorb II 2300 Flowsorber. ^{1}H NMR spectra were obtained on a Varian VXR-500 NMR spectrometer operating at 500 MHz, using pyridine-d_5 as the solvent and tetramethylsilane (TMS) as the internal standard. All ^{1}H NMR spectra were recorded at 80°C. The sample tube size was 5 mm and the sample concentrations were *ca.* 10 mg/ml. Assignment of the three peaks of methyl groups was made on the basis of known results [8].

The carbamoylated β-CD bonded to silica gels was packed into 250 × 4.6 mm I.D. stainless-steel columns by conventional high-pressure slurry-packing procedures. The chromatographic experiments were performed using a Jasco BIP-I HPLC pump, a Jasco 875 UV detector (254 nm), a Jasco DIP-181C polarimeter (Hg lamp, no filter, 5 × 0.3 cm I.D.) and a Jasco RC-228 recorder at room temperature. About 1–5 μl of a solution of a racemate was injected into the chromatographic system (20-μl loop) with a Rheodyne Model 7125 injector. *n*-Hexane–2-propanol (90:10, v/v) was usually used as the eluent at a flow-rate of 0.5 ml/min. The dead time (t_0) was determined with 1,3,5-tri-*tert.*-butylbenzene.

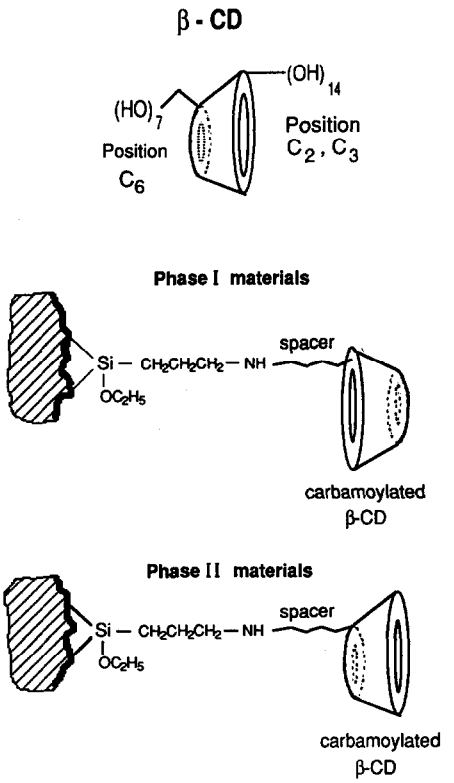

Fig. 1. Schematic model of β-cyclodextrin and phase I and phase II chiral stationary phases.

RESULTS AND DISCUSSION

Characterization of prepared materials

The chiral stationary phases consisting of 3,5-dimethylphenylcarbamoylated β-cyclodextrin bonded to silica particles were prepared by different methods. The materials obtained were divided into two groups of CSPs, phase I and phase II materials. In phase I materials, probably a large fraction of the immobilized β-CD molecules are bonded to the silica surface by the larger opening of the truncated cone of β-CD. In phase II materials, probably the smaller opening of the cone is bonded to the silica surface (Fig. 1). The CSPs are named according to the orientation of the immobilized β-CD molecule, using the roman numerals I or II, and according to the spacer used. Six different difunctional spacers, A, B, C, D, E and F, shown in Fig. 2, were used to immobilize β-CD. However, depending on the preparation method used, materials IIB and IIE contain identical linkages between the silica surface and the carbamoylated β-CD and are therefore comparable. The number of spacers per β-CD, in Tables I and II, were calculated on the assumption that all added spacers during the preparation of the CSPs were used as spacers. The carbon contents of phase I materials are approximately comparable to the immobilized carbamoylated β-CD contents determined by gravimetric analysis (Table I).

The different phase I and II materials were prepared by utilizing the commonly accepted fact that the seven primary hydroxy groups, located on the smaller opening of the truncated cone (position C-6), have higher reactivities than the fourteen secondary hydroxy groups (positions C-2 and C-3) (Fig. 1) [1]. The higher reactivity of the primary hydroxy groups was confirmed by NMR analysis on three different carbamoylated β-CD samples. Three peaks were detected for the three different positioned carbamoylated groups (Fig. 3a). The posi-

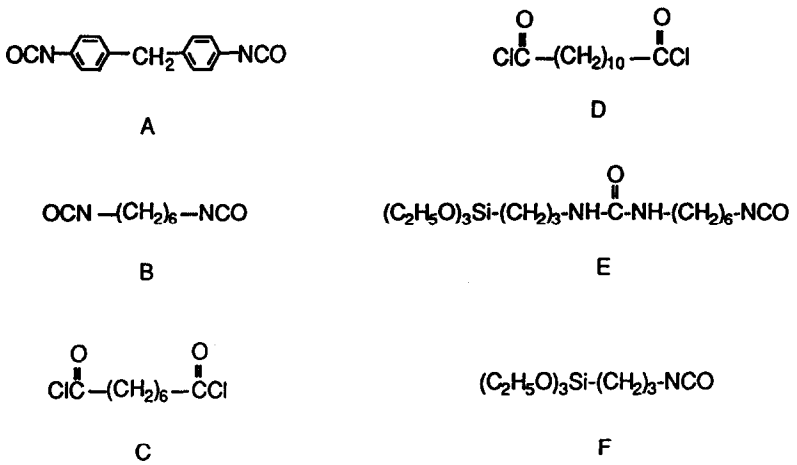

Fig. 2. Difunctional spacers used for immobilization of β-cyclodextrin on silica particles.

tions of the primary *versus* the secondary carbamoylated groups were distinguished by using triphenylmethyl chloride (trityl chloride) as a selective protecting reagent for the primary hydroxy groups [8], thus obtaining predominately position C-2- and C-3-carbamoylated β-CD (Fig. 3b). The higher reactivity of primary hydroxy groups was confirmed by analysis on a partly carbamoylated β-CD sample where about four (20%) of the total available twenty-one hydroxy groups on β-CD were carbamoylated (see *Preparation of NMR samples*). The resulting NMR spectra show only one peak at the position for primary hydroxy carbamoylated groups (Fig. 3c).

Phase I materials were prepared by carbamoylation of about 85–95 mol% of the available hydroxy groups on β-CD (18–20 OH per β-CDs) with 3,5-dimethylphenyl isocyanate, according to preparation method 1. The remaining unreacted secondary hydroxy groups were reacted with a difunctional spacer and the spacer-functionalyzed β-CD obtained were immobilized on amino-functionalized silica particles. The degree of carbamoylation on β-CD was confirmed by elemental analysis of nitro-

TABLE I

CHARACTERIZATION OF PHASE I MATERIALS PREPARED BY METHOD 1

Parameter	Chiral stationary phase						
Material	IA1	IA2	IB1	IB2	IC1	IC2	ID1
Spacer	A	A	B	B	C	C	D
No. of spacers per β-CD[a]	3–4	1	3	2	3	1–2	1
β-CD (mass %)[b]	6	11	14	17	10	13	12
C_{tot} (%)[c]	5.5	9.3	10.1	11.9	7.0	10.9	9.5
β-CD (mass %)[d]	8	14	16	19	11	17	14
β-CD (μmol/m²)[e]	0.05	0.10	0.13	0.16	0.09	0.12	0.11

[a] Molar ratios of spacer to β-CD used.
[b] Based on gravimetric analysis on silica-bonded carbamoylated β-CD.
[c] Due to functionalized phase, including spacer and carbamoylated CD but excluding carbon content on the silica surface.
[d] Based on C elemental analysis.
[e] Surface concentration of carbamoylated β-CD based on gravimetric results for mass% immobilized β-CD.

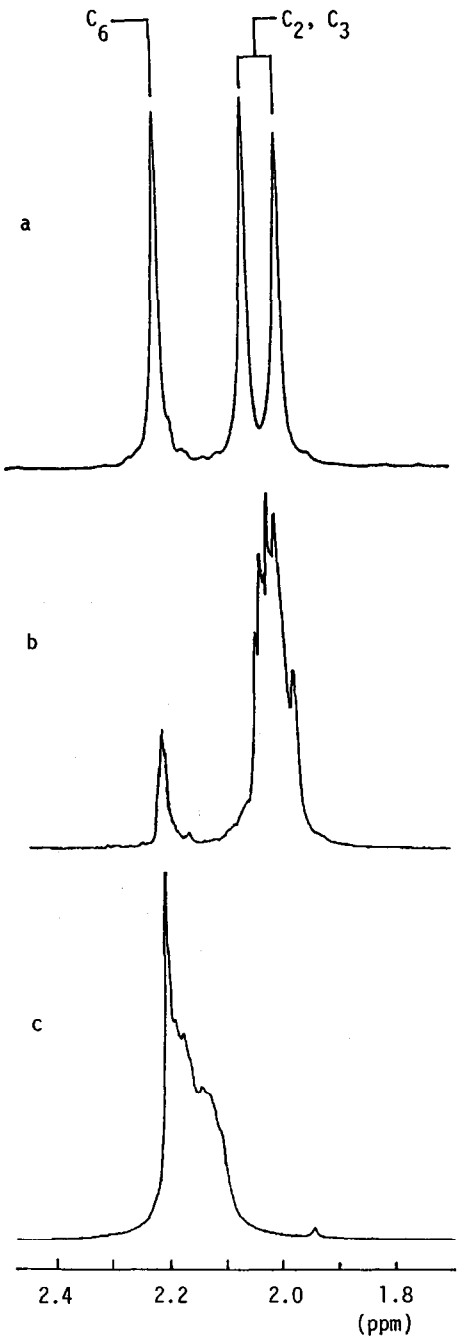

Fig. 3. ^1H NMR spectra of the CH_3 protons of 3,5-dimethylphenylcarbamate of β-cyclodextrines: (a) 100 mol% carbamoylated; (b) C-2-, C-3-carbamoylated; (c) 20 mol% carbamoylated. Pyridine-d_5, 80°C, 500 MH.

gen for a 95% carbamoylated β-CD, the calculated and found nitrogen contents being 6.87 and 6.88%, respectively. The surface concentration of silica-bonded carbamoylated β-CD for phase I materials is about 0.12 μmol/m^2 calculated from gravimetric results (Table I). This is comparable to reported results [9] of 0.12 and 0.19 μmol/m^2 of hydroxypropyl-functionalized β-CD, depending on the specific surface area of the bare silica, 170 and 100 m^2/g, respectively. As the surface area of the amino-functionalized silica gel used in this work is 290 m^2/g (bare silica 345 m^2/g), high absolute amounts of bonded carbamoylated β-CD are prepared by method 1 (Table I).

Phase II materials were prepared by two different methods. In preparation method 2, spacers were first bonded to amino-functionalized silica particles, then unmodified β-CD was immobilized by reaction between the spacers and the primary hydroxy groups on the β-CD. The remaining hydroxy groups on the immobilized β-CD were finally carbamoylated with an excess of the derivatizing agent. In method 3, a small amount of spacer (about two spacers per β-CD) was first reacted with the primary hydroxy groups on β-CD followed by carbamoylation of the remaining unreacted hydroxy groups. The functionalized β-CD obtained was then immobilized on unmodified silica particles and the materials obtained were finally end-capped with trimethylchlorosilane.

Preparation of phase II materials results in small amounts of immobilized carbamoylated β-CD compared with phase I materials (Table II). The gravimetrically determined values of the amount of immobilized carbamoylated β-CD, prepared according to method 2, are influenced by the formation of urea groups as a fraction of the added amount of 3,5-dimethylphenyl isocyanate reacts with amino groups on the silica surface. In an experiment in which amino-functionalized silica particles were reacted with a twofold molar excess of 3,5-dimethylphenyl isocyanate, based on moles of amino groups, about 0.03 g of urea groups per gram of silica particles was formed. The amount of immobilized carbamoylated β-CD of CSPs prepared by method 2 is probably smaller because not all of the available hydroxy groups were carbamoylated, even though a twofold excess of the derivatizing agent was used. Similar results of low degrees of

TABLE II

CHARACTERIZATION OF PHASE II MATERIALS PREPARED BY METHODS 2 AND 3

Parameter	Method 2			Method 3		
Material	IIA	IIB	IIC	IID	IIE	IIF
Spacer	A	B	C	D	E	F
No. of spacers per β-CD[a]	1	1	1	1	2	2
β-CD (mass%)[b]	7–9	7–9	6–8	5–7	6	3

[a] Molar ratio of spacer to β-CD used.
[b] Based on gravimetric analysis of silica-bonded carbamoylated β-CD.

substitution on immobilization of unfuntionalized β-CD have been reported [3,10]. A simple calculation, assuming that the same number of unfunctionalized β-CD molecules as carbamoylated β-CD molecules by preparation method 1 were immobilized by preparation method 2, indicates that only about ten of the available twenty-one hydroxy groups of β-CD are carbamoylated. This is comparable to values of seven and ten reported for the degree of substitution of β-CD bonded to silica with naphthylethyl isocyanate and 2,6-dimethylphenyl isocyanate, respectively [3]. By using preparation method 3, completely carbamoylated β-CD (calculated and found nitrogen contents 7.4 and 7.6%, respectively) was immobilized on the silica particles. The small amount (3%, w/w) of immobilized β-CD on material IIF can be ascribed to steric difficulties with the relative short spacer arm of spacer F reacting with the silica surface. A satisfactory explanation for the small amount of immobilized β-CD on material IIE cannot be given.

Different batches of amino-functionalized silica gels were prepared. The surface concentration of bonded amino-groups, which was calculated on results obtained from on C and N elemental analysis, was 2.7 μmol/m^2. The average number of reacted ethoxy groups per 3-aminopropyltriethoxysilane was calculated to be 1.9 which corresponds to 3.0 hydroxy groups/nm^2 having reacted on the silica surface. These results can be compared with reported values of chemically reactive hydroxy groups on silica surfaces, based on reactions of monosilanes, of 2.3 [11] and 2.7 [12] hydroxy groups/nm^2.

Most columns exhibited a theoretical plate number of 3000 per column with benzene as solute. No improvements in the theoretical plate number by varying the packing procedure were observed.

Chromatographic evaluation

The chromatographic results for the optical resolution of fourteen racemic solutes (Fig. 4) are presented in Tables III, IV and V. Thirteen CSPs were prepared and chromatographically evaluated, seven phase I materials and six phase II materials. Also, different spacers were used for the immobilization of β-CD, the amount of carbamoylated β-CD bonded to silica and the number of spacers bonded to each carbamoylated β-CD being varied. As a general conclusion, phase I materials show superior enantioselectivity to phase II materials. On phase I materials, eleven of thirteen racemates were separated whereas on phase II materials only five of fourteen racemates were separated.

Phase I materials. For most racemates an increase in selectivity was observed as the amount of immobilized carbamoylated β-CD increased. For racemate **5**, however, a higher selectivity was observed on the materials containing smaller amounts of carbamoylated β-CD (materials IB and IC, Table III). A reverse elution order was also observed for racemate **5** depending on whether large or small amounts of β-CD were immobilized. An increased amount of bonded carbamoylated β-CD resulted in higher capacity factors. A few exceptions from these observations are for racemates **7** and **11** eluted on mateials IA and IB, for which the capacity factors decrease with increasing amount of bonded carbamoylated β-CD (Table III). This can be ex-

TABLE III

NORMAL-PHASE HPLC SEPARATION DATA FOR PHASE I MATERIALS

Separation data for a variety of racemic solutes (Fig. 4). k'_1 = Capacity factor with optical rotation (in parentheses) of the first-eluted enantiomer. Separation factor $\alpha = k'_2/k'_1$. Mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 0.5 ml/min.

Solute No.	IA1		IA2		IB1		IB2		IC1		IC2		ID	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	3.32(−)	*ca.* 1	3.60(−)	*ca.* 1	3.93(−)	*ca.* 1	4.82(+)	*ca.* 1	1.46(−)	*ca.* 1	1.60(−)	*ca.* 1	1.85(−)	*ca.* 1
2	0.50(+)	*ca.* 1	0.72(+)	1.16	0.53(+)	*ca.* 1	0.63(+)	1.16	0.40(+)	*ca.* 1	0.85(−)	*ca.* 1	0.53(−)	*ca.* 1
3	1.45(+)	1.25	2.31(+)	1.44	2.56(+)	1.23	2.94(+)	1.45	2.54(+)	1.14	3.17(+)	1.42	1.57(+)	1.54
4	2.27	1.0	4.83	1.0	3.47	1.0	2.76	1.0	2.62	1.0	4.4	1.0	1.67	1.0
5	0.99	1.0	0.99(−)	1.16	0.84(+)	1.14	1.33(−)	*ca.* 1	0.60(+)	1.14	1.10(−)	1.09	0.90(−)	*ca.* 1
6	0.99(−)	*ca.* 1	1.35(−)	1.10	1.12(−)	*ca.* 1	1.68(−)	1.05	0.92(−)	*ca.* 1	1.42 (−)	1.09	1.01(−)	*ca.* 1
7	(k' > 26)	–	8.56(−)	*ca.* 1	8.84	1.0	4.13(+)	*ca.* 1	5.06	1.0	5.39(−)	1.05	2.52(−)	*ca.* 1
8	0.99	1.0	3.98(+)	1.03	3.0(+)	*ca.* 1	1.65	1.0	2.49(+)	*ca.* 1	3.96(+)	1.03	2.27(+)	*ca.* 1
9	1.42(+)	1.39	2.24(+)	1.98	1.46(+)	1.07	2.22(+)	1.27	1.05(+)	1.13	2.19(+)	1.69	1.28(+)	1.73
10	1.27(−)	1.07	1.95(−)	1.12	1.33	1.0	1.45	1.0	0.97	1.0	1.79(−)	1.09	0.97(−)	*ca.* 1
11	(k' > 19)	–	(k' > 23)	–	(k' > 19)	–	11.8(+)	1.07	10.1	1.0	12.7(+)	1.02	4.76(+)	1.04
12	4.36(+)	*ca.* 1	6.57(−)	1.08	3.92	1.0	7.30(−)	1.03	3.19(+)	*ca.* 1	6.79(−)	1.13	4.50(−)	1.08
14[a]	–	–	8.84(−)	1.40	7.26(−)	1.04	12.4(−)	1.27	4.38(−)	1.08	9.95(−)	1.35	6.36(−)	1.23

[a] Mobile phase = *n*-hexane–2-propanol (70:30, v/v).

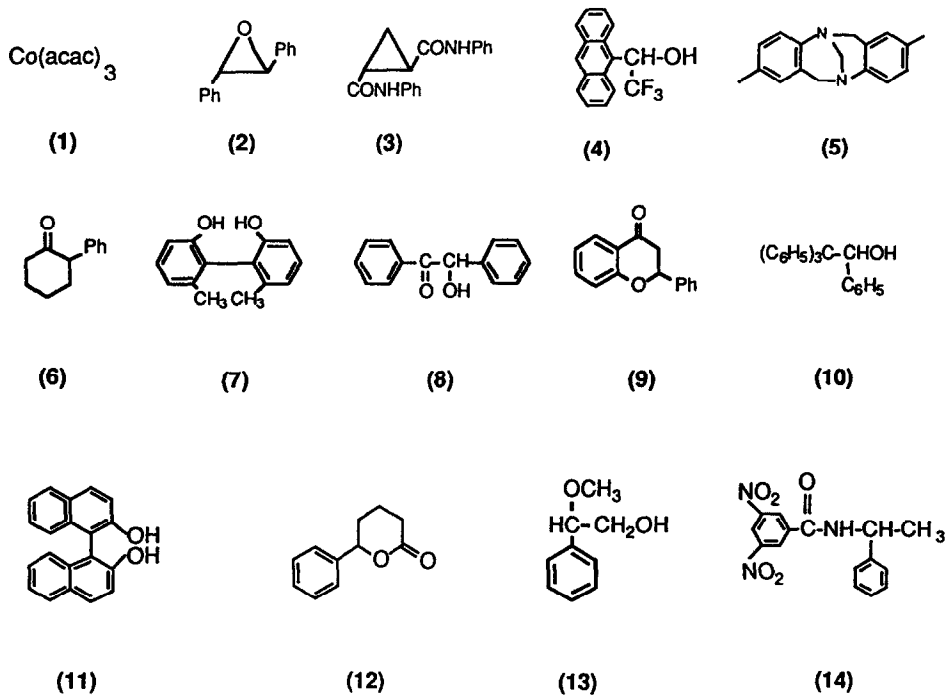

Fig. 4. Structures of racemic solutes.

plained by the strong interaction of the racemates with amino groups on the silica surface. As the amount of bonded carbamoylated β-CD increases, the effect of the strong interaction between racemates **7** and **11** and the amino groups decreases.

The four spacers A, B, C and D (Fig. 3) can be divided into two reactive functional groups, isocyanate and acyl chloride groups. The spacers bonds to β-CD through carbamate or ester bonds and to the amino groups on the silica surface by either urea or amide bonds. No significant difference in enantioselectivity, depending on the type of spacer used, could be observed between the different materials even though a reversed elution order could be detected for racemate **2** depending on the functionality of the spacers used (Table III). On the other hand, a greater influence on the enantioselectivity of the materials could be observed between the two isocyanate spacers and the two acid chloride spacers. Higher enantioselectivity was usually observed for the shorter acid chloride spacer C than the longer acid chloride spacer D. Similarly, higher enantioselectivity was usually observed for the aromatic, more rigid, space A than spacer B.

The influence of the number of bonded spacers per β-CD on the enantioselectivity is difficult to establish as the amounts of immobilized carbamoylated β-CD on the materials IA, IB and IC are different.

Phase II materials. As mentioned before, phase II materials usually have lower enantioselectivity than phase I materials. The four phase II materials, prepared according to method 2, have very similar enantioselectivities and capacity factors (Table IV); hence no significant effect of the spacers can be observed for these materials.

The two phase III materials prepared according to method 3 show an improved enantioselectivity compared with the other phase II materials in spite of the small amounts of immobilized carbamoylated β-CD (Table V). This probably depends on the higher degree of substitution of carbamate groups on β-CD achieved by method 3. The enantioselectivities of the two materials are comparable except for racemate **11**, which on material IIF exhibited the highest selectivity among all the materials prepared.

Influence of functionality of silica surface. Most of

TABLE IV

NORMAL-PHASE HPLC SEPARATION DATA FOR PHASE II MATERIALS PREPARED BY METHOD 2

Separation data for a variety of racemic solutes (Fig. 4) obtained on phase II materials prepared by method 2. k'_1 = Capacity factor with optical rotation (in parentheses) of the first-eluted enantiomer. Separation factor $\alpha = k'_2/k'_1$. Mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 0.5 ml/min.

Solute No.	IIA		IIB		IIC		IID	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	5.59(+)	*ca.* 1	8.76(+)	*ca.* 1	3.82(+)	*ca.* 1	4.89(+)	*ca.* 1
2	0.28(+)	*ca.* 1	0.31(+)	*ca.* 1	0.29(+)	*ca.* 1	0.29(+)	*ca.* 1
3	0.69(+)	1.20	0.64(+)	1.17	0.86(+)	1.23	0.67(+)	1.31
4	1.08	1.0	1.17	1.0	1.59	1.0	1.29	1.0
5	0.57	1.0	0.62(−)	*ca.* 1	0.55(−)	*ca.* 1	0.60(−)	*ca.* 1
6	0.71(−)	*ca.* 1	0.72(−)	*ca.* 1	0.74(−)	*ca.* 1	0.70(−)	*ca.* 1
7	0.94	1.0	1.10	1.0	1.54	1.0	1.17(+)	*ca.* 1
8	1.41	1.0	1.45(+)	1.05	1.50(+)	1.07	1.37(+)	1.07
9	0.71(+)	*ca.* 1	0.79(+)	*ca.* 1	0.75(+)	1.13	0.74(+)	1.04
10	0.56	1.0	0.60	1.0	0.66	1.0	0.58	1.0
11	1.99(+)	1.05	2.40(+)	*ca.* 1	3.15(+)	*ca.* 1	2.38(+)	1.06
12	2.39(+)	*ca.* 1	2.86(+)	*ca.* 1	2.78(+)	*ca.* 1	3.03(+)	*ca.* 1
14[a]	–	–	4.40(−)	1.09	4.35(−)	1.12	–	–

[a] Mobile phase = *n*-hexane–2-propanol (70:30, v/v).

TABLE V

NORMAL-PHASE HPLC SEPARATION DATA FOR PHASE II MATERIALS PREPARED BY METHOD 3

Separation data for a variety of racemic solutes (Fig. 4). k'_1 = Capacity factor with optical rotation (in parentheses) of the first-eluted enantiomer. Separation factor $\alpha = k'_2/k'_1$. Mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 1.0 ml/min.

Solute No.	IIE[a]		IIE[b]		IIF	
	k'_1	α	k'_1	α	k'_1	α
1	2.23	1.0	0.82	1.0	0.41	*ca.* 1
2	0.20(+)	*ca.* 1	0.15(+)	*ca.* 1	0.15(+)	*ca.* 1
3	0.53(+)	1.36	0.38(+)	1.43	0.28(+)	1.47
4	0.62	1.0	0.46	1.0	0.29	1.0
5	1.63(−)	*ca.* 1	0.25(−)	*ca.* 1	0.38(−)	*ca.* 1
6	0.50(−)	*ca.* 1	0.56	1.0	0.41(−)	*ca.* 1
7	0.63(−)	1.09	0.50(−)	*ca.* 1	0.35(−)	*ca.* 1
8	0.88(+)	*ca.* 1	0.50	1.0	0.44(+)	*ca.* 1
9	0.46(+)	1.47	0.34(+)	1.82	0.35(+)	1.60
10	0.37	1.0	0.31	1.0	0.23	1.0
11	1.00(+)	1.06	0.69(+)	1.09	0.47(+)	1.25
12	2.03	1.0	0.79(−)	*ca.* 1	0.88(−)	*ca.* 1
13	1.00(+)	*ca.* 1	0.32(+)	*ca.* 1	0.29	1.0
14	6.20(−)	1.29	1.40(−)[c]	1.23	0.82(−)	1.36

[a] Before end-capping with trimethylchlorosilane.
[b] After end-capping with trimethylchlorosilane.
[c] Mobile phase = *n*-hexane–2-propanol (70:30, v/v).

the racemates show significantly lower capacity factors (k') on phase II than phase I materials. This can be explained by the functionality of the silica surface and to some extent also by the smaller amounts of immobilized carbamoylated β-CD on the phase II materials. During the preparation of phase II materials by method 2, some of the available amino groups are partly converted into urea residues, which should influence the surface functionality on the silica gel. The effect of a partly urea-functionalized silica surface on the capacity factors of the racemates was compared with capacity factors obtained on the pure amino-functionalized silica gel. About 30 mol% of the available amino groups on the silica surface reacted with 3,5-dimethylphenyl isocyanate. The results in Table VI confirm that the functionality of the silica surface influences the capacity factors of the racemates, especially for materials with a small amount of bonded carbamoylated β-CD. Some racemates, **7** and **11**, interact very strongly with amino groups on the silica surface, and racemate **1** interacts strongly with the partly isocyanate-reacted silica surface. These results explain the higher capacity factors for racemate **1** on phase IIA, B, C and D materials compared with phase I materials and the lower capacity factors for racemates **7** and **11** on phase IIA, B, C and D materials compared with those on phase I materials (Tables III and IV).

The capacity factors for the racemates are lower on the trimethylsilane end-capped materials IIE and IIF than on the other materials. The influence of end-capping on material IIE is presented in Table V. The capacity factors for the racemates decrease after the end-capping reaction. The selectivities for racemates **7** and **9** are affected by the end-capping reaction. The separation factor (α) for racemate **9** increases whereas α decreases for racemate **7** after the end-capping reaction.

Chiral separation mechanism. There are many reasons for believing that the enantiomeric separation mechanism is not due to the formation of inclusion complexes in the relatively hydrophobic interior of the truncated cone on completely carbamoylated β-CD. As the normal-phase chromatographic mode was used to evaluate the materials, probably the interior is occupied with the non-polar eluent (hexane) [13]. Each glucose unit contains five chiral centres. Two of these chiral centres are on the C-2 and C-3 atoms. The carbamate groups on C-2 are located at the entrance of the cone and point in a clockwise direction whereas those on C-3 are located on the outside of the cyclodextrin molecule and point in a counter-clockwise direction [14]. Hence the number and orientation of carbamate groups on β-CD have a large effect on the enantioselectivity of the materials.

TABLE VI

NORMAL-PHASE HPLC SEPARATION DATA FOR FUNCTIONALIZED SILICA PARTICLES

Separation data for a variety of racemic solutes (Fig. 4) obtained on amino-functionalized and partly isocyanate-treated amino-functionalized silica gel. k' = Capacity factor. Mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 0.5 ml/min.

Solute No.	k'	
	Amino-silica	Isocyanate-treated silica
1	0.58	10.99
2	0.12	0.12
3	0.72	0.25
4	2.01	0.47
5	0.28	0.71
6	0.34	0.41
7	Not eluted	0.42
8	0.30	0.27
9	0.23	0.33
10	0.59	0.23
11	Not eluted	0.79
12	1.21	1.78

Comparison with other CSPs functionalized with 3,5-dimethylphenylcarbamate groups. Recently CSPs have been prepared by complete carbamoylation of α, β- or γ-CD with 3,5-dimethylphenyl isocyanate, followed by adsorption of the carbamoylated cyclodextrins on amino-functionalized silica gel [7]. The CSPs prepared were chromatographically evaluated with racemates **1**, **2**, **4**, **5** and **6**. The only racemates that were better or comparably separated on the materials presented in this paper are **5** and **6**; the latter racemate was not separated at all on the adsorbed β-CD CSP. The three other racemates (**1**, **2** and **4**) were all separated on the adsorbed β-CD CSP; in fact, **1** and **4** were the only racemates that could not be separated on the materials presented in this paper.

Racemates **1**–**10** have been separated on CSPs

containing the tris(3,5-dimethylphenylcarbamate) of cellulose or amylose [5]. Of the ten racemates, only racemate **9** has a higher separation factor (α) on the phase I materials presented in this paper.

CONCLUSIONS

Many factors influence the separation mechanism on the materials considered here. The influences of the degree of substitution of carbamate groups on β-CD and the amount of immobilized carbamoylated β-CD seem to be the most important factors for the enantioselectivity. Other factors such as the functionality and chain length of the spacer arm, the orientation of immobilized β-CD and the functionality of the silica surface all contribute to the total interaction possibilities and thus also influence the separation mechanism. More work remains to be done before more convincing explanations can be presented for the separation mechanism. The inclusion complexation mechanism, however, does not appear to play an important role in the separation mechanism.

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