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Thermal treatment of supports for chiral stationary phases and its influence on enantioselectivity

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ABSTRACT

Thermal treatment of silica at temperatures above 800°C leads to a significant decrease in the silanol surface concentration Chiral modification of such thermally treated silicas gives derived stationary phases with lower specific surface areas and lower surface concentrations of ligands The resolution of chiral amides was in some instances better with thermally treated π-donor chiral stationary phases based on (R)-1-(1-naphthyl)ethylamine in comparison with the corresponding untreated phases Correlation experiments with two homologous series indicate that retention also depends on other hydrogen-bond acceptors such as secondary hydroxyl groups near the chiral selector

INTRODUCTION

Pirkle and co-workers [1–3] synthesized π-donor chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) with a lower surface loading of chiral ligands by using smaller amounts of reactants These "diluted" CSPs were developed and tested with a view to clarifying chiral recognition mechanisms

Dappen *et al* [4] obtained CSPs with various surface coverages depending on the order of connection of the spacer, 3-glycidoxypropyltrimethoxysilane, with the chiral selector molecule and the surface silanol groups A decrease in the number of active polar sites on the silica support can increase the enantioselectivity of the CSP for polar solutes

When using ordinary "diluted" chiral stationary phases for the separation of enantiomers it is necessary to consider that residual silanol groups can cause disturbing polar adsorption (non-chiral inter-action with polar functional groups of the solute) which lower the chiral recognition by peak broadening A thermal treatment prior to a chemical modification deactivates these surfaces so much that such polar adsorptions are mostly suppressed For this treatment, temperatures up to 1000°C for several hours have been applied Thermal treatment reduces the surface area of the silica to an extent dependent on the temperature applied and the purity of the silica [5–9]

The aim of the first part of this study was to investigate whether a thermal treatment of silica prior to the chemical reaction decreases the surface density of chiral ligands We also compared these thermally treated and lightly loaded chiral stationary phases (TCSPs) with the CSPs previously described [4,10,11] The basic structure of the CSPs is given in Fig 1 The only differences between slightly and heavily loaded CSPs are the surface density of the chiral ligands, the number of remaining silanol groups and the structure of the support

EXPERIMENTAL

Two chiral stationary phases, one with an N-pivaloyl group, R_1, [CSP I (PIV)] and another with an

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☆ This paper is part of the Dissertation of R Straub [10]

Fig 1 Chiral stationary phases (CSPs and TCSPs) I and II N-Acyl derivatives of (R)-4-(N-1-alkyloylamino-1-ethyl)-1-naphthylamine

N-cyclohexylcarbonyl group, R_2, [CSP II (CYH)] were synthesized starting from (R)-1-(1-naphthyl)ethylamine according to a procedure described earlier [4,10,11] Similar phases, TCSP I (PIV) and TCSP II (CYH), but from thermally treated supports were also made and are discussed below

The elemental analyses were done with a routine analyser in the microanalytical department of Ciba-Geigy (Basle, Switzerland) and specific surface area (S_{BET}) measurements with a Carlo Erba Sorptomatic Series 1800 instrument All samples used were synthesized according to common laboratory methods

Materials

LiChrospher Si 100 (Merck, Darmstadt, Germany) with a particle size of 5 μm and a specific surface area S_{BET} = 264 4 ± 5 4 m^2/g was dried at 150°C and 0 01 mbar for 6 h prior to the bonding procedure All chemicals were purchased from Merck or Fluka (Buchs, Switzerland) All solvents used for reactions or for washing were dried according to general laboratory procedures For liquid chromatography, HPLC-grade solvents from Romil Chemicals (Shepshed, UK) were used

Silylation of thermally treated silica

Thermal treatment Three 0 55-g portions of silica in covered porcelaine crucibles were heated at 600, 800 and 1000°C for 4 h in a muffle furnace A further portion of silica was heated for the same period in a Buchi GKR-51 Kugelrohr oven at 150°C and 0 01 mbar The differently thermally treated solids were cooled in a desiccator containing phosphorus pentoxide and then moved into a dry-box, further

preparations were carried out in a dry nitrogen atmosphere

Silylation Samples of 0 18–0 5 g of the thermally treated silica were refluxed with 0 1 ml of dry pyridine and 0 8 mmol of n-octadecyldimethylchlorosilane (ODCS) per gram of silica in 6 ml of dry toluene for 137 h The derived silicas were filtered and washed successively with toluene, methanol, methanol–water (1 1), water, methanol and diethyl ether and dried for 6 h at 150°C and 0 01 mbar No 1 (150°C) weight increase 16 7% Analysis found, C 13 98, H 2 56, Cl <0 1% No 2 (600°C) weight increase 9 5% Analysis found, C 13 83, H 2 70, Cl <0 2% No 3 (800°C) weight increase 10 5% Analysis found, C 13 76, H 2 83, Cl <0 2% No 4 (1000°C) weight increase 6 08% Analysis found, C 10 42, H 1 89, Cl <0 1% Data on surface coverages calculated from elemental analysis are given in Table I

CSPs I and II

CSP I (PIV) Weight increase 9 0% Analysis found, C 6 99, H 1 51, N 0 69% Calculated 0 25 mmol of (R)-ligand/g stationary phase (based on N) and 0 23 mmol of (R)-ligand/g stationary phase (based on C)

CSP II (CYH) Weight increase 7 8% Analysis found, C 6 43, H 1 34, N 0 83% Calculated 0 22 mmol of (R)-ligand/g stationary phase (based on N) and 0 20 mmol of (R)-ligand/g stationary phase (based on C)

TCSPs I and II

Thermal treatment In a covered porcelain crucible, 2 3–2 4 g of silica were heated at 1000°C for 6 h

in a muffle furnace, cooled in a desiccator over phosphorus pentoxide and moved for further treatment into a dry-box

Coating Samples of 1 9–2 0 g of thermally treated silica were reacted with 0 7 mmol of (R)-4-(N-1-pivaloylamino-1-ethyl)-1-naphthylamine or (R)-4-(N-1-cyclohexylcarboxamido-1-ethyl)-1-naphthylamine and an equimolar amount of 3-glycidoxypropyltrimethoxysilane in 15 ml of dry toluene according to the procedure reported previously [4,10,11]

All calculations are based on the thermally treated silica gel

TCSP I (PIV) Weight increase 5 1% Analysis found, C 4 71, H 0 67, N 0 3% Calculated 0 11 mmol of (R)-ligand/g stationary phase (based on N) and 0 16 mmol of (R)-ligand/g stationary phase (based on C)

TCSP II (CYH) Weight increase 4 9% Analysis found, C 4 91, H 0 73, N 0 3% Calculated 0 11 mmol of (R)-ligand/g stationary phase (based on N) and 0 15 mmol of (R)-ligand/g stationary phase (based on C)

Liquid chromatography

To eliminate fines, the CSPs and the TCSPs were sedimented five times in methanol Stainless-steel tubes (25 cm × 3 2 mm I D) were used as columns A slurry prepared from 1 9 g of the phase and 30 ml of dibromomethane–n-hexane (8 2) was packed into the columns with a Haskel (Burbank, CA, USA) Model 27486-4 airdriven fluid pump at a pressure of 680 bar The columns were conditioned with methanol and n-hexane

Chromatography was performed using an Altex (Berkeley, CA, USA) Model 110 solvent metering pump, a Hitachi Model 100-10 variable-wavelength UV detector (Kontron, Zurich, Switzerland) with detection at 254 nm, a Rheodyne (Berkeley, CA, USA) Model 7125 syringe-loading sample injector with a 20-μl loop, and a Tarkan W & W Model 600 recorder (Kontron) and an HP 3396 A integrator (Hewlett-Packard, Widen, Switzerland)

The mobile phases were n-hexane–tetrahydrofuran (3 1) and n-hexane–2-propanol (78 22) at a flow-rate of 1 ml/min The columns and the mobile phase container were held at 20°C (Assistant WTE var 3185 thermostat, R C Kuhn, Berne, Switzerland) Toluene as a non-retained standard, dissolved in the appropriate mobile phase, was used to deter-

mine the dead time, t_0, and number of theoretical plates, N_0 The results are given in Table II

RESULTS AND DISCUSSION

Thermal treatment

The number of silanol groups per unit area of porous glass or silica has been estimated or measured by numerous workers and values in the range 4–8/nm^2 were found [12–14] The generally accepted value is 4 6/nm^2 for surface silanol groups of a properly dried silica Davydov [15] has shown that the surface concentration of silanol groups dropped from 5 3/nm^2 at 100°C to 2 6/nm^2 at 400°C and <1 0/nm^2 at about 850°C or higher temperatures of thermal treatment The rehydration of the siloxane groups formed is usually slow and often the thermally treated silica or porous glass must be heated in hot acids [16]

In preliminary experiments with bonded chiral stationary phases we attempted to react thermally treated silica, from the same batch as the support used for chiral modification, with n-octadecyldimethylchlorosilane in dry toluene Table I shows the results of the elemental analyses A measurable decrease in the n-octadecyldimethylsilyl (ODS) surface concentration was obtained only after thermal treatment of the silica at above 800°C prior to chemical reaction In contrast, a 4-h treatment at 1000°C diminished the active silanol groups very effectively so that a significant decrease to 0 47 mmol ODS/g was measured The bulky ODS was used to obtain almost comparable conditions to the bonding procedure with chiral ligands

TABLE I

SILYLATION OF THERMALLY TREATED SILICA RESULTS FROM ELEMENTAL ANALYSES

T = Temperature of thermal treatment, $O_M(C)$ = surface concentration of ODS, based on C, from elemental analysis data

No	T (°C)	$O_M(C)$ (mmol/g)
1	150	0 74
2	600	0 71
3	800	0 66
4	1000	0 47

TABLE II

COLUMN PARAMETERS OF STATIONARY PHASES I AND II

O_M(C) = surface concentration based on C, O_M(N) surface concentration based on N, S_{BET} = specific surface area, t_0 = dead time, N_0 = number of theoretical plates, ε = total porosity, mobile phases, n-hexane–2-propanol (78 22) and n-hexane–tetrahydrofuran (3 1), flow-rate, 1 ml/min, column, 25 cm × 3 2 mm I D , 5 μm Mean results from ten measurements with each mobile phase

Stationary phase	O_M(C) (mmol/g)	O_M(N) (mmol/g)	S_{BET} (m²/g)	t_0[a] (min)	N_0[a]	ε[a]
CSP I	0 23	0 25	290	1 72	4300	0 85
TCSP I	0 16	0 11	60	1 51	7900	0 75
CSP II	0 20	0 22	290	1 71	2300	0 85
TCSP II	0 15	0 11	20	1 46	7900	0 73

[a] Toluene as non-retained standard

For the "diluted" CSPs, silica was heated at 1000°C for 6 h in the muffle furnace, cooled and reacted with 3-glycidoxypropyltrimethoxysilane and one of the chiral selectors under dry atmospheric conditions Both elemental analyses and weight difference measurements indicated a twofold lower coverage with organic molecules than the 0 25 mmol/g for heavily loaded CSPs Table II shows relevant physical characteristics of the four tested stationary phases

Heavily loaded silicas have specific surface areas (S_{BET}) that differ only slightly from that of the unmodified support Thermal treatment of silica at 1000°C with a subsequent surface reaction causes a severe decrease in S_{BET} to below 60 m²/g [8,17,18] Therefore, it can be assumed that at 1000°C significant changes in the structure of silica occur

The chromatographic data (dead time, t_0, number of theoretical plates, N_0, and total porosity, ε [19]) in Table II indicate a good packing quality of the columns made from these "diluted" phases

TABLE III

PHENYL- AND NAPHTHYL-CONTAINING SAMPLES 1–6 USED IN CHROMATOGRAPHIC EXPERIMENTS

DNB = 3,5-Dinitrobenzoyl
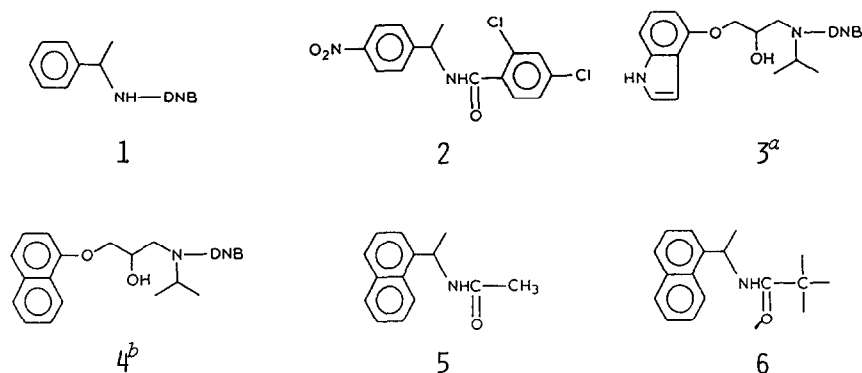
[a] 3 = DNB amide of pindolol
[b] 4 = DNB amide of propranolol

TABLE IV

RESOLUTION OF SAMPLES 1–6 ON STATIONARY PHASES I AND II

Conditions mobile phase, n-hexane–tetrahydrofuran (3 1), flow-rate, 1 ml/min, column, 25 cm × 3 2 mm I D , 5 μm, detection, UV at 254 nm k'_1 = Capacity factor of the first-elute enantiomer, α = separation factor

Stationary	Sample											
	1		2		3		4		5		6	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
CSP I	4 92	2 17	2 82	1 01	6 66	1 05	2 86	1 10	10 30	1 05	0 50	1 06
TCSP I	5 63	2 14	2 23	1 03	6 95	1 17	2 48	1 18	4 30	1 08	0 75	1 13
CSP II	4 14	2 15	3 56	n r[a]	6 19	1 12	3 24	1 09	8 18	1 04	0 51	n r[a]
TCSP II	7 11	2 26	3 01	1 08	8 44	1 16	2 33	1 10	5 28	1 07	0 75	1 25

[a] No resolution

Samples

The phases were tested with aromatic amides derived with N-acyl groups of different π-acidity and bulkiness Table III shows the structures and Table IV gives the chromatographic results for six samples

The thermally treated chiral stationary phases separate virtually all test enantiomers better than the corresponding heavily loaded phases, the separation factors, α, are about 0 1 unit higher The capacity factors, k'_1, follow no distinct tendency In fact, for the amides 1, 3 and 6 longer retention times were measured on TCSPs I and II than on the corresponding CSPs without thermal treatment of the support Mauss and Engelhardt [8] examined the influence of thermal treatment on chromatographic selectivity and reported similar effects for aromatic molecules which can act as hydrogen-bond acceptors They also claimed that the retention of solutes with hydrogen-bond acceptors is not affected by the removal of vicinal silanols

Correlation methods

To compare the phases and to obtain more information on the recognition mechanisms involved, two homologous series with different alkyl chain lenghts were used for chromatographic correlation experiments, the racemic 1-phenylalkyl amines 1a–l and 1-phenylglycine derivatives 7a–j, both derived as 3,5-dinitrobenzoyl (DNB) amides, were eluted with n-hexane-2-propanol (78 22) Fig 2 shows the structures of the test homologues The results are

listed in Tables V and VI and in Figs 3–6

For the separation of 1a–l, all four CSPs show similar selectivity depending on the chain length (Fig 3) The separation factors, α, are without exception higher for the type II phases TCSP I (PIV) has better separation properties than the heavily loaded CSP I and the type II phases show just the opposite behaviour Apart from this difference in selectivity, all the tested phases point to the same chiral recognition mechanism recently described for correlation experiments with heavily loaded phases [11]

The plots of k'_1 versus carbon number n in the Fig 4 indicate differences in the retention behaviour The thermally treated and lightly loaded TCSPs I and II show a large decrease in capacity factors with increasing hydrophobicity (solutes 1 with longer n-alkyl chains) than the CSPs I and II Only after n-propyl (TCSP I) or n-pentyl (TCSP II)

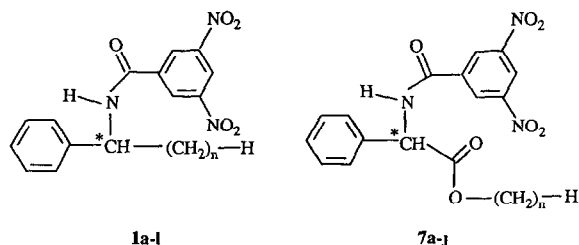1a-l   7a-j

Fig 2 N-3,5-Dinitrobenzoyl (DNB)-derived homologous series 1a–l and 7a–j For carbon numbers n see Tables V and VI and Figs 3–6

TABLE V

RESOLUTION OF N-3,5-DINITROBENZOYL-1-PHENYLALKYLAMINES **1a–l** ON STATIONARY PHASES I AND II

HPLC conditions mobile phase, n-hexane–2-propanol (78 22), flow-rate, 1 ml/min, column, 25 cm × 3 2 mm I D , 5 μm, detection, UV at 254 nm n = Number of carbon atoms in the alkyl chain, k'_1 = capacity factor of the first-eluted enantiomer, α = separation factor, Conf = configuration of last-eluted enantiomer

n	CPS I (PIV)		TCSP I (PIV)		CSP II (CYH)		TCSP II (CYH)		Conf
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	
1	9 48	2 08	11 65	2 04	8 54	2 19	11 23	2 21	R
2	9 94	2 50	12 58	2 55	8 76	2 61	12 28	2 62	R
3	9 69	2 52	12 77	2 53	9 08	2 67	12 49	2 62	R
4	10 27	2 38	12 08	2 40	10 22	2 55	12 72	2 52	R
5	10 29	2 50	11 75	2 45	10 69	2 64	12 87	2 66	
7	9 29	2 47	10 49	2 57	9 62	2 77	11 62	2 72	
8	8 77	2 52	9 44	2 59	8 77	2 85	10 92	2 78	
9	8 43	2 57	9 17	2 62	8 61	2 94	8 98	2 86	
10	8 14	2 59	8 20	2 64	8 56	2 99	8 98	2 87	
13	7 57	2 67	5 52	2 73	7 75	3 08	7 97	2 93	
15	7 00	2 68	4 81	2 77	7 16	3 14	7 34	2 98	
17	5 53	2 70	4 42	2 79	6 96	3 19	6 96	3 01	

was a decrease in retention with increasing chain length of the DNB-amides **1d–l** observed

For an interpretation of this behaviour it must also be considered that in addition to the remaining silanol groups on the silica surface, there are likewise polar secondary hydroxyl groups on the spacer (Fig 1) formed in the amination step of the epoxide [4,11] In the heavily loaded CSPs I and II the resid-
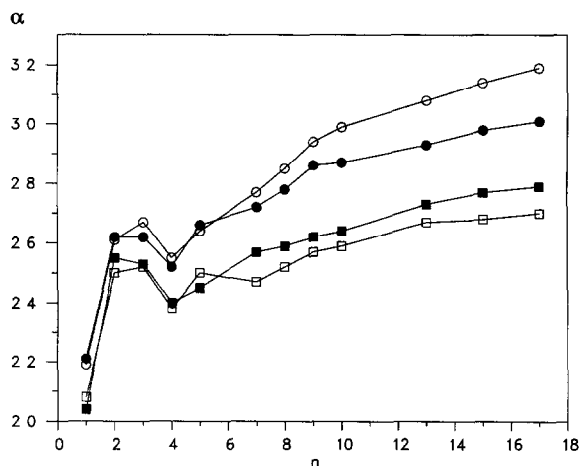

Fig 3 Separation of phenylalkyl DNB derivatives **1a–l** on CSP and TCSP I and II using 2-propanol–n-hexane (22 78) as mobile phase Separation factor α versus carbon number n □ = CSP (PIV), ■ = TCSP I (IV), ○ = CSP II (CYH), ● = TCSP II (CYH)
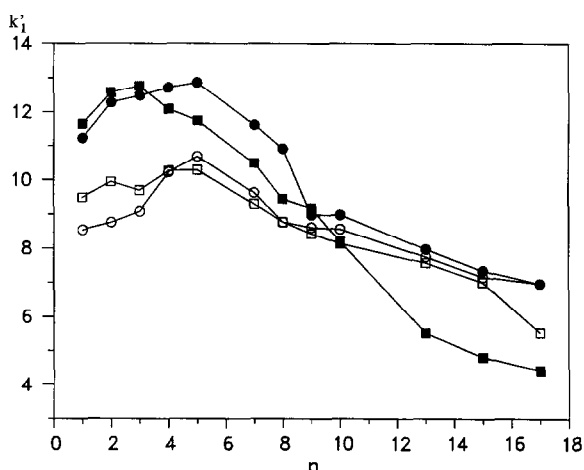

Fig 4 Separation of phenylalkyl DNB derivatives **1a–l** on CSP and TCSP I and II using 2-propanol–n-hexane (22 78) as mobile phase Capacity factor k'_1 versus carbon number n ○ = CSP I (PIV), ■ = TCSP I (PIV), ○ = CSP II (CYH), ● = TCSP II (CYH)
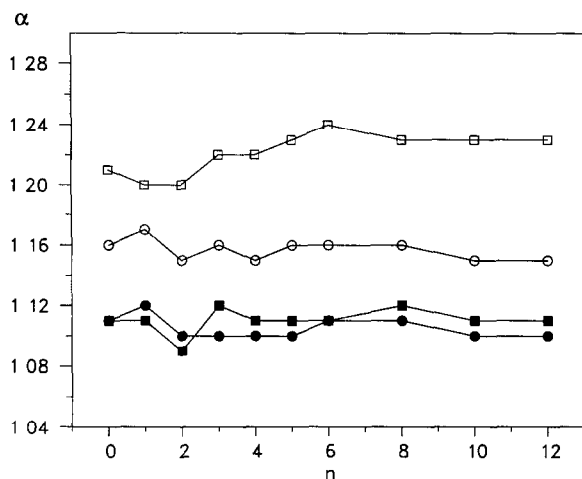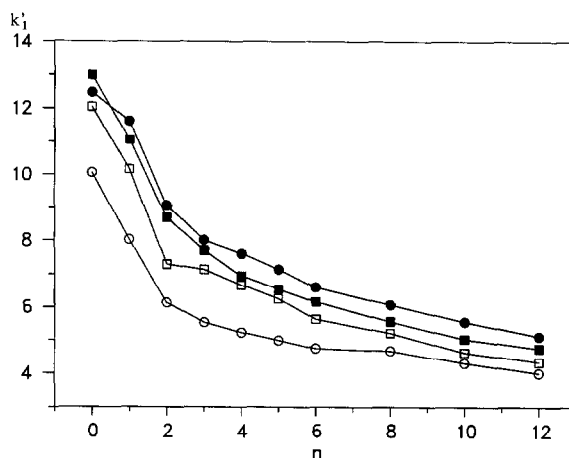
Fig 5 Separation of phenylglycine DNB derivatives 7a–j on CSP and TCSP I and II using 2-propanol–n-hexane (22 78) as mobile phase Separation factor α versus carbon number n □ = CSP I (PIV), ■ = TCSP I (PIV), ○ = CSP II (CYH), ● = TCSP II (CYH)

Fig 6 Separation of phenylglycine DNB derivatives 7a–j on CSP and TCSP I and II using 2-propanol–n-hexane (22 78) as mobile phase Capacity factor k'_1 versus carbon number n □ = CSP I (PIV), ■ = TCSP I (PIV), ○ = CSP II (CYH), ● = TCSP II (CYH)

ual silanol groups are very effectively screened by the bulky organic molecules Hence, they excert a smaller contribution to the retention of polar DNB-amides On the other hand, higher surface loadings with chiral ligands increase the number of secondary polar hydroxyl groups The high k'_1 values of samples 1a–h with TCSPs I and II support the assumption that polar adsorptive effects take place

TABLE VI

RESOLUTION OF nALKYL ESTERS 7a–j OF N-3,5-DINITROBENZOYL-1-PHENYLGLYCINE ON STATIONAY PHASES I AND II

HPLC conditions mobile phase, n-hexane–2-propanol (78 22), flow-rate, 1 ml/min, column, 25 cm × 3 2 mm I D , 5 μm, detection, UV at 254 nm n = Number of carbon atoms in the alkoxy chain, k'_1 = capacity factor of the first-eluted enantiomer, α = separation factor, Conf = configuration of last-eluted enantiomer

| n	CPS I (PIV)		TCSP I (PIV)		CSP II (CYH)		TCSP II (CYH)		Conf
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	
0	12 05	1 21	12 99	1 11	10 06	1 16	12 48	1 11	
1	10 16	1 20	11 06	1 11	8 02	1 17	11 61	1 12	R
2	7 29	1 20	8 69	1 09	6 13	1 15	9 06	1 10	R
3	7 13	1 22	7 70	1 12	5 53	1 16	8 01	1 10	R
4	6 67	1 22	6 94	1 11	5 23	1 15	7 61	1 10	
5	6 25	1 23	6 54	1 11	4 99	1 16	7 14	1 10	R
6	5 63	1 24	6 16	1 11	4 75	1 16	6 60	1 11	
8	5 21	1 23	5 56	1 12	4 68	1 16	6 07	1 11	
10	4 63	1 23	5 03	1 11	4 31	1 15	5 55	1 10	
12	4 34	1 23	4 75	1 11	4 01	1 15	5 11	1 10	
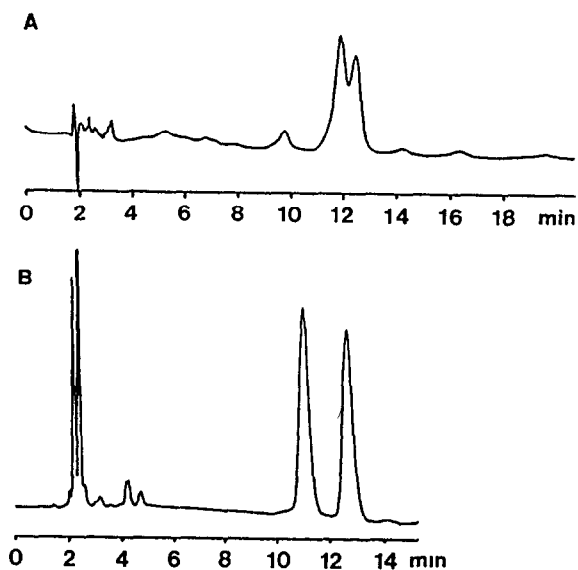
Fig 7 Separation of DNB amide of (R/S)-pindolol (3) on (A) CSP I (PIV) and (B) TCSP II (PIV) Mobile phase, n-hexane–tetrahydrofuran (3 1), flow-rate, 1 ml/min, temperature, 20°C

Fig 8 Separation of (R/S)-1-(N-1-pivaloylamino-1-ethyl)naphthalene (6) on (A) TCSP I (PIV) and (B) TCSP II (CYH) Mobile phase, n-hexane–tetrahydrofuran (3 1), flow-rate, 1 ml/min, temperature, 20°C

from residual silanol groups and also from secondary hydroxyl groups For samples with long n-alkyl chains, where an intercalation between the strands of the chiral ligands is excluded for steric reasons, the role of the secondary hydroxyl group on the spacer is predominant Remaining silanols on the silica matrix may not act as strong adsorption sites for such solutes This leads to similar or even lower k'_1 values in comparison with CSPs I and II

Samples with dominant hydrogen bonding such as the homologous 1-phenylglycine derivatives **7a–j** whose n-alkoxy chain does not intercalate between the strands are less resolved on TCSPs I and II than on the appropriate CSPs I and II, as shown in Fig 5 The lower enantioselectivity of these phases is due to a smaller number of available chiral selectors The retention times of the 1-phenylglycine derivatives **7a-j** are higher for both diluted phases than for the more highly loaded phases (Fig 6)

Examples of the DNB-amide of (R/S)-pindolol (3) (Table III) separated on CSP I (PIV) and TCSP I (PIV) are given in Fig 7 The diluted phase has a higher resolution for the racemate, shown in the baseline-separated peaks in chromatogram B Similar observations with thermally treated silica were reported for various aromatic solutes by Mauss and
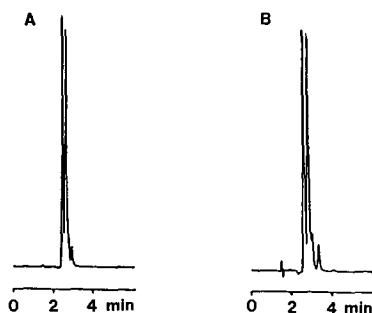
Engelhardt [8] The CSPs I and II resolved the naphthyl-containing sample **6** (Table III) only very incompletely (1 0 ⩽ α ⩽ 1 06) In comparison with this lack of selectivity, TCSPs I and II resolved this sample better (1 13 ⩽ α ⩽ 1 25), as can be seen in the chromatograms in Fig 8

CONCLUSIONS

Chromatographic selectivity with chiral stationary phases is not only a function of the chiral selector used to form diastereomeric solute–CSP complexes but also depends on non-enantioselective interactions of surface silanol groups or other polar sites on the phase

The retention of solutes which are able to interfere directly by hydrogen bonding is strongly affected by thermal treatment of silica

The observed retention of various samples suggests a more polar silica surface than would be expected after thermal treatment at 1000°C This treatment lowers te specific surface area severely but in some instances increases the selectivity of the chiral stationary phase owing to the lower surface concentration of silanols The reason is fewer polar interaction sites, which leads to additional peak broadening In the tested phases, the polar secondary hydroxyl group near the chiral selector part can influence the retention of samples

surface area measurements of silica, Dr W Padowetz of Ciba-Geigy, Basle, for elemental analysis data and Mrs H Murray for linguistic advice

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