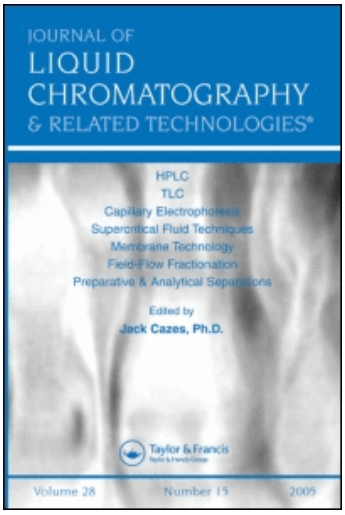


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chiral Recognition Based on Hydrophobic Entanglement of Enantiomers with Chiral Diamide Phases in Aqueous Media

Akira Dobashi[a]; Yasuo Dobashi[a]; Tamami Ono[a]; Ken'ya Ishida[b]; Norio Oshida[c]; Shoji Hara[a]
[a] Tokyo College of Pharmacy, Tokyo, Japan [b] Central Research Laboratory, Takasago International Co., Tokyo, Japan [c] Nisshin Flour Milling Co.-Ltd., Pharmaceutical Research Center, Saitama, Japan

To cite this Article Dobashi, Akira , Dobashi, Yasuo , Ono, Tamami , Ishida, Ken'ya , Oshida, Norio and Hara, Shoji(1993) 'Chiral Recognition Based on Hydrophobic Entanglement of Enantiomers with Chiral Diamide Phases in Aqueous Media', Journal of Liquid Chromatography & Related Technologies, 16: 4, 825 — 841
To link to this Article: DOI: 10.1080/10826079308020937
URL: http://dx.doi.org/10.1080/10826079308020937

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(4), 825-841 (1993)

CHIRAL RECOGNITION BASED ON HYDROPHOBIC ENTANGLEMENT OF ENANTIOMERS WITH CHIRAL DIAMIDE PHASES IN AQUEOUS MEDIA

AKIRA DOBASHI*, YASUO DOBASHI, TAMAMI ONO, KEN'YA ISHIDA†, NORIO OSHIDA§, AND SHOJI HARA

Tokyo College of Pharmacy
1432-1 Horinouchi
Hachioji, Tokyo 192-03, Japan

ABSTRACT

Chiral stationary phases (CSPs) from *N*-(10-undecenoyl)-L-valine *tert*-butylamide (**1**) and *N*-(5-hexenoyl)-L-valine *tert*-butylamide (**2**) were each found to afford a hydrophobic interfacial phase by which hydrogen-bond association could be induced for the resolution of enantiomeric *N*-acylated amino acid esters in aqueous phase liquid chromatography.

This was shown using the fluorescence fine structure of pyrene sorbed on the interfacial phase in three kinds of wetting water-organic solvent mixtures: water-methanol, water-acetonitrile, and water-tetrahydrofuran (THF). The intensity ratios of pyrene vibronic emission peaks gave strong indication of changes in microenvironment polarity around pyrene as a function of overlaying solvent composition.

Increase in organic solvent concentration enhanced polarity due to solvent intercalation in the interfacial phase. A less polar organic solvent, such as THF, was caused to become distributed to a greater degree within the phase than a more polar methanol and consequently, thus giving rise to greater polarity. Enantioselective association between the chiral moiety and enantiomers was hindered by increased intercalation of the less polar organic solvent, thus lessening the degree of chiral recognition.

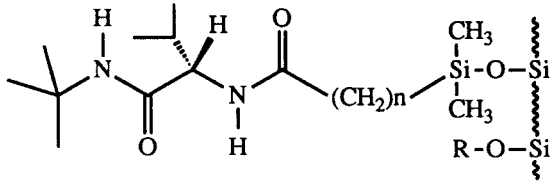
†Current address: Takasago International Co., Central Research Laboratory, 36-31, 5-chome Kamata, Ohta-ku, Tokyo 144, Japan

§Current address: Nisshin Flour Milling Co.-Ltd., Pharmaceutical Research Center, 3-1, 5-chome Turugaoka, Oi-machi, Iruma, Saitama 354, Japan

The relationship between the polarity of the interfacial phase and extent of enantiomer resolution in liquid chromatography is discussed in regard to the difference in the spacer length between CSP**1** and **2** and that between CSP**1b** trimethylsilylated to remove remaining surface silanols and CSP**1** without trimethylsilylation.

INTRODUCTION

Hydrophobic interactions contribute most to the formation of molecular assemblies that are shielded from bulk aqueous phase in aqueous media. When other recognition forces such as hydrogen bonding enter the hydrophobic microenvironment, these forces become effective for the chiral recognition of enantiomers. Alkylsilyl-modified silicas commonly used in aqueous phase liquid chromatography form hydrophobic interfacial phases so as to diminish the liquid-solid interfacial area under aqueous media (*1-3*). Each such phase is a medium having potential for introducing the recognition force in the solubilization of hydrophobic substances.



	n	R
1	10	H
1b	10	$(CH_3)_3Si$
2	4	H

Chiral stationary phases (CSPs) derived from ω-alkenoyl-L-valine *tert*-butylamides afford a hydrophobic microenvironment in which hydrogen-bond association is induced for the resolution of enantiomers in aqueous phase liquid chromatography. CSP**1** and **2** differ in the length of their spacer units by which the chiral moiety is partitioned off from the silica surface. CSP**1** has a decamethylene and CSP**2** a tetramethylene unit. Both units contribute to the formation of a hydrophobic interfacial phase, along with amino acid side-chains and the *tert*-butyl group of the chiral moiety. CSPs depend on two amide functions for entrapping enantiomers through hydrogen bonding. CSP**1** is capable

of separating various enantiomers possessing at least two hydrogen bonding sites in nonaqueous phase operation and higher enantioselectivity for enantiomeric *N*-acylated amino esters than for any other types of enantiomers (*4*). That CSP is produced through an association mode that mimics mutual hydrogen bonding between amide functions in linear peptides may be the reason for this. In an aqueous phase experiment, preliminary results were obtained on amino acid derivatives as solutes.

The hydrogen bonding mode of *N*-acetyl-L-valine *tert*-butylamide (**1c**), a soluble analogue of CSP**1**, for the amino acid derivatives that would lead to separation on CSP**1** was clarified based on IR and NMR studies using a solution of **1c** and *N*-4-nitrobenzoylleucine isopropyl ester (**3**) (*5*). A more stable (L-L) diastereomeric complex was induced through the simultaneous formation of bidentate NH--O=C hydrogen bonds between **1c** and **3**, and hydrogen bonding sites in **1c** were the *N*-terminal carbonyl group and *C*-terminal amide proton, designated as the C_7 side.

Formation of the hydrophobic interfacial phase and influence of overlaying solvents, *i.e.*, water-organic solvent mixtures, on CSPs were evident by the fluorescence of pyrene sorbed onto the phases, as have been observed in alkylsilyl-modified silicas of which octadecylsilyl-modified silica is representative (*1-3*). In the following is discussed relationship between the microenvironment polarity of the interfacial phase and extent of enantiomer separation in liquid chromatography as determined by two different chiral moiety-grafting spacers in length and three kinds of organic solvents, and trimethylsilylation to remove remaining surface silanols on CSP**1**. Enantioselective hydrogen-bond association between the chiral moiety and amino acid derivatives has also been demonstrated in a hydrophobic interfacial phase using dansylated amino acid esters as alternative fluorescence probes.

EXPERIMENTAL SECTION

Synthesis of CSP1

N-(10-Undecenoyl)-L-valine *tert*-butylamide (**1**). To a solution of L-valine *tert*-butylamide hydrochloride (1.98 g, 8.24 mmole; subliming at ca. 148°C (recrystallized from ethanol-ether)), prepared from catalytic hydrogenation (2%

palladium-on-charcol) of *N*-benzyloxycarbonyl-L-valine *tert*-butylamide (mp 112-113°C; lit.(*6*) mp 108.5-109.5°C) in HCl-methanol solution, in 10 mL of dry $CHCl_3$ (distilled from P_2O_5) were added 1.83 g of triethylamine and 1.84 g of 10-undecenoyl chloride (Tokyo Kasei; distilled under reduced pressure (bp 143.5°C (24.5 Torr))) in an ice-water bath under an atomosphere of argon. After 1 h of stirring, the solvent was removed under reduced pressure. The residual oil was dissolved in diethyl ether. The organic layer was washed successively with water, 7%(v/v) hydrochloric acid and saturated salty water and dried over anhydrous sodium salfate. The solvent was removed under reduced pressure and the residue chromatographed with Dowex 1-X8 (20 mL) using methanol as the effluent. The combined eluent was concentrated to dryness. The residual oil was further purified on 20 g of a silica gel (Wako C-100) column and ethyl acetate-*n*-hexane (1:8, v/v) mixture to yield 2.82 g (82%) of pure diamide as a colorless semisolid: IR(KBr) 3300, 1660, 1640, 1545 cm^{-1}; ^{1}H-NMR (90MHz; $CDCl_3$) δ 0.90 (3H, d, J=6.6 Hz), 0.94 (3H, d, J=6.6Hz), 1.28 (10H, brs), 1.33 (9H, s), 1.48-1.80 (2H, m), 1.80-2.35 (5H, m), 4.12 (1H, dd, J=8.6Hz, J=8.6Hz), 4.80-5.12 (2H, m), 5.55-6.02 (1H, m), 6.15 (1H, brs), 6.45 (1H, brd, J=8.6Hz); MS(chemical ionization) Calcd. for $C_{22}H_{38}O_2N_2$ 338, Found 339(MH^+); $[\alpha]^{28}_D$ = -24.0° (c 0.986, $CHCl_3$). Anal. Calcd: C, 70.96; H, 11.31; N, 8.27, Found: C, 70.89; H, 11.30; N, 8.19.

N-(11-(Chlorodimethylsilyl)undecenoyl)-L-valine *tert*-butylamide (**2**). To a solution of olefin **1** (453 mg, 1.34 mmole) in 5 mL of dry $CHCl_3$ was added 0.1 mL of a 2-propanol solution of chloroplatinic acid (0.25 mol/L) at room temperature. After stirring the mixture for 5 min, 2.2 mL of dimethylchlorosilane were added, followed by heating 60°C for 15h. The solvent and excess silane reagent were removed under reduced pressure and the residue was coevaporated twice with dry $CHCl_3$ to afford the desired silane as a slightly yellowish oil. This material was used for the next stage without further purification.

Silica gel modified with chlorodimethylsilane **2** (**3**). Porous silica (1.0 g, Nucleosil 100-5 (5 μm, 100 Å) Marcherey-Nagel, Düren) was dried at 200°C under reduced pressure for 24 h, and treated with a solution of silane **2** in 2 mL of pyridine (distilled from CaH) at room temperature. After the mixture had been gently stirred for 24 h, the modified gel was collected by filtration and washed successively with methanol and acetone: IR (diffuse reflection method with the

modified gel and KBr mixture) 3288, 1642, 1556 cm^{-1}. Anal. Found: C,14.38; N, 1.48.

Silane **2** was also grafted on Develosil 100-5 (5 μm, 100 Å; Nomura Chemical Co., Tokyo) in the same manner described above. Anal. Found: C, 14.72; N, 1.27.

For some of the modified gels, trimethylsilylation of the remaining surface silanols was conducted.

Trimethylsilylated gel (**4**). To a suspension of 1.56 g of modified gel **3** (dried at 70°C under reduced pressure for 4 h) in 6 mL of dry pyridine were added 3 mL of trimethylsilyl chloride (Tokyo Kasei). The mixture was gently stirred at room temperature for 12 h and the silica gel was collected by filtration and washed successively with $CHCl_3$, methanol and acetone. The trimethylsilylated gel **4** was dried over P_2O_5 under reduced pressure for 3 h. Gel **4** derived from Nucleosil 100-5, Anal. Found: C, 14.89; N, 1.39.

Synthesis of CSP2

N-(5-hexenoyl)-L-valine *tert*-butylamide (**5**) Olefin **5** was prepared from 5-hexenoyl chloride (bp 110-118°C), obtained by treating 5-hexenoic acid with a mixture of thionyl chloride and catalytic amount of dimethylformamide, and L-valine *tert*-butylamide hydrochloride by the procedure for olefin **1**: mp 206-206.5°C (recrystallized from ethyl acetate-*n*-hexane); ^{1}H-NMR (90MHz; $CDCl_3$) δ 0.93 (6H, d, J=6.9Hz), 1.33 (9H, s), 1.80-2.17 (1H, m), 2.27-2.45 (4H, m), 4.09 (1H, dd, J=6.9Hz, J=6.6Hz), 4.89-5.21 (2H, m), 5.54-6.07 (2H, m; $CONHC(CH_3)_3$ + CH_2=C<u>H</u>CH_2-), 6.27 (1H, brd, J=6.9Hz); MS (chemical ionization) Calcd. for $C_{14}H_{26}N_2O_2$ 254, Found 255 (MH^+); $[\alpha]^{22}D$= -28.21° (c=1.003, chloroform); Anal. Calcd: C, 66.11; H, 10.30; N, 11.01, Found: C, 65.91; H, 10.30; N, 10.95.

The modified gel via hydrosilylation of olefin **5** was prepared according to the procedure for modified gel **3**: IR (the diffuse reflaction method) 3304, 1646, 1550 cm^{-1}; Anal. Found: C, 11.72, N, 1.63.

Chromatographic Runs

Two types of laboratory-constructed liquid chromatography were conducted. One system consisted a Jasco Model LCP-350 pump, Rheodyne Model 7125

injector with a 20-μL loop and Jasco UVIDEC-100-II variable-wavelength detector; the other was comprised of a Simazu LC-5A pump, Rheodyne Model 7413 injector with a 0.5-μL loop, and variable-wavelength UV detector, Simazu SPD-2AM equipped with a 0.5-μL cell. The wavelength was set at 254 nm. The column, 25 X 0.46 (i.d.) cm and packed with CSP**1** derived from Develosil 100-5, was used for each chromatographic run. The column (50 X 0.1 (i.d.) cm) slurry packed by the usual technique (slurry solvent, chloroform; pressurizing with carbon tetrachloride at 11000 psi) with CSP**1**, **1b** and **2** from Nucleosil 100-5, were also used.

Methanol and tetrahydroruran (THF), as mobile phase components, were fluorescence spectroscopic and of HPLC-grade, respectively. Mixtures of each organic solvent and deionized water were filtered through a 0.45 μm pore-membrane filter and degassed by bubbling helium gas through them for 10 min. The amino acid derivatives to be resolved were the same as those reported previously except that *N*-dansyl amino acid isopropyl esters (6) were also used. These derivatives were prepared from the corresponding amino acid isopropyl ester hydrochloride by treatment with dansyl chloride (Wako Pure Chemical Co., Tokyo) in the presence of triethylamine.

Fluorescence Studies

Steady-state fluorescence spectra were obtained with a Hitachi 650-60 spectrometer, the excitation and emission slit widths both being 2 nm. Emission intensity was measured during excitation at 337 nm and at both 373 and 383 nm, using pyrene (Tokyo Kasei; recrystallized from ethanol (mp 152-152.5°C)) as the probe in a suspension of chemically modified silica gel, according to literature method (*1*). The modified gels for fluorescence measurement were CSP**1** and **2** present on the surfaces of remaining silanols and trimethylsilylated CSP**1b**, all from Nucleosil 100-5, and the octylsilyl-modified silica gel (Nucleosil 100-5 C_8 (5 μm, 100 Å); Marchery-Nagel) whose surface silanols were also still present. The suspension was prepared by adding 2.5 mL of 0.052 M $CuSO_4$ and 0.1 mL of 0.043 M sodium dodecyl sulfate (SDS) to 5 mg of particles in a 10-mm quartz cell. The mixture was vigorously shaken to ensure the complete suspension of all particules, followed by immersion in an ultrasonic bath for 1 min. To the suspension were added 20 μL of 2.5 X 10^{-4} M pyrene-methanol solution. After

sonication for 1 min, emission intensity was then measured as quickly as possible at both wavelengths, followed by the addition of 125 μL of the organic solvent to the cell. All measurments were made for three different cells containing the same sample with different organic solvent-water mixtures. Pyrene fluorescence was corrected by subtracting the intensity of scattered light by the particles and that of emission from the supernatant obtained by centrifuging a suspension having the same composition as that in the cells. The intensity ratio of the emission peak at 383 nm relative to that at 373 nm was calculated from the corrected data.

Entanglement of dansylamino acid isopropyl esters into CSP1 was studied by the above procedure except that the probe concentration was 10^{-3} M in methanol. Fluorescence spectra were recorded with excitation at 343 nm in *N*-dansylphenylalanine isopropyl ester and 345 nm in the corresponding leucine derivative.

RESULTS AND DISCUSSION

Formation of the hydrophobic interfacial phase on CSPs was detected by the fluorescence of pyrene sorbed onto the phases. The intensity ratio of absorption at 383 nm relative to that at 373 nm (I_{383}/I_{373}) indicated microenvironment polarity around pyrene as the probe and ranges from 0.51 in water to 1.63 in *n*-hexane (*8*). Figure 1 shows the intensity ratio in CSP1 in the presense of three wetting water-organic solvent mixtures: water-methanol, water-acetonitrile and water-THF. The fractions of organic solvents ranged from 0.8 to 30 parts in methanol and 0.8 to 25 parts in THF by volume ratio to 100 parts of water. A significant concentration of the probe molecule on the chiral layer was maintained in these concentration ranges. Microenvironment polarity in CSP1 was noted to depend on the particular organic solvent used and its fraction, as indicated in Figure 1. With water-methanol mixtures, the intensity ratio, following a slight initial increase from 1.08 (0.8) to 1.10 (10 parts of methanol), gradually decreased with increase in the methanol and was still 1.08 at 30 parts of methanol. With THF as the organic solvent, intensity ratio of pyrene in the chiral layer was less than that with methanol throughout the concentration region examined and dropped markedly with increase in THF fraction. At 25 parts of THF, the intensity ratio was 0.90. Being a less polar organic solvent, THF would necessarily become distributed to a greater extent within the interfacial phase, resulting in higher polarity, as evident from Figure 1.

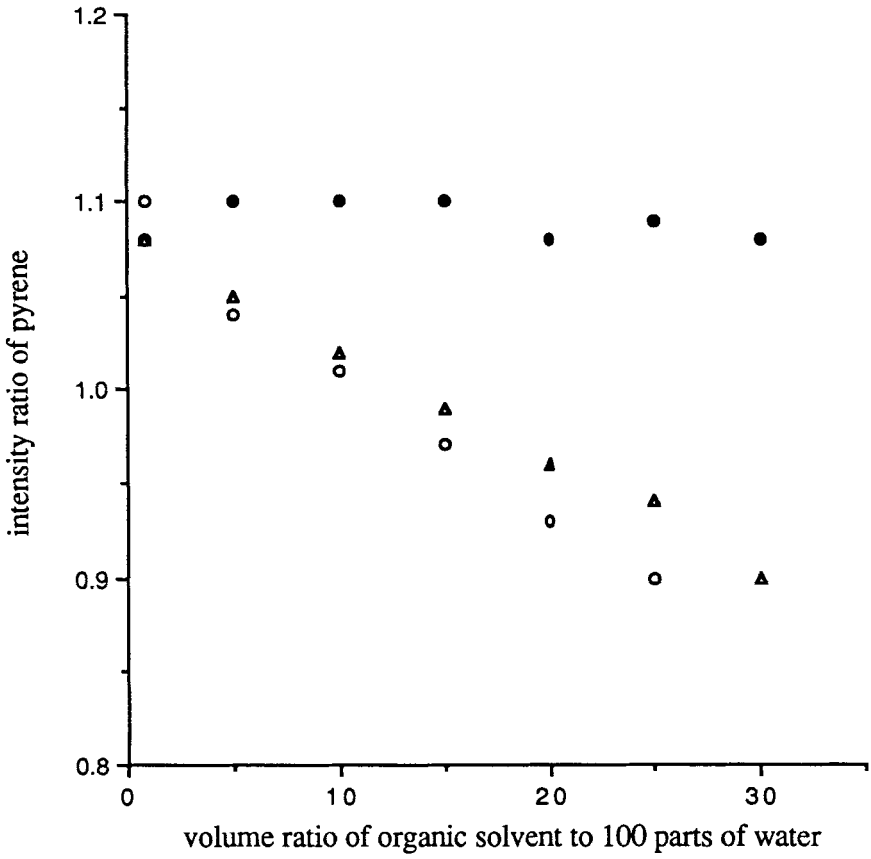


FIGURE 1 Microenvironment Polarity of Pyrene Sorbed in CSP1 as a Function of Solvent Composition: (a) methanol-water mixtures; ●, (b) acetonitrile-water mixtures; Δ, and (c) THF-water mixtures; o.

Of the three organic solvents examined, acetonitrile exhibited intermediate behavior near that of THF. This order relevant to phase polarity is similar to that expected based on the isotherms of distribution between the bulk aqueous phase and octadecylsilyl-modified gel (*9*). The interfacial phase formed with the water-methanol mobile phase continued to have lower polarity which depended on solvent composition to a lesser extent than that of the water-THF mobile phase. The

former thus provides a favorable hydrophobic interfacial phase for inducing diastereomeric hydrogen-bond association and maximum chiral separation of enantiomers thus becomes possible.

Hydrophobic entanglement of enantiomers in the chiral interfacial phase was demonstrated by fluorescence using dansylated amino acid isopropyl esters capable of resolving on CSP**1**, as shown in Table I. The wavelength and intensity of the emission peak of the dansyl derivative are influenced by solvent polarity (*10, 11*); its emission is very weak in water and shifts toward a shorter wavelength with increase in emission intensity in solvents whose polarity is less than that of water.

Thus, the position of maximum emission makes possible assessment of accessibility to the hydrophobic chiral layer of the dansyl derivative. The dansyl-L-phenylalanine derivative sorbed onto CSP**1** in methanol-water (0.8:100, v/v) mixture had an emission maxima at 470 nm, indicating a blue shift of about 85 nm, in contrast with the peak (555 nm) observed for its solution in the same methanol-water mixture. The emission maxima shifted to 482 nm in the presence of 30 parts of methanol, corresponding to an increase in phase polarity and the relative intensity of the fluorescence reduced gradually from 0.49 in 0.8 to 0.34 in 30 parts with increase in methanol fraction. This behavior was also observed for the leucine derivative (471 nm in 0.8 (relative intensity, 1.01) and 478nm in 30 parts of methanol (0.70)). Difference in relative intensity between the enantiomers, as would be expected from that in their retentivity on CSP**1**, could not be observed by the above suspension fluorescence study owing to difficulty in maintaining the experimental conditions for the enantiomers. However, the L enantiomer showed greater decrease in the relative intensity than the D enantiomer for both dansylated derivatives when the methanol fraction was increased from 0.8 to 30 parts (0.154 for the L-phenylalanine and 0.083 for D-phenylalanine derivatives; 0.310 for the L-leucine and 0.224 for D-leucine derivatives). It thus follows that the dansyl derivative is entangled in the hydrophobic interfacial phase where enantioselective hydrogen-bond association occurs. The same situation would also apply to the resolution of the 4-nitrobenzoylated amino acid derivatives which are the most effectively resolved derivatives on CSP**1** under nonaqueous phase operation.

Table II shows the resolution of racemic *N*-4-nitrobenzoyl amino acid isopropyl esters on CSP**1** and **2** when using a 40% water-methanol mixture as the mobile phase solvent. Methanol concentration in the mobile phase exceeded that in phase polarity measurement on CSP**1** in order to achieve adequate retention of the

TABLE I
Optical Resolution of Enantiomeric *N*-Dansylamino Acid Isopropyl Esters on CSP1 with an Aqueous Mobile Phase[1)]

Amino acid	k'_D[2, 3)]	α[4)]
Ala	3.20	1.03
Val	5.42	1.03
Leu	7.83	1.10
Phe	8.15	1.12

1) Chromatographic conditions: column, 25 X 0.46 (i.d.) cm packed with CSP1 derived from Develosil 100-5; mobile phase solvent, 40%(v/v) water in methanol; flow-rate, 1 mL/min; column temperature, ambient; detection, UV at 254 nm. 2) Capacity factor of the first-eluted D enantiomer. 3) Retention time of a vacant peak of methanol-enriched samples was used for the nominal hold-up time. 4) $\alpha = k'_L/k'_D$.

TABLE II
Optical Resolution of Enantiomeric *N*-(4-Nitrobenzoyl)amino Acid Isopropyl Esters on CSP1 and 2 with an Aqueous Mobile Phase[1)]

	CSP1		CSP2	
Amino acid[2)]	k'_D	α	k'_D	α
Leu	2.88	1.34	1.25	1.19
Ile	2.78	1.20	1.19	1.10
Val	1.89	1.17	0.84	1.11
Ala	1.00	1.17	0.52	1.00
Phe	3.05	1.25	1.27	1.13
Thr	4.26	1.11	1.68	1.04
Ser	3.34	1.09	1.37	1.03
Tyr	6.52	1.19	2.53	1.10
Glu	1.82	1.18	0.89	1.10
Asp	1.62	1.08	0.83	1.00
Trp	3.52	1.21	1.52	1.13
Cys	4.55	1.10	1.79	1.03
Met	1.81	1.22	0.82	1.15
Pro	0.70	1.00	0.42	1.00

1) Chromatographic conditions the same as in Table I, except for a column packed with CSP2 derived from Nucleosil 100-5; 50 X 0.1 (i.d.) cm; flow-rate, 40 μL/min. 2) Hydroxyl group of serine, threonine, and tyrosine were protected as 4-nitrobenzoyl esters and the carboxyls of aspartic and glutamic acid were protected fully as isopropyl esters.

enantiomers. Sufficient resoution, however, was possible even at methanol concentrations exceeding 50%, which should lead to greater phase polarity.

Figure 2 shows a typical resolution of racemic *N*-4-nitrobenzoylamino acid isopropyl esters on CSP1. The L enantiomer, rather than corresponding D enantiomer, was retained the most in a series of amino acid derivatives, as also noted for dansylated derivatives. This elution order was also identical to that in nonaqueous phase experiments though α was less (α of the leucine derivative that separated the most efficiently was 3.27 with a 1% 2-propanol-*n*-hexane mobile phase (*4*)). The elution order of each amino acid derivative was determined by the degree of hydrophobicity of the amino acid side-chain in the solute. Table III indicates the retentivity and separability of enantiomers observed for the leucine and alanine derivatives as a function of the water fraction. An increase in the water fraction in the mobile phase solvent led to greater solute retention, as has generally been observed in reversed phase liquid chromatography, accompanied by slight increase in α between enantiomers. It thus follows that hydrophobic interactions are the major factors determining the degree of retention of solute enantiomers on CSP1.

Exhaustive trimethylsilylation of CSP1 was noted to actually cause the intensity ratio to increase throughout the region examined (increase: 0.08 at 0.8% methanol), as illustrated in Figure 3. In a chromatographic study, trimethylsilylation led to greater enantiomer retention, as expected from this observation, while α slightly decreased. By trimethylsilylation in the case of CSP1 the α and k' of the first-eluted D enantiomer with 40% water-methanol mobile phase were: 1.28, 3.65 for leucine; 1.14, 2.42 for valine; 1.13, 1.22 for alanine; and 1.46, 3.80 for the phenylalanine derivative. Reduced enantioselectivity in aqueous mobile phases may be explained as due to intervention of achiral hydrophobic interactions in the overall retention process as well as competition between mobile phase components penetrating the interfacial phase and solute enantiomers for CSPs. The retentivity and separability of enantiomers are likely affected by achiral and nondiscriminating interactions which do not contribute to enantioselectivity between CSP and enantiomers in overall retention along with chiral interactions. In our hydrogen bonding systems, elimination of remaining surface silanols on CSP1, which possibly compete with the recognition force to a considerable extent and lessen enantioselectivity, led to greater separability of the

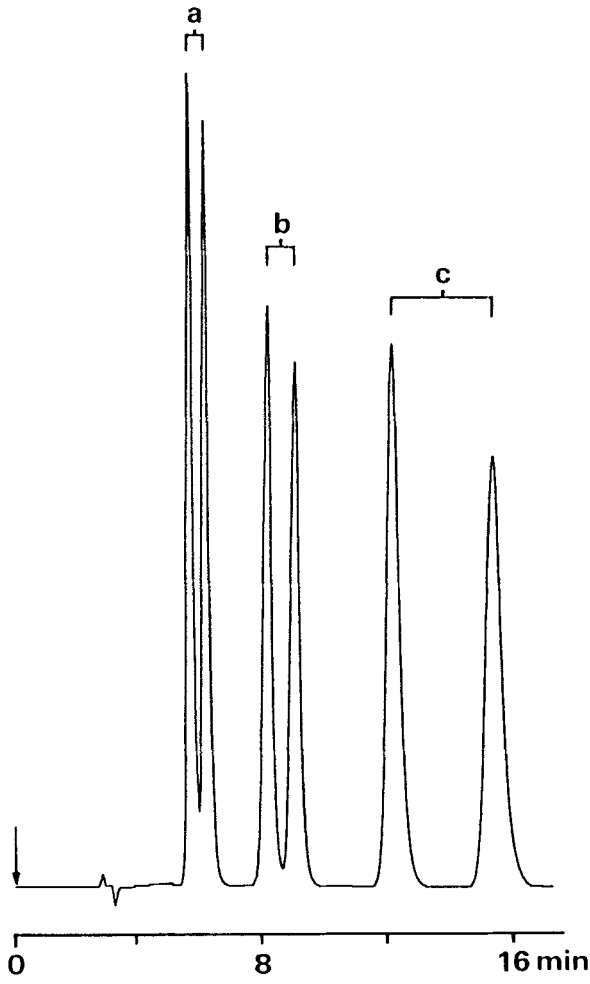


FIGURE 2 Optical Resolution of a Mixture Containing Three Enantiomeric Pairs of *N*-4-Nitrobenzoylamino Acid Isopropyl Esters on CSP1: (a) alanine, (b) valine, and (c) leucine derivatives. Chromatographic conditions the same as in Table II.

TABLE III
Retentivity and Enantioselectivity for *N*-(4-Nitrobenzoyl)leucine Isopropyl Ester and *N*-(4-Nitrobenzoyl)alanine Isopropyl Ester on CSP1 as a Function of Water Composition[1)]

Leucine derivative						
	Organic solvent					
	Methanol		Acetonitrile		THF	
Water (%)	k'D	α	k'D	α	k'D	α
30	1.49	1.26				
40	2.88	1.34	1.57	1.16	1.08	1.00
50	9.75	1.33	3.39	1.16	4.17	1.00
60			9.15	1.17	11.00	1.07

Alanine derivative						
	Organic solvent					
	Methanol		Acetonitrile		THF	
Water(%)	k'D	α	k'D	α	k'D	α
30	0.96	1.10				
40	1.00	1.17	0.75	1.08	0.76	1.00
50	2.44	1.18	1.40	1.08	1.87	1.00
60			2.87	1.06	3.34	1.07

1) Chromatographic conditions the same as in Table I.

amino acid derivatives in the nonaqueous phase operation (*5, 12*). In an aqueous phase experiment, subsequent trimethylsilylation, in contrast, caused adverse effects, possibly due to additional hydrophobic interactions brought on by a trimethylsilyl group on the silica surface.

When the spacer length bearing the chiral moiety was shortened from decamethylene in CSP1 to tetramethylene units in CSP2, hydrophobicity of the interfacial phase observed in the intensity ratio decreased from 1.10 to 0.99 in the maximum ratio though CSP2 showed a profile of changes in the intensity ratio similar to that observed in CSP1 throughout the methanol concentration region examined, as illustrated in Figure 3. This may possibly have been due to water

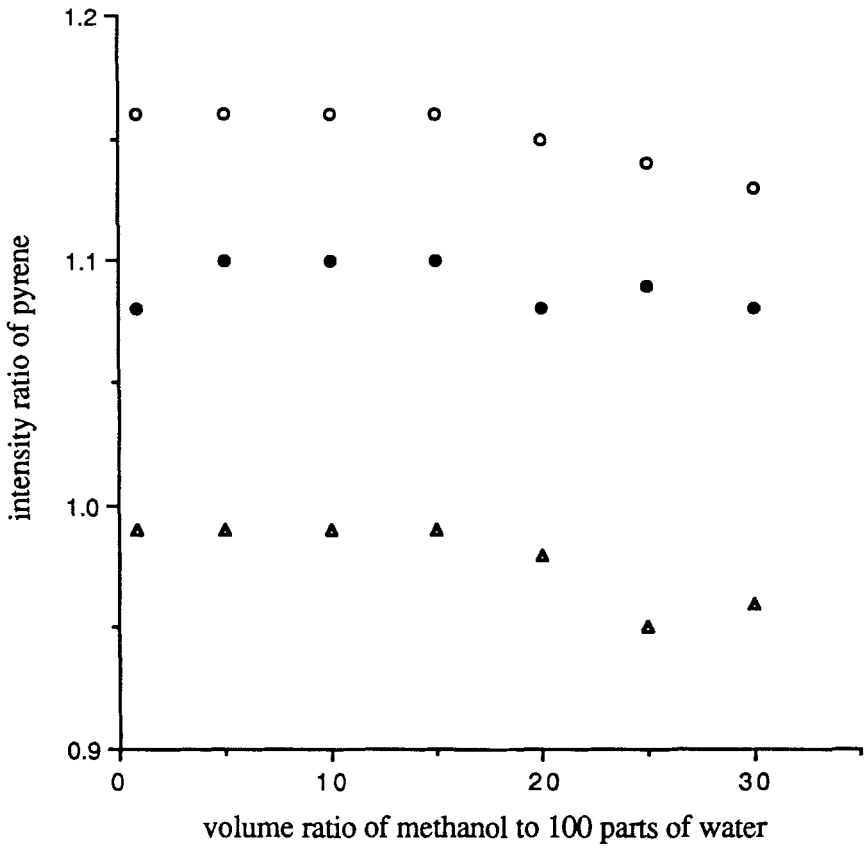


FIGURE 3 Microenvironment Polarity of Pyrene in CSP1, **1b**, and CSP2 as a Function of Methanol Composition: (a) CSP1; ●, (b) CSP**1b**; o, and (c) CSP2; Δ

penetration into the interfacial phase to a greater extent than that for CSP**1**, followed by consequently lower retentivity and separability of enantiomers for the amino acid derivatives than those on CSP**1**.

The hydrophobic features of the interfacial phase that cause enantioselective hydrogen bonding between the terminated chiral moiety and enantiomers should be hindered by increased intercalation of the less polar THF. Chiral recognition using methanol-water mixtures was thus prevented by 40% water-THF mixtures in all

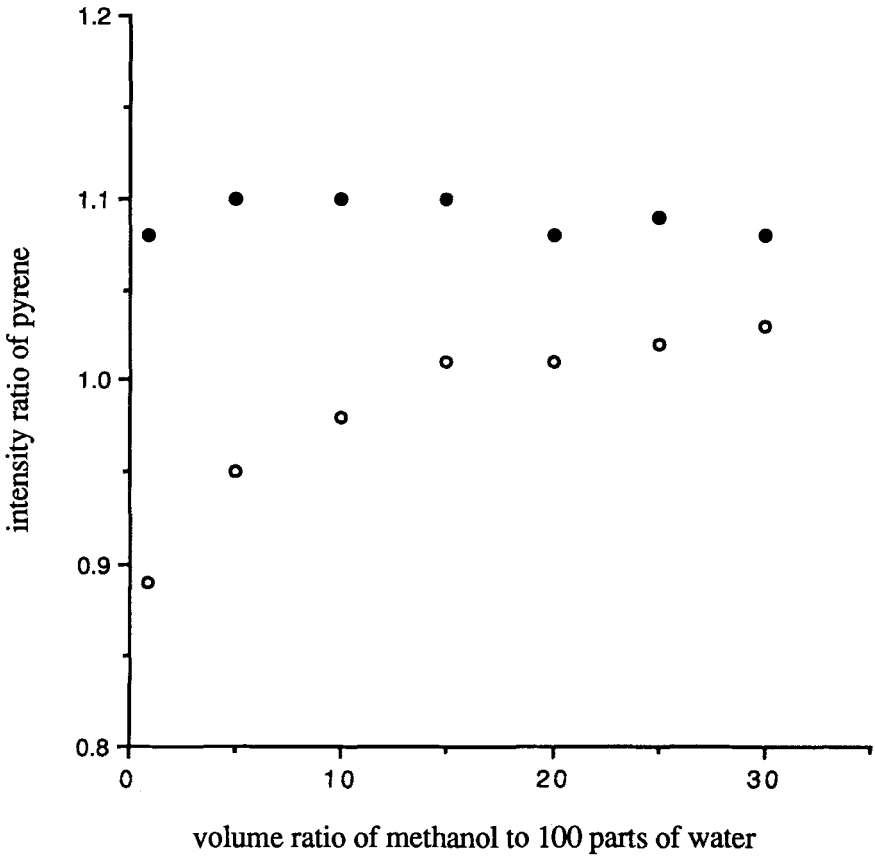


FIGURE 4 Microenvironment Polarity of Pyrene in CSP1 and Octylsilyl-modified Silica as a Function of Methanol Composition: (a) CSP1; ● and (b) octylsilyl-modified silica; o.

cases. Elution was faster than with the water-methanol mobile phase, the maximum capacity factor being 1.89 for the tyrosine derivative. An increase in the water fraction in the mobile phase caused the leucine, serine and metionine derivatives to be resolved again. However, α was only 1.07 (k'_D 11.00) for the leucine derivative with 60% water-THF mixture, as shown in Table III. Intermediate polarity of CSP1 obtained with overlaying water-acetonitrile mixtures

was achieved by the intermediate separability of enantiomers but shorter retentivity comparable to that obtained with water-THF mixtures. Differences in competition of organic solvents intercalated into the chiral layer with association between the chiral moiety and enantiomers, *i.e.*, differences in hydrogen bonding affinity between acetonitrile and THF, would possibly be the reason for this.

With octylsilyl-modified silica having only a hydrocarbon layer (C_8) comparable to the chiral layer (C_{10} at the spacer unit linking the chiral diamide moiety as a polar head group to the silica surface), increase in the methanol fraction caused the intensity ratio to increase rather than decrease in the same methanol concentration range, as indicated in Figure 4. This tendency for increase in the intensity ratio to occur for high water fractions has also been observed on a octadecylsilyl-modified silica surface (*2, 3*). This modified surface attains minimum polarity at about 50% methanol (maximum intensity ratio, about 1.3, this being less than that in alkane solution, about 1.7), followed by increase in methanol concentration. Phase polarity increased again with the methanol concentration exceeded 50%, due to intercalation of methanol in the bonded layer. For CSP1, no such difference transition could be detected in the two regions of solvent composition. Collapse of the hydrocarbon-bonded phase on exposing the probe to the mobile phase and silica surface would appear to account for the reduction in phase polarity for high water fractions (*3*). Possibly, CSP1 provides a hydrophobic environment of sufficient size as to adequately shield pyrene from the solvent and silica surface polarity even in a region where the water fraction is high due to the chiral layer terminated with the valine diamide moiety as a polar head group.

ACKNOWLEDGEMENTS

We gratefully acknowledge support of this research from the Ministry of Education of Japan (Grant-in-Aid No. 03771716)

REFERENCES

1. Stahlverg, J.; Almgren, M. *Anal. Chem.* 1985, *57*, 817.

2. Carr, J. W.; Harris, J. M. *Anal. Chem.* 1986, *58*, 626.

3. Carr, J. W.; Harris, J. M. *Anal. Chem.* 1987, *59*, 2546.

4. Dobashi, A.; Dobashi, Y.; Kinoshita, K.; Hara, S. *Anal. Chem.* 1988, *60*, 1985.

5. Dobashi, A.; Dobashi, Y.; Hara, S. *ACS Sympo. Ser.* 1991, *471*, 164.

6. Beitler, U; Feibush, B. *J. Chromatogr.* 1976, *123*, 149.

7. Dobashi, A.; Hara, S. *Anal. Chem.* 1983, *55*, 1805.

8. Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* 1977, *99*, 2039.

9. Karger, B. L.; NcCormik, R. M. *Anal. Chem.* 1980, *52*, 2249.

10. Chen, R. F. *Arch. Biochem. Biophys.* 1967, *120*, 609.

11. Lochmüller, C. H.; Marshall, D. B.; Wilder, D. R. *Anal. Chim. Acta* 1981, *130*, 31.

12. Dobashi, Y.; Hara, S. *J. Am. Chem. Soc.* 1985, *107*, 3406