Journal of Chromatography, 592 (1992) 309–316
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2420

Investigations of the influence of silanol groups on the separation of enantiomers by liquid and supercritical fluid chromatography

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ABSTRACT

Chiral stationary phases based on (*R*)-N-(1-phenylethyl)-N′-(propylsilyl)urea covalently bonded to silica at different coverage densities with or without end-capped silanol groups were tested by liquid and supercritical fluid chromatography. Greater enantioselectivities and in most instances unexpectedly greater retentions were observed with the end-capped phases. This indicates that silanol groups may influence the interactions between the analyte and a chiral stationary phase.

INTRODUCTION

The direct chromatographic separation of enantiomers by liquid chromatography has become an important tool for the separation of optical isomers [1]. Chiral stationary phases based on small molecules covalently bonded to silica are often used because they combine good enantioselectivity with high efficiency. Different phases consist of molecules with π-donor or π-acceptor groups as chiral selectors. The chiral recognition mechanism for such donor–acceptor phases has been reviewed [2]. A complex between the chiral selector and the more retained enantiomer caused by different interactions was proposed in order to discuss the enantioselectivity of such donor–acceptor phases.

Only a few retention mechanisms lead to enantioselectivity. The retention on the chiral stationary phase (CSP) can be described as a sum of non-chiral and chiral interactions [3]. Silanol groups may influence the separation of enantiomers by non-chiral retention [4,5]. The influence of silanol groups on the chiral retention has also been investigated recently by Pirkle and Readnour [6].

To investigate the influence of silanol groups, we synthesized seven CSPs with different coverage densities. Three CSPs were end-capped by reaction of the residual silanol groups with hexamethyldisilazane. As a chiral selector we used (*R*)-N-(1-phenylethyl)-N′-(propylsilyl)urea covalently bonded to silica. This CSP is extremely easy to prepare by the reaction of isocyanate with aminopropylsilane and is also commercially available as Supelcosil LC)-(*R*)-phenyl urea (Supelco, Gland, Switzerland). Such urea-linked CSPs were introduced by Ôi *et al.* [7]. The separation mechanisms of related CSPs were investigated by Pirkle and co-workers [8–10].

EXPERIMENTAL

General

LiChrospher Si 100 (Merck, Darmstadt, Germany) with a particle size of 5 μm was used. All other chemicals were purchased from Fluka (Buchs, Switzerland).

Chiral stationary phases were slurry-packed into 250 × 3.2 mm I.D. stainless-steel columns as a dibromomethane–hexane slurry.

(R)-N-(1-Phenylethyl)-N′-(diethoxymethylsilylpropyl)urea

A 7.48-g (36-mmol) amount of 3-aminopropylmethyldiethoxysilane was dissolved in 40 ml of dry

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diethyl ether. To the stirred solution 5 ml (36 mmol) of (*R*)-(+)-1-phenylethyl isocyanate were added dropwise. After stirring the reaction mixture under a nitrogen atmosphere for 12 h at room temperature, the solvent was removed and the urea was purified by flash column chromatography and identified by ^{1}H NMR spectrometry.

Chiral stationary phases

Different amounts of (*R*)-N-(1-phenylethyl)-N′-(diethoxymethylsilylpropyl)urea in 15 ml of dry toluene were added to 2.20 g of LiChrospher Si 100 (dried at 180°C and 0.1 mbar for 4 h). The mixture was heated at reflux for about 4 h. After cooling, the gel was washed with toluene, methanol and hexane.

End-capping

Three chiral stationary phases with different coverage densities were heated at reflux with an excess of hexamethyldisilazane in 20 ml of dry toluene under a nitrogen atmosphere. After cooling, they were washed with toluene, methanol and hexane.

Liquid chromatography

Chromatography was performed using a Kontron (Zürich, Switzerland) LC 410 pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injection valve with a 20-μl loop, a Uvikon LCD 725 variable-wavelength UV detector (Kontron) at 254 nm and a HP 3396A integrator (Hewlett-Packard, Widen, Switzerland).

Packed column supercritical fluid chromatography

The laboratory-constructed apparatus for supercritical fluid chromatography (SFC) consisted of a System Gold 116 high-performance liquid chromatographic (HPLC) pump (Beckman Instruments, Basle, Switzerland) controlled by a NEC PC-8201A computer. The pump head was cooled by an ethanol cooling bath at −10°C. The cooling jacket was laboratory-built. The sample was introduced by a Rheodyne Model 7125 HPLC injection valve with a 5-μl loop. Temperature control for the column was provided by a Sigma 2 oven (Perkin-Elmer, Küsnacht, Switzerland). The outlet pressure was regulated by a Tescom restrictor (Matkemi, Therwil, Switzerland) at 40°C. The inlet and outlet pressures were controlled by a laboratory-built pressure controller. A Uvikon 720 LC UV detector (Kontron) was used at 254 nm. The results were recorded with a HP 3396A integrator (Hewlett-Packard).

Carbon dioxide (48-grade) and carbon dioxide (40-grade) containing 5% methanol were obtained from Carbagas (Berne, Switzerland).

RESULTS AND DISCUSSION

CSPs were prepared according to the scheme in Fig. 1. Isocyanate treated with 3-aminopropylsilane yielded a silylurea. Different amounts of the silylurea were bonded to silica to give CSPs with different coverage densities. Three CSPs with different coverage densities were additionally treated with hexamethyldisilazane to eliminate silanol groups (Fig. 2). The coverage densities were calculated from the nitrogen content obtained by elemental analysis (Table I).

In the following chromatographic experiments the capacity factors, k', and separation factors, α, are given as means of three measurements. The standard deviation was less than 3% for the capacity factors and less than 0.5% for the separation factors.

The phases were first tested with 3,5-dinitrobenzoyl (DNB) derivatives of several amines and amino acid esters (Tables II and III). Figs. 3 and 4 show the relationship between the separation factor and the coverage density of the chiral urea. For the non-end-capped CSPs the separation factor decreases with lower coverage densities. This is not the case if end-capped CSPs are used; the separation factor does not decrease with lower coverage densities and

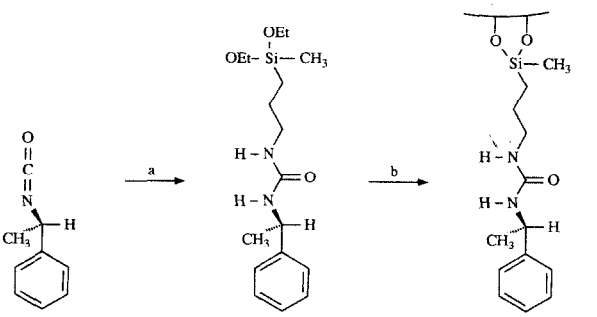

Fig. 1. Preparation of the chiral stationary phases. Conditions: (a) 3-aminopropylmethyldiethoxysilane, diethyl ether, room temperature; (b) 5-μm silica gel, toluene, reflux.

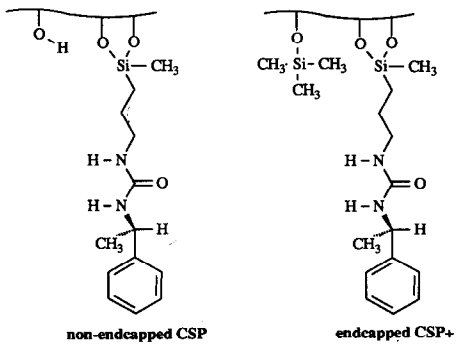

Fig. 2. Structure of the non-end-capped and the end-capped chiral stationary phases.

is even slightly greater at lower coverage densities. On the end-capped CSPs a lower coverage density leads to reduced chiral and non-chiral interactions. Hence the retention decreases but not the separation factor because the ratio of chiral and non-chiral interactions remains more or less constant. In contrast, on the non-end-capped CSPs additional non-chiral interactions with silanol groups become possible so that the ratio of chiral and non-chiral interactions changes with lower coverage densities, which leads to lower separation factors.

To show the reduction of accessible silanol groups by the end-capping, the phases were tested with acetophenone using carbon dioxide as a supercritical mobile phase.

Fig. 5 shows that the retention of acetophenone on the CSPs is mainly affected by silanol interactions. The retention increases with lower coverage densities where more silanol groups are accessible.

TABLE I

CHARACTERISTICS OF THE NON-END-CAPPED (CSP) AND THE END-CAPPED (CSP+) CHIRAL STATIONARY PHASES

CSP	Carbon content (%)	Nitrogen content (%)	Concentration of chemically bonded groups (mmol/g)	Groups/nm^2 [a]
CSP1	3.40	0.57	0.20	0.33
CSP2	4.79	0.76	0.27	0.45
CSP3	6.92	1.06	0.38	0.65
CSP4	7.79	1.29	0.46	0.81
CSP5	9.67	1.64	0.58	1.07
CSP6	10.83	1.75	0.62	1.16
CSP7	11.63	2.05	0.73	1.40
CSP1+	6.69	0.45		0.33
CSP3+	9.03	1.00		0.65
CSP6+	12.46	1.78		1.16

[a] Concentration of chemically bonded chiral groups calculated with a specific surface area of 392 m^2/g.

TABLE II

RESOLUTION OF N-(3,5-DINITROBENZOYL)-1-PHENYLETHYLAMINE (DNB-PEA) AND N-(3,5-DINITROBENZOYL)-1-NAPHTHYLETHYLAMINE (DNB-NEA)

LC conditions: mobile phase, *n*-hexane–2-propanol (80:20); flow-rate, 1 ml/min; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; k'_2 = capacity factor of the second-eluted enantiomer; α = separation factor; absolute configuration of the second-eluted enantiomer is *R*.

Stationary phase	DNB-PEA			DNB-NEA		
	k'_1	k'_2	α	k'_1	k'_2	α
Silica	0.2	0.2	1.00	0.1	0.1	1.00
CSP1	0.5	0.6	1.15	0.5	0.8	1.47
CSP2	0.9	1.1	1.18	0.9	1.4	1.56
CSP3	1.2	1.4	1.21	1.2	1.9	1.67
CSP4	1.4	1.7	1.23	1.4	2.4	1.70
CSP5	1.7	2.1	1.25	1.7	3.0	1.76
CSP6	1.8	2.3	1.26	1.9	3.4	1.78
CSP7	2.2	2.7	1.27	2.3	4.1	1.79
CSP1+	0.6	0.9	1.57	0.5	1.2	2.41
CSP3+	1.8	2.8	1.58	1.6	3.9	2.47
CSP6+	2.2	3.4	1.53	2.1	4.9	2.36

TABLE III

RESOLUTION OF SOME DNB AMIDES OF AMINO ACID METHYL ESTERS

LC conditions: mobile phase, *n*-hexane–2-propanol (80:20); flow-rate, 1 ml/min; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; α = separation factor; absolute configuration of the second eluted enantiomer is *R*.

CSP	Alanine		Leucine		Phenylalanine	
	k'_1	α	k'_1	α	k'_1	α
CSP1	0.9	1.13	0.4	1.19	0.6	1.18
CSP3	1.3	1.25	0.7	1.29	1.1	1.28
CSP6	1.7	1.33	1.1	1.31	1.7	1.29
CSP1+	0.5	1.59	0.3	1.50	0.4	1.40
CSP3+	1.3	1.63	1.0	1.54	1.2	1.45
CSP6+	1.7	1.58	1.2	1.48	1.7	1.44

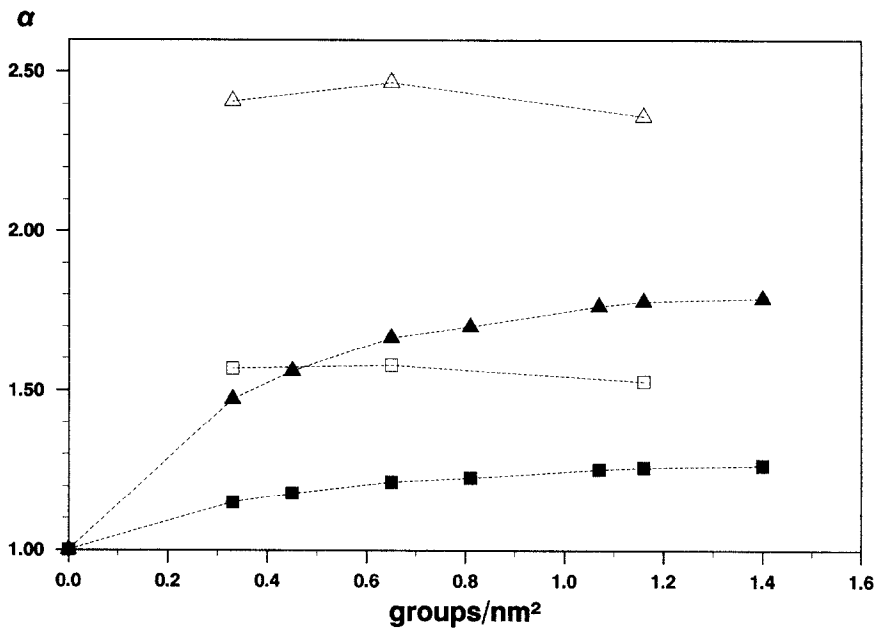

Fig. 3. Separation factor, α, of (■) N-(3,5-dinitrobenzoyl)-1-phenylethylamine and (▲) N-(3,5-dinitrobenzoyl)-1-naphthylethylamine on the (□, △) end-capped and (■, ▲) non-end-capped CSPs as a function of the coverage density. The chromatographic conditions are described in Table II.

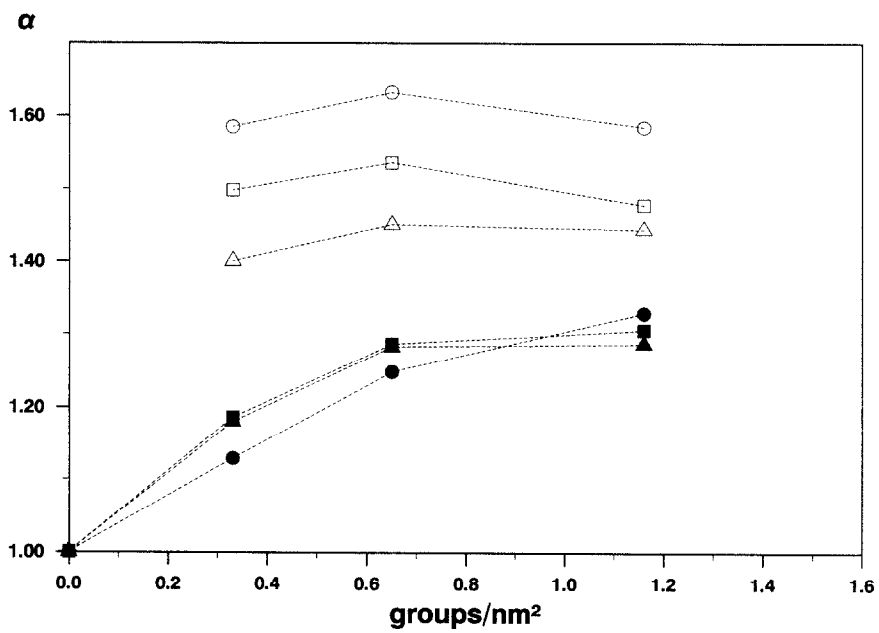

Fig. 4. Separation factor, α, of (■) N-(3,5-dinitrobenzoyl)leucine methyl ester, (▲) N-(3,5-dinitrobenzoyl)phenylalanine methyl ester and (●) N-(3,5-dinitrobenzoyl)alanine methyl ester on the (□, △, ○) end-capped and (■, ▲, ●) non-end-capped CSPs as a function of the coverage density. The chromatographic conditions are described in Table III.

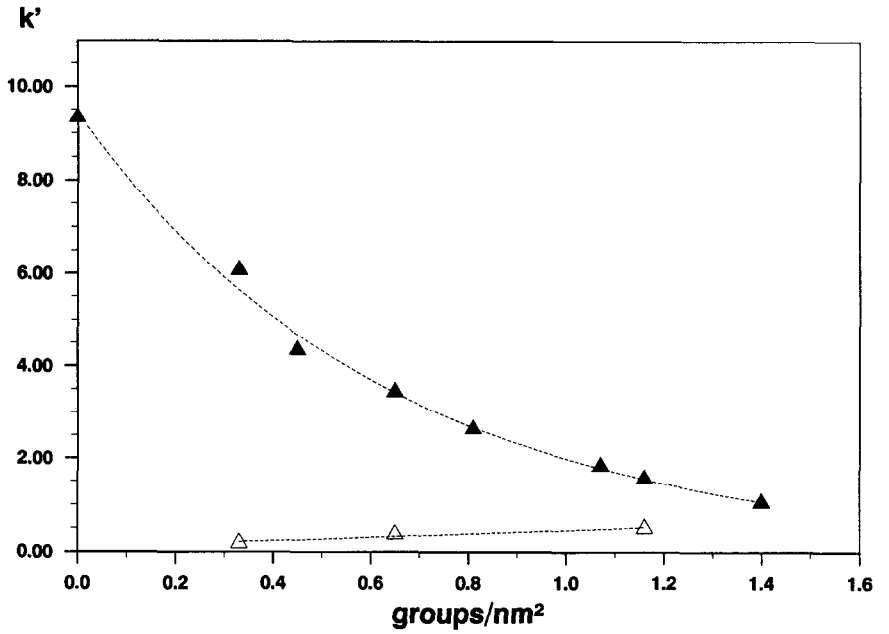

Fig. 5. Capacity factor, k', of acetophenone on (△) end-capped and (▲) non-end-capped CSPs as a function of the coverage density. SFC conditions: mobile phase, carbon dioxide; flow-rate, 3 ml/min; temperature, 50°C; column inlet pressure, 220 bar; column outlet pressure, 180 bar.

A totally different effect can be seen with the end-capped CSPs. In this instance, the retention decreases with lower coverage densities. This indicates that most of the accessible silanol groups are converted into inactive species.

For the separation of enantiomers with *n*-hexane–2-propanol (80:20) as mobile phase, the retention should also be smaller on the end-capped CSPs if a reduction of interactions with silanol groups were the only reason for the greater separation factors. However, surprisingly, the separation factors and the retentions are greater on the end-capped CSPs (Figs. 3 and 4), so an additional effect must cause the greater enantioselectivity.

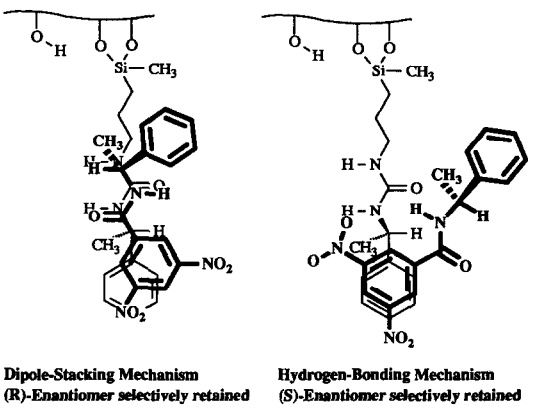

Fig. 6. Competing chiral recognition mechanisms.

Previously, Pirkle *et al.* [8] proposed the occurrence of two competing chiral recognition processes which have opposite senses of enantioselectivity for the resolution of 3,5-dinitrobenzoylamide enantiomers on amide or urea chiral stationary phases. The degree of chiral recognition is determined by the extent of each competing chiral recognition process. The two mechanisms are shown in Fig. 6. According to the results of Pirkle *et al.*, who investigated series of α-arylalkylamines as their 3,5-dinitrobenzoyl derivatives, the dipole-stacking mechanism dominates for the sepration of analytes with short alkyl tails, such as 3,5 dinitrobenzoyl-1-phenylethylamine. Hence the *R*-enantiomer is retained more when (*R*)-N-(1-phenylethyl)-N'-(propylsilyl)urea is used as a chiral stationary phase. The hydrogen-bonding mechanism would dominate for analytes with longer alkyl tails, where the *S*-enantiomer is retained more. In contrast, the

TABLE IV

RESOLUTION OF SOME DNB AMIDES OF 1-PHENYLALKYLAMINES

LC conditions: mobile phase, *n*-hexane–2-propanol (80:20); flow-rate, 1 ml/min; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; k'_2 = capacity factor of the second-eluted enantiomer; α = separation factor; n = carbon number of the alkyl group. In case of resolution the last eluted enantiomer is *R* for $n < 7$ and *S* for $n > 7$.

CSP	n	CSP			CSP+		
		k'_1	k'_2	α	k'_1	k'_2	α
CSP1	1	0.5	0.6	1.15	0.6	0.9	1.57
	2	0.5	0.6	1.16	0.6	0.9	1.51
	3	0.5	0.5	1.00	0.5	0.7	1.36
	4				0.5	0.6	1.23
	5				0.4	0.5	1.14
	7				0.4	0.4	1.00
	8				0.4	0.4	1.00
	9				0.4	0.4	1.00
	10				0.4	0.4	1.00
	13				0.3	0.3	1.00
	17	0.2	0.2	1.00	0.3	0.3	1.00
CSP3	1	1.3	1.6	1.22	1.7	2.7	1.57
	2	1.3	1.6	1.21	1.7	2.6	1.50
	3	1.2	1.4	1.16	1.6	2.2	1.34
	4	1.1	1.2	1.09	1.6	1.9	1.21
	5	1.1	1.1	1.00	1.4	1.6	1.13
	7	0.9	0.9	1.00	1.4	1.4	1.00
	8	0.9	0.9	1.00	1.2	1.2	1.00
	9	0.8	0.8	1.00	1.2	1.2	1.00
	10	0.7	0.7	1.00	1.1	1.1	1.00
	13	0.6	0.6	1.00	0.9	0.9	1.00
	17	0.5	0.5	1.00	0.9	0.9	1.00
CSP6	1	1.8	2.3	1.26	2.2	3.4	1.53
	2	1.8	2.3	1.22	2.3	3.3	1.44
	3	1.7	1.9	1.15	2.1	2.7	1.29
	4	1.6	1.7	1.07	1.9	2.2	1.15
	5	1.5	1.5	1.00	1.9	2.0	1.06
	7	1.3	1.3	1.00	1.7	1.7	1.00
	8	1.2	1.2	1.00	1.5	1.5	1.00
	9	1.1	1.2	1.07	1.4	1.5	1.08
	10	1.0	1.1	1.08	1.3	1.4	1.09
	13	0.8	0.9	1.10	1.3	1.4	1.09
	17	0.8	0.9	1.12	1.0	1.2	1.14

3,5-dinitrobenzoyl derivatives of amino acid esters show a preference for the hydrogen-bonding mechanism because of the additional hydrogen bonding site (*i.e.*, the ester carbonyl oxygen) [8]. In this instance the *R*-enantiomer is retained more (inversion of substituent priority).

To investigate if the separation factor is greater on the end-capped CSPs because the distribution of the two competing chiral recognition processes is different for end-capped and non-end-capped CSPs, we tested CSPs with a homologous series of 3,5-dinitrobenzoyl derivatives of 1-phenylalkylamines (Table IV). For the analytes with alkyl tails longer than five carbons, inversion of the elution order was observed with the end-capped and non-end-capped CSP6 (Fig. 7). According to Pirkle *et al.* [8], this result can be explained by an increasing contribution of the hydrogen-bonding mechanism with increasing length of the alkyl tail, which results in the opposite sense of enantioselectivity. As a result, the *S*-enantiomer is retained more. For CSP1 and CSP3 the enantioselectivity is too low to cause a separation of analytes with longer alkyl tails if the retention is so short. In any case, the end-capped CSPs afford greater retention and greater enantioselectivity for both mechanisms. Hence a different distribution of the two competing chiral recognition processes is not the reason for the greater separation factors on the end-capped CSPs.

The results indicate that the mechanisms which afford retention and enantioselectivity are suppressed on CSPs with non-end-capped silanol groups. Probably the chiral selector is blocked by interactions with silanol groups. A reduction of non-chiral retention caused by silanol groups may yield a greater enantioselectivity but would also yield shorter retentions. As we found greater retention on the end-capped CSPs, we consider that the

TABLE V

RESOLUTION OF N-(3,5-DINITROBENZOYL)-1-PHENYLETHYLAMINE BY SFC

SFC conditions: mobile phase, carbon dioxide–methanol (95:5); flow-rate, 3 ml/min; temperature, 40°C; column inlet pressure, 220 bar; column outlet pressure, 180 bar; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; k'_2 = capacity factor of the second-eluted enantiomer; α = separation factor.

CSP	CSP			CSP+		
	k'_1	k'_2	α	k'_1	k'_2	α
CSP1	2.1	2.2	1.06	1.4	1.9	1.30
CSP3	4.3	4.8	1.11	4.3	5.7	1.33
CSP6	7.4	8.6	1.16	6.4	8.4	1.30

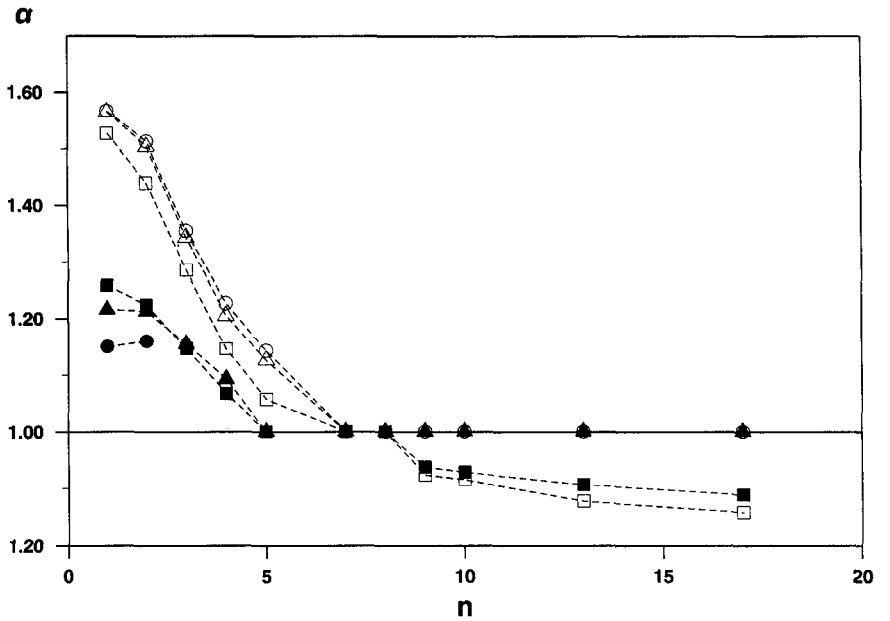

Fig. 7. Separation factor, α, of N-(3,5-dinitrobenzoyl)-1-phenylalkylamines on the end-capped (○) CSP1+, (△) CSP3+ and (□) CSP6+ and non-end-capped (●) CSP1, (▲) CSP3 and (■) CSP6 as a function of the carbon number, *n*, of the alkyl group. The chromatographic conditions are described in Table IV.

reason for the greater separation factors is probably a better accessibility of the chiral selector when the silanol groups are end-capped.

Pirkle and Hyun [10] also found greater enantioselectivities for the separation of 3,5-dinitrobenzoyl-1-phenylethylamine on an amide CSP filled with alkyl groups. Because the retention was shorter on this CSP, they ascribed this phenomenon to the end-capping of residual silanol groups which afford retention without enantioselectivity. In contrast to their results, we observed in most instances greater retention on the end-capped CSPs (Table III and IV).

Table V shows the separation of N-(3,5-dinitrobenzoyl)-1-phenylethylamine by SFC with carbon dioxide–methanol (95:5) as the mobile phase on different CSPs. The enantioselectivity is still greater with the end-capped CSPs, but the retention is smaller in some instances. The solvation interactions of the polar urea group with the methanol modifier are stronger than with 2-propanol. As a result, the urea group is less accessible and so chiral interactions are reduced. Therefore, the enantioselectivity is lower when carbon dioxide–methanol (95:5) is used as the mobile phase, but there is still a difference between end-capped and non-end-capped phases.

CONCLUSIONS

Silanol groups may influence the separation of enantiomers by non-chiral retention. In addition, they may also interfere with the interactions between the analyte and the chiral selector. The enantioselectivity may be substantially greater when the silanol groups are end-capped. This is the case for analytes which are separated on (*R*)-N-(1-phenylethyl)-N′-(propylsilyl)urea. Moreover, the retention is in most instances greater on the end-capped CSPs when hexane–2-propanol is used as the mobile phase. We assume that analyte–chiral selector interactions are suppressed by interactions between the chiral selector and the silanol groups and that the end-capping prevents this suppression. Similar effects were recently observed by Pirkle and Readnour [6].

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