Enantiomer separation by high-performance liquid chromatography on polysiloxane-based chiral stationary phases

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Abstract

The synthesis of two polysiloxane-based chiral stationary phases (CSPs) derived from a π-acidic N-(3.5-dinitrobenzoyl)-β-amino acid (JEM-1) and a π-basic N-(1-naphthyl)-leucine selector is described as is their systematical comparison with the corresponding "brush"-type CSPs. The enantioselectivity of the polysiloxane-based CSPs is higher under both normal- and reversed-phase conditions. In the normal-phase mode, the greater enantioselectivity stems from smaller retention factors for the least retained enantiomers, presumably because of a reduction of analyte interactions with the support silanols owing to effective shielding of the surface by the polymer. The retention factors of the second-eluted enantiomers are shifted to higher values on the π-basic CSP and to lower values on the π-acidic CSP. The latter CSP shows but a small increase in enantioselectivity relative to the corresponding "brush"-type CSP having a comparable selector loading. The silanophilic interactions can be further reduced by end-capping with hexamethyldisilazane (HMDS). When lower amounts of polar modifier are used, the resolution of the polymeric CSPs approaches that of the corresponding brush-type CSP. Under reversed-phase conditions enantioselectivity is reduced but not to the extent generally found for brush-type CSPs. The presence of the non-polar polymeric backbone can introduce hydrophobic interactions which may alter enantioselectivity. It would seem advantageous to use dimethylpolysiloxanes having a high selector concentration in order to reduce the extent of any non-chiral contribution by the polysiloxane backbone to analyte retention while enhancing the favorable chiral recognition properties of the polymer.

1. Introduction

During the past decade the development of non-volatile, non-leachable polysiloxane-based chiral stationary phases (CSPs) has proven to be of importance in the separation of enantiomers by gas chromatography (GC). The linkage of chiral molecules to silicones having the potential for cross-linking and/or immobilization leads to gum-like CSPs with low volatility and high physical and chemical stability, all favorable properties for coatings of porous (e.g. silica gel) or non-porous (e.g. glass or fused-silica capillaries) support surfaces. Since the introduction of Chirasil-Val [1,2] into capillary GC, many similar chiral polysiloxanes have been prepared mainly for applications in GC [3,4].

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The spectrum of possible applications in GC was greatly extended by the attachment of permethylated β-cyclodextrin [5] or metal complexes [6] to a polysiloxane backbone. The capability for cross-linking and simultaneous attachment to the support surface enables chiral polysiloxanes to be used for enantiomer separation by supercritical fluid chromatography (SFC) [7–9], LC [10], and, more recently, by electrochromatography using Chirasil-Dex coated capillaries [11]. The synthesis of polysiloxanes containing chiral selectors such as 3,5-dinitrobenzoyl (DNB)-phenylglycine, naphthylalanine and naphthylethylamine and their subsequent coating onto silica gel or into fused-silica capillaries for enantiomer separation by HPLC and SFC was reported about five years ago by Ruffing et al. [10]. However, such CSPs have not yet found much application in the field of enantiomer separation even though such CSPs potentially could provide some advantages relative to the conventional method of silanization of surface silanols with monomeric chiral silicon derivatives. These advantages can be summarized as follows: (i) The preparation of the CSP can be carried out externally under controlled conditions. This allows characterization by spectroscopic or other methods before coating. (ii) The loading of the selector, which is randomly distributed along the polymer chains, can easily be varied either during synthesis of the CSP or by using different film thickness of the coating. (iii) The homogeneous, non-leachable films thus created largely suppress mixed retention mechanisms which might arise from residual silanol groups on the surface of the support.

In this paper, we report on our investigations upon the use of CSPs based on dimethylpolysiloxanes, analogous to those previously introduced by Ruffing et al. [10]. The synthesis of two polysiloxane-anchored chiral selectors, a π-acidic and a π-basic one is described and their behavior in HPLC is compared to the corresponding "brush"-type CSP (which was obtained by the silanization of surface Si–OH of silica gel).

2. Experimental

2.1. Instrumentation

Chromatography was performed using an Anspec–Bischoff Model 2200 isocratic HPLC pump, equipped with a Rheodyne 7125 injector (20 ml) and a Milton Roy LDC UV Monitor D fixed-wavelength (254 nm) detector. The columns were immersed in a constant-temperature bath to regulate temperatures. Integration was carried out with a Hewlett-Packard 3394A recording integrator, using tri-*tert.*-butylbenzene (normal phase) and sodium iodide (reversed phase) as void volume markers.

2.2. Materials

Polymethylhydrosiloxane (PMHS), china clay and hexachloroplatinic acid were purchased from Aldrich (Milwaukee, WI, USA). All other silicon containing reagents were obtained from Petrarch Systems (Bristol, MA, USA). The silica, spherical Rexchrom 5 μm, 300 Å was from Regis (Morton Grove, IL, USA). The ω-olefinated chiral selectors were prepared according to literature [12,13]. All solutes were available from prior studies.

2.3. Synthesis of a dimethylpolysiloxane containing 20% hydromethylsiloxane units

Under an atmosphere of nitrogen 10.0 g (4 mmol polymer, 166 mmol Si–H) of PMHS and 49.2 g (166 mmol) octamethylcyclotetrasiloxane were mixed with 0.7 g of china clay by vigorous stirring. Then 1 ml of concentrated sulfuric acid was added and the mixture held at 70°C for 20 h. After cooling the mixture, the china clay was removed by suction through a frit, which was accelerated by diluting with 150 ml of diethyl ether. The solution was washed with water, the organic layer dried over sodium sulfate, filtered and evaporated. The resulting polymer then was submitted to vacuum (10^{-4} Torr; 1 Torr = 133.322 Pa) at 120°C, yielding 45.6 g (77%) of a

clear viscous fluid. ^{1}H NMR indicates that the ratio of dimethylsiloxane to methylhydrosiloxane units is 3.8:1 and alkaline hydrolysis shows 2.8 mmol Si–H per gram of pre-polymer.

2.4. General procedure for the synthesis of polysiloxane based CSPs

A 500-mg amount (1.4 mmol Si–H) of the pre-polymer and 1.05 mmol of the selector were dissolved in 40 ml of dry toluene under an atmosphere of nitrogen. The solution was heated to 70°C (for CSP **1P**, and to reflux for CSP **2P**) and half of a solution of ca. 0.2 mg of $H_2PtCl_6 \cdot 6H_2O$ in 10–15 ml dry tetrahydrofuran was added under nitrogen. After 1 h, the second half of the solution was added and heating was continued for 20 h (for CSP **1P** temperature was reduced to 50°C). The progress of the reaction was monitored by evaporating small aliquots of the solution to dryness and examining them by IR and ^{1}H NMR spectroscopy. The absence of olefinic protons and the loss of Si–H signals from the ^{1}H NMR and IR spectra indicate complete reaction of the olefinic selector. This leaves ca. 0.2 mmol g^{-1} Si–H in the resulting chiral polymer.

2.5. Synthesis of a chiral polysiloxane derived from (2R,3R)-undecenyl-N-3.5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)propanoate (JEM-1) (CSP 1P)

Following the general procedure, 570 mg (1.05 mmol) of the JEM-1 selector was treated with 500 mg (1.4 mmol Si–H) of the pre-polymer at 70°C. After 2 h, the reaction temperature was lowered to 55°C and reaction was continued for 18 h. After evaporation of the toluene at 30°C the resulting dark yellow polymer was washed with several 10-ml portions of anhydrous methanol which were removed by decantation. Residual methanol was evaporated at 30°C in vacuo (10^{-3} Torr) yielding 1.04 g of CSP **1P**, which was directly used for the coating procedure. Found: 47.07% C, 7.64% H, 3.38% N (calculated: 48.14% C, 7.64% H, 3.66% N).

2.6. Synthesis of a chiral polysiloxane derived from N-(1-naphthyl)-leucine undecenyl ester (N1N-leucine) (CSP 2P)

Preparation was carried out analogous using 550 mg (1.54 mmol Si–H) of the prepolymer and 470 mg (1.17 mmol) of the N1N-leucine derived selector, which were refluxed for 20 h. After evaporation of the toluene at 30°C the resulting brown polymer was washed with several 10-ml portions of anhydrous methanol, which were removed by decantation. Residual methanol was evaporated at 30°C in vacuo (10^{-3} Torr) yielding 1.0 g of CSP **2P**, which was directly used for the coating procedure. Found: 50.10% C, 9.00% H, 1.39% N (calculated: 50.98% C, 8.66% H, 1.41% N).

2.7. Immobilization of the chiral polymers onto silica gel

A 1.0-g amount of the polymeric CSP **1P** or **2P** was diluted in 20 ml methylene chloride and 4.0 g of silica gel (5 mm, 300 Å), freshly dried by azeotropic distillation with benzene using a Dean–Stark trap) was added. The resulting slurry was sonicated for not more than 10 min after which the solvent was slowly evaporated. Finally, the coated silica gel was heated to 120°C under reduced pressure (10^{-4} Torr) for 24 h. Before packing of the silica gel into a 250 × 4.6 mm I.D. column by conventional methods, it was washed with methylene chloride and methanol (200 ml each), to remove all soluble material (40–100 mg) from the support. As the surface area of the employed silica gel is 100 m^2 g^{-1}, the film thickness can be calculated to be somewhat less than 2.5 μm, assuming that the density of the CSPs is 1 g cm^{-3}.

3. Results and discussion

Chiral recognition processes in chromatography are influenced not only by the non-racemic selector chosen but also by the local environment of the selector. The nature of the underlying

support, the manner in which the selector is attached, the absence or presence of extraneous polar (or non-polar) sites at which additional interactions occur with either the analyte or the selector, and the spacing between the chiral sites of the CSP also influence the chromatographic behavior of a given pair of enantiomers.

The aim of this study was to investigate the differences in enantioselectivity and efficiency of CSPs due to variations in the mode of attachment of the selector to the support. For this purpose chiral selectors of demonstrated utility in "brush"-type CSPs, the π-acidic JEM-1 selector (CSP 1) and the π-basic N1N-leucine derived selector (CSP 2), were incorporated into polysiloxanes via hydrosilylation [13] of the olefinic precursors, yielding the polymeric CSPs 1P and 2P (cf. Fig. 1).

In this way, the selectors are randomly distributed along the polymer chains. After coating the polymer onto silica gel, it is subsequently cross-linked/immobilized by heating. This results in

non-leachable CSPs. As pointed out previously by Ruffing et al. [10], the ratio of platinum catalyst (needed for the hydrosilation step) to the number of Si–H left after this reaction plays an important role in the thermally induced cross-linking and surface bonding. Immobilization and the extent of cross-linking then is controlled by the number of Si–H left after the hydrosilylation step. In analogy to the preparation of other CSPs [5,6] the amount of platinum catalyst needed for hydrosilation of the JEM-1 or N1N-leucine derived selectors can be reduced to about 0.2 mg per mmol of olefin by raising the temperature and employing aromatic hydrocarbons as solvents. The amount of polymer bonded to the silica gel can be calculated from microanalysis data (see Table 1). It is well known that polymeric stationary phases cannot compete with the high speed of mass transfer found in "brush"-type phases [14] (cf. also the resolution R_s in Table 4). This effect, in conjunction with the adsorption/desorption kinetics of the desired

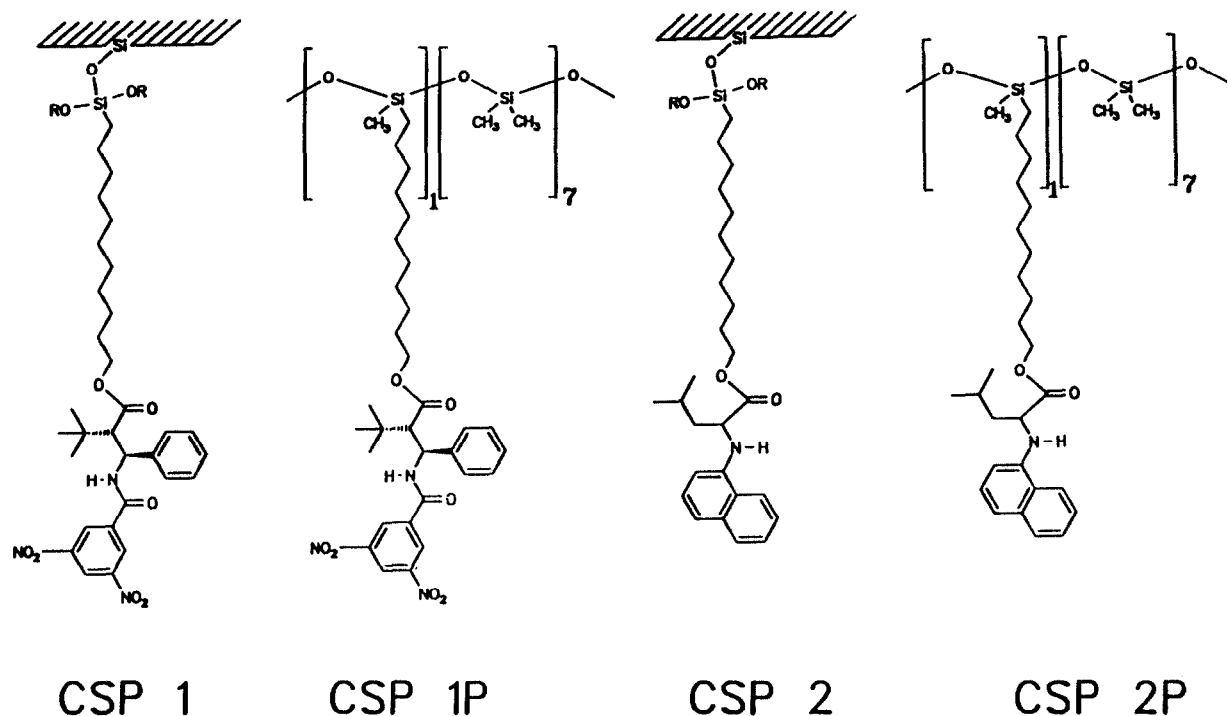CSP 1　　　　　CSP 1P　　　　　CSP 2　　　　　CSP 2P

Fig. 1. Structures of "brush"-type CSPs 1 and 2 and their polysiloxane-based analogues CSPs 1P and 2P.

Table 1
Elemental analysis data for silica coated with CSP 1P and CSP 2P

CSP	Coating (%), mg/100 mg SiO$_2$	Found			Calculated, mmol/g SiO$_2$	
		% C	% H	% N	by C	by N
1P	25	10.00	1.62	0.84	0.181	0.199
2P	25	10.56	1.84	0.29	0.209	0.207

diastereomeric interaction leads to large C terms in the Van Deemter height equivalent to a theoretical plate (HETP) vs. flow-rate curves. This was assumed to be caused principally by the cross-linking of the polymer [15]. The upper branch of the Van Deemter curves, measured for the least-retained enantiomer of the 3.5-dimethylanilide derivative of ibuprofen on CSP 1 and CSP 1P, are depicted for different mobile phase systems in Fig. 2. Interestingly, the relatively large loss in efficiency observed at higher flow-rates in mobile phase system 1 (n-hexane–2-propanol, 80:20; compare curve B_1 with A_1), can nearly be compensated by reducing the amount of the polar modifier (system 3: n-hexane–2-propanol, 95:5; compare curve B_3 with A_1) thus producing similar efficiency at comparable retention factors. This makes a proper choice of the modifier concentration very important for enantiomer separations on polymeric CSPs since resolution can be affected much more strongly than it is found for the corresponding "brush"-type CSPs. It is assumed that the lower concentration of the polar modifier affords greater swelling of the polysiloxanes with consequently improved mass transfer characteristics.

The retention and enantioselectivity shown are affected by a number of variables, e.g. the concentration of the selector and the length of the spacer, the film thickness of the polysiloxane, the porosity of the support, the number of residual surface silanols, the mobile phase employed, and the overall polarity of the analyte as determined by polar substituents both near or remote from the stereogenic center(s). This leads to a variety of interactions between the analyte and the stationary phase, not all of which are easily specified [16]. Under normal-phase conditions, silanophilic or other polar interactions which occur in addition to those involved in the chiral recognition process may reduce the observed separation factor by producing unwanted retention [17]. In order to evaluate the extent to which residual surface silanols on the support have been shielded by the polymeric backbone, solutes with remote polar functions in addition to those required for chiral recognition (cf. Table 2) were injected before and after hexamethyldisilazane (HMDS) treatment [18] of CSP 2P. The chromatographic behavior of these analytes was compared to their behavior on a HMDS-endcapped column containing CSP 2. The data indicate that a number of silanols are still accessible after the polysiloxane coating and show that endcapping is advantageous, especially so for those solutes having one or more acidic hydrogen bond donor sites. The endcapping procedure also reduces the C term slightly, as can be seen from Fig. 3 for both enantiomers of the 3,5-dinitrophenylcarbamate of 1-phenylethanol be-
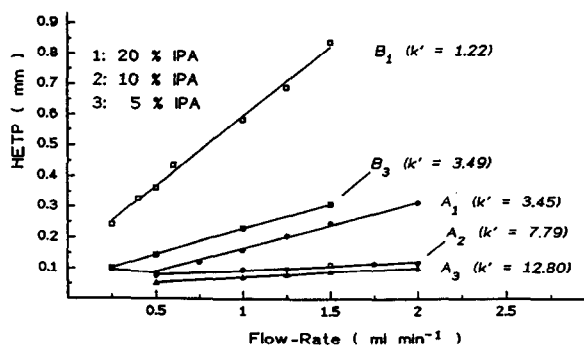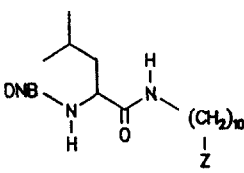

Fig. 2. HETP versus flow-rate for the least-retained enantiomer of the 3,5-dimethylanilide of ibuprofen on CSP 1 (curves A) and CSP 1P (curves B) at different concentrations of 2-propanol (IPA) in n-hexane.

Table 2
The influence of additional polar groups

Analyte	CSP 2, encapped[a]			CSP 2P					
				Not endcapped			Endcapped[a]		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
Z = H	1.11	26.56	23.9	0.13	6.33	48.7	0.19	7.63	40.2
Z = CON(ethyl)$_2$	2.92	68.38	21.7	0.18	7.05	39.8	0.17	6.73	39.0
Z = CONH(n-butyl)	2.39	51.19	21.7	0.15	5.40	35.1	0.12	4.65	37.5
Z = CONH$_2$	–	–	–	0.37	11.72	31.7	0.14	7.12	50.8

[a] By purging the column slowly with a solution of 10 ml HMDS in 40 ml dry methylene chloride.

fore ("NOT") and after HMDS treatment ("HMDS").

The chromatographic behavior of the polymer-coated columns was evaluated by comparing the retention factors obtained on CSP 2 at different loadings of the silica gel (5 μm, 100 Å) with the values obtained on CSP 2P, coated onto 5 μm, 300 Å silica gel. Representative results for five solutes, all chromatographed under the same normal-phase conditions, are given in Table 3. As expected for the brush-type CSPs (columns A–C), a linear relationship between the retention factors, k', and the selector loading was

found. Column D, coated with CSP 2P at a selector loading comparable to column A, always gives less retention for the first-eluted enantiomer but more retention for the second-eluted enantiomer. Hence, CSP 2P affords higher enantioselectivities than even heavily loaded type 2 "brush"-type CSPs (columns B and C). Coated also onto 5 μm, 300 Å silica gel, CSP 1P shows significantly reduced retention factors of *both* enantiomers as can be seen from Table 4. The corresponding separation factors, α, under normal-phase conditions are found to be only slightly higher than those shown by CSP 1, bonded to
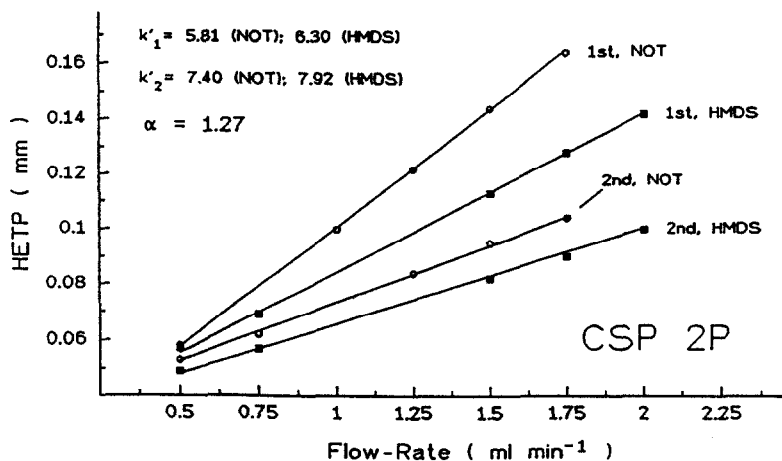
Fig. 3. HETP versus flow rate for the enantiomers of the 3,5-dinitrophenylcarbamate of 1-phenylethanol on CSP 2P before (NOT) and after treatment with hexamethyldisilazane (HMDS). Mobile phase: 2% (v/v) IPA in n-hexane.

Table 3
Comparison of "brush"-type (CSP 2) with different loadings to a polymer (CSP 2P)-coated column (D)

Analyte	Parameter	Column[a]			
		A	B	C	D
N-3,5-Dinitrobenzoyl-	k_1'	0.46	1.19	0.96	0.29
leucine-	k_2'	7.90	30.84	21.67	11.11
n-butylamide	α	17.1	26.0	22.7	38.5
N-3,5-Dinitrobenzoyl-	k_1'	0.72	2.27	1.62	0.54
phenylalanine-	k_2'	6.34	25.53	33.78	15.56
n-butylamide	α	8.7	11.2	20.8	30.3
Most retained		L	L	L	L
N-3,5-Dinitrobenzoyl-	k_1'	0.69	1.63	1.20	0.34
alanine-	k_2'	6.42	29.55	18.71	8.31
n-butylamide	α	9.4	18.1	15.5	24.2
N-3,5-Dinitrobenzoyl-	k_1'	0.95	2.54	1.99	0.91
leucine-	k_2'	7.02	29.31	20.99	13.08
methyl ester	α	7.4	11.5	10.5	14.4
N-3,5-Dinitrobenzoyl-	k_1'	1.58	5.12	3.94	1.49
phenylalanine-	k_2'	8.65	48.21	33.00	16.30
methyl ester	α	5.5	9.4	8.4	10.9
Most retained		L	L	L	L

Temperaturee 22°C; n-hexane–2-propanol (80:20, v/v); flow-rate 2 ml min^{-1}.
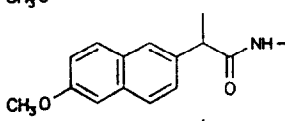[a] Columns: A = low loading, 0.22 mmol g^{-1} (by C), 0.20 mmol g^{-1} (by N); B = high loading, 0.32 mmol g^{-1} (by C), 0.31 mmol g^{-1} (by N); C = commercially available, 0.27 mmol g^{-1}; D = polymer, 0.21 mmol g^{-1} (both by C and by N).
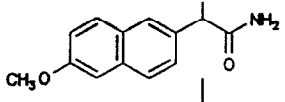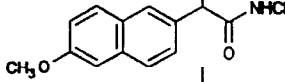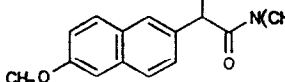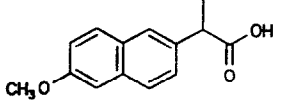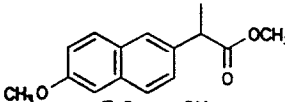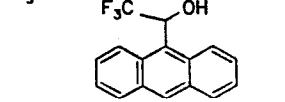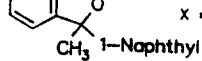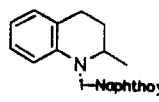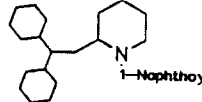
5 μm, 100 Å silica gel. The lower retention afforded by the polymeric CSPs is partly due to the larger pore size silica gel (300 Å instead of 100 Å) employed for these columns. This material was chosen not only because there are fewer surface silanols, but also to reduce adsorption, especially of small molecules, into the pores. It is important to note that owing to the lower surface area of the 5 μm 300 Å silica gel (100 m^2 g^{-1}) the same film thickness is already possible at half of the amount of polymer needed for 5 μm 100 Å silica gel (200 m^2 g^{-1}). Fig. 4 shows a chromatogram o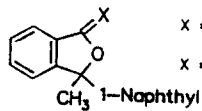btained of an N-(1-naphthoyl) derivative of a heterocyclic amine on CSP 1P. A flow-rate of 2 ml min^{-1} afforded an observed efficiency of about 2500 effective plates for the 250 × 4.6 mm column, a value which can be increased through use of lower mobile phase velocities. The enantiomer separation of the methyl ester of N-(3,5-DNB)-leucine on CSP 2P

(Fig. 5) shows that bandshapes can often easily be improved by increasing the analysis temperature.

Another interesting aspect of polymeric CSPs is their application in reversed-phase systems. The selectors, which are able to form highly selective hydrogen bonds to the analytes, are now part of a non-polar polysiloxane and should be less solvated by the polar mobile phase additives, thus leading to a somewhat greater enantioselectivity than is found with "brush"-type CSPs under reversed-phase conditions. Besides the usual "polar effects" which account for chiral recognition, there are also hydrophobic interactions. In some instances, these can influence enantioselectivity when an alkyl substituent of one enantiomer selectively intercalates between the hydrocarbon spacers connecting the selectors to the polysiloxane backbone [19]. In polysiloxane-based CSPs, silanophobic

Table 4
Comparison of "brush"-type CSP **1** with polymer-coated CSP **1P**

Analyte	CSP **1**				CSP **1P**			
	k_1'	k_2'	α	R_s [a]	k_1'	k_2'	α	R_s [a]
(structure: naphthyl-CH_3O, NH_2)	–	–	–	–	11.33	16.50	1.4	2.1
(structure: naphthyl-CH_3O, NHCH₃)	73.31	141.10	1.9	6.9	31.46	60.78	1.9	5.0
(structure: naphthyl-CH_3O, N(CH₃)₂)	14.84	37.51	2.5	7.2	5.76	16.55	2.9	6.9
(structure: naphthyl-CH_3O, NH–t-Bu)	11.34	30.13	2.6	8.9	4.46	11.73	2.6	8.4
(structure: naphthyl-CH_3O, OH)	–	–	– [b]	–	–	–	–	– [b]
(structure: naphthyl-CH_3O, OCH₃)	2.00	2.33	1.2	1.0	0.91	1.11	1.2	1.0
(structure: F_3C, OH, anthracene)	1.72	2.24	1.3	2.0	0.83	1.06	1.3	1.0
(structure: X = O, 1-Naphthyl)	15.55	34.83	2.2	5.5	6.58	15.99	2.4	6.6
(structure: X = S, 1-Naphthyl)	2.33	5.81	2.5	4.1	0.96	2.56	2.6	3.5
(structure: tetrahydroquinoline, 1-Naphthoyl)	14.04	31.49	2.2	6.9	5.99	14.42	2.4	5.6
(structure: piperidine dicyclohexyl, 1-Naphthoyl)	15.25	28.75	1.9	5.0	4.19	8.22	2.0	3.2

Temperature 22°C; hexane–2-propanol (95:5, v/v); flow-rate 2 ml min[1].

[a] $R_s = 2\Delta t/[w_{base}\,(1) + w_{base}\,(2)]$, where t = retention time and w = peak width.

[b] The separation of underivatized Naproxen is only marginal under these conditions. An improved mobile phase system will be discussed elsewhere.
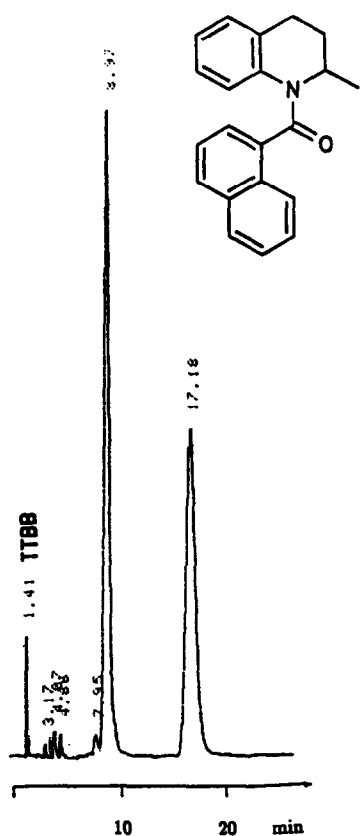
Fig. 4. Enantiomer separation of N-(1-naphthoyl)-1,2,3,4-tetrahydro-5,6-benzo-α-picoline on CSP 1P. Conditions: 1% (v/v) methanol in n-hexane at 2.0 ml min⁻¹, 250 × 4.6 mm column endcapped with HMDS; 22°C; UV 254 nm. TTBB = tri-tert.-butylbenzene.

Fig. 5. Enantiomer separation of N-(3,5-DNB)-leucine methyl ester on CSP 2P at different temperatures. Conditions: 20% (v/v) IPA in n-hexane at 1.0 ml min⁻¹, 250 × 4.6 mm column endcapped with HMDS; UV 254 nm.

interactions caused by the presence of alkyl groups in the polysiloxane backbone are superimposed upon the prior effects. These interactions may be enantioselective since they take place in a chiral environment. However, they may also lower enantioselectivity by causing additional retention. It is therefore instructive to examine homologous series of racemates in order to evaluate the non-polar contributions to retention and enantioselectivity under reversed-phase conditions. Some of the racemates tested are depicted in general form in Fig. 6 as 3, 4, 5 and 6.

When chromatographed with normal phases on CSPs 2 and 2P, type 3 analytes show the expected decrease in retention with an increase

in the number of methylene units (n), whereas the inverse occurs under reversed-phase conditions. The effect of the value of n on the magnitude of the separation factor, α, is shown for these analytes in Fig. 7. The generally observed reduction in enantioselectivity when going from normal to reversed phases is less pronounced on the polysiloxane-based CSPs than for the "brush"-type counterparts as pointed out before. The contribution of non-polar interac-

Fig. 6. General structures for the racemates tested.

tions to the enantioselectivity of CSP **2P** towards N-DNB-α-aminoalkylphosphonates is higher than is found on the corresponding CSP **2** as evidenced by the greater slope of the relevant plot in Fig. 7. Here, the more retained enantiomers experience the greater hydrophobic bonding owing to selective intercalation of their a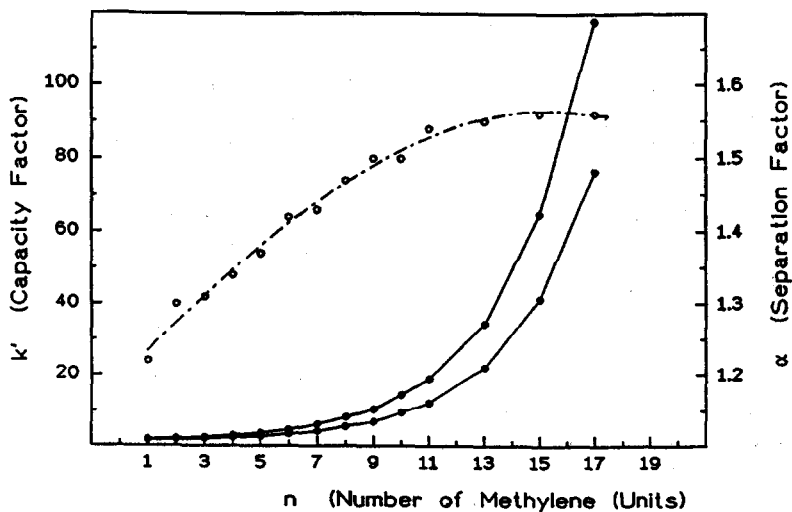lkyl substituents between adjacent strands of bonded phase. The reversed-phase behavior of other analytes on CSP **1P** is shown in Fig. 8. The reversed-phase behavior of CSP **1P** for different

analytes is shown in Fig. 8 (type 4), Fig. 9 (type 5) and Fig. 10 (type 6). Note from Figs. 7–9 that both retention and enantioselectivity increase as the length of the alkyl substituent increases. Eventually however, enantioselectivity no longer increases. This seems to occur when the length of the intercalated alkyl substituent is approximately the same as the length of the tether which connects the selector to the silica. Alkyl substituents longer than this presumably begin to encounter the polysiloxane backbone and/or the silica support. The resulting steric interactions

Fig. 7. Separation factor α versus number of methylene units (n) for N-3,5-DNB α-aminoalkylphosphonates (analyte type 3) on CSPs **2** and **2P** in normal and reversed mobile phases. ● = n-Hexane–2-propanol (80:20); ○ = methanol–water (80:20).

Fig. 8. Retention factor k' (●) and separation factor α (○) versus number of methylene units (n) for p-alkylanilides of ibuprofen (analyte type 4) on CSP **1P** in reversed mobile phase (acetonitrile–water, 70:30; flow-rate 2 ml min^{-1}).

Fig. 9. Retention factor k' (●) and separation factor α (○) versus number of methylene units (n) for N-3,5-DNB-1-(p-methoxyphenyl)-aminoalkanes (analyte type 5) on CSP 1P in reversed mobile phase (acetonitrile–water, 70:30; flow-rate 2 ml min^{-1}).

offset any additional hydrophobic bonding which would normally accrue to the more retained enantiomer as the alkyl substituent is lengthened. Similar behavioral trends have been noted for brush-type CSPs. This would seem to suggest that the neighboring selector strands are spatially oriented in similar fashions for both types of CSPs. It is not intuitively obvious that this should necessarily be the case. Further studies of the effects of film thickness, selector concentration, and selector structure are needed to better understand whether this apparent similarity in spatial orientation is pervasive or occasional. Fig. 9 provides another example of the
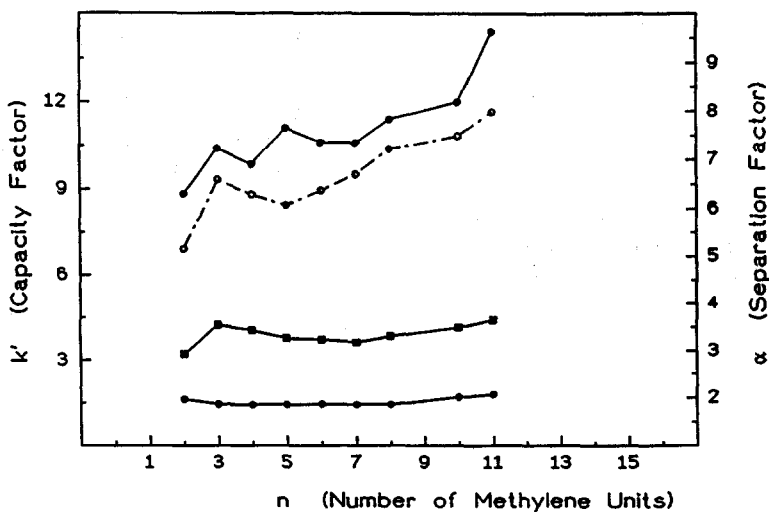
Fig. 10. Retention factor k' (● = $k'_{D,L}$; ■ = k'_{meso}) and separation factor α (○) versus number of methylene units (n) for bis-amides of dicarboxylic acids and α-(1-naphthyl) ethylamine (analyte type 6) on CSP 1P in reversed mobile phase (methanol, flow-rate 2 ml min^{-1}).

resolution of π-acidic enantiomers on a π-acidic CSP.

The plots shown in Fig. 10 demonstrate that both ends of the type 6 bis-amide analytes are capable of simultaneously bonding to neighboring strands of the bonded phase and that the retention (and consequently the enantioselectivity) of the more retained enantiomer is strongly affected by the number of methylene groups connecting the two amide units together. Although the mobile phase is methanol, the retentions of the less retained enantiomers and of the *meso*-diastereomers are but little affected by the number of connecting methylene units.

It has been shown that the enantioselectivity of a chiral selector may be increased by incorporating modest concentrations of the chiral selector into a polysiloxane. It seems to be advantageous to coat relatively thin films of a polysiloxane containing a high concentration of the selector onto the silica gel. This appears to reduce the contributions of the polymeric backbone to reversed-phase retention without losing the desirable properties of the polysiloxane. Because these polysiloxanes are anchored to the silica support, the are likely to be sufficiently robust as to make them useful as CSPs for both analytical- and preparative-scale separation of enantiomers using supercritical fluids as mobile phases in addition to the more traditional liquid mobile phases.

Acknowledgements

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