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Comparison of monomeric and polymeric chiral stationary phases

Kwang-Pill Lee[a,*], Seong-Ho Choi[a], Soo-Yeon Kim[a], Tae-Hyuk Kim[a], Jae Jeong Ryoo[a], Kazutoku Ohta[b], Ji-Ye Jin[c], Toyohide Takeuchi[d], Chuzo Fujimoto[e]

[a]*Department of Chemistry Graduate School, Kyungpook National University, Daegu 702-701, South Korea*
[b]*Ceramics Research Institute, National Institute of Advanced Industrial Science and Technology, 2266-98 Anagahora, Simoshidami, Moriyama-ku, Nagoya 463-8560, Japan*
[c]*Instrumental Analysis Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[d]*Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[e]*Department of Chemistry, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan*

Abstract

Two-type polymeric chiral stationary phases (pCSPs) were prepared by surface grafting of chiral acryl-type monomers on a silica gel surface modified with 3-(trimethoxysilyl)propylmethacrylate. The prepared pCSPs were characterized by IR, FT-Raman, scanning electron microscopy, and elemental analysis. In addition, two-type monomeric chiral stationary phases (mCSPs) were also prepared. The racemic analytes were separated using the prepared mCSPs and pCSPs. The separation factor (α) and capacity factor (k_1) of the racemic analytes for the pCSP and mCSP were compared. The α and k_1 values of the mCSP were higher than those of the pCSP.

Keywords: Chiral stationary phases, LC; Surface grafting; Retention factor; Separation factor; Amino acids; Naphthylene methylethyl propanamide

1. Introduction

A large number of chiral stationary phases (CSPs) have been developed during the past decade to satisfy the challenge of enantiomer separations, and numerous CSPs are now commercially available [1–4]. Most commercial CSPs contain chiral selectors supported by silica gel. Silica-based chromatography supports have numerous qualities, such as high mechanical stability, resistance to swelling, and excellent efficiency [5–8].

On the other hand, the synthetic polymer beads have stability over an entire range of pH, the variety of possible surface chemistries makes them well suited for chiral HPLC. Despite the many advantages of polymeric supports, there are only a few examples of polymeric stationary phases because the polymeric stationary phases have a low mechanical stability and low resistance to eluents as a standpoint of the swelling [9–11].

In a previous paper [12], the synthetic polymeric micro beads were prepared by radiation-induced polymerization of glycidyl methacrylate (GMA), and, subsequently, synthetic polymeric micro beads were immobilized by lipase in an alkaline medium for chiral separation. However, the synthetic polymeric micro bead was not packed for stainless steel column because the polymeric micro beads prepared

*Corresponding author. Tel.: +82-53-950-5901; fax: +82-53-952-8104.
E-mail address: kplee@knu.ac.kr (K.-P. Lee).

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by radiation-induced polymerization have soft properties.

In order to improve soft properties, the polymeric (p) CSPs were prepared by surface grafting of chiral acryl-type monomers on a silica gel surface with 3-(trimethoxysilyl)propylmethacrylate (γ-MAPS) with a vinyl group. The prepared pCSPs were characterized by IR, FT-Raman, scanning electron microscopy (SEM), and elemental analysis (EA). In addition, the monomeric (m) CSPs were also prepared. The racemic analytes were separated using mCSPs and pCSPs. From the results, the separation factors (α) and capacity factors (k_1) of the mCSP and pCSP were compared.

2. Experimental

2.1. Materials

γ-MAPS was obtained from Tokyo Kasei (Japan). 3,5-Dinitrobenzyl chloride (DNBCl), (*R*)-(−)-2-phenylglycinol, 2-amino-2-phenylethanol, 2-isocyanatoethyl methacrylate, and 3-(triethoxysilyl)propyl isocyanate were purchased from Aldrich. Silica gel (Article 7734, silica gel 60) was obtained from Merck. Toluene, benzene, and dichloromethane (CH_2Cl_2) were distilled before use. The analytes, amide derivatives were synthesized as previously described [13]. The chemical structure of the analytes were shown in Table 1. The chiral monomer, monomeric chiral stationary phase (mCSP-3) in Fig. 1 and three amino acid derivatives in Table 2 were obtained from K-MAC (South Korea).

2.2. Preparation of mCSP-1, pCSP-2, and pCSP-4

2.2.1. Preparation of mCSP-1

Fig. 2 shows the preparation procedure of mCSP-1. To a solution of (*R*)-(−)-2-phenylglycinol (2.24 g, 16.0 mmol) and triethylamine (2.25 ml, 16.2 mmol) in dry dichloromethane (100 ml), 3,5-DNBCl (3.95 g, 16.8 mmol) was added under nitrogen at 0 °C. After the mixture was stirred at room temperature for 12 h, it was washed with 2.0 N HCl, saturated $NaHCO_3$ solution, and then finally with H_2O. The product was purified by an open silica column

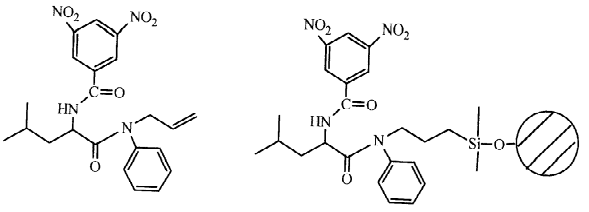


Fig. 1. Structures of the chiral monomer (**5**, left) and mCSP-3 right).

chromatography using hexane–CH_2Cl_2 (1:1.5, v/v) and then dried in vaccuo at 30 °C for 4 h. The yield of (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycinol (**1**) was 1.94 g (43.4%). mp 162–164 °C; ^{1}H NMR (C^2Hd_3): δ 4.30 (m, 1H), 4.66–4.73 (q, 1H), 5.39–5.46 (m, 1H), 7.32–7.38 (m, 5H), 7.60 (d, 1H), 8.97 (d, 2H), 9.16 (t, 1H); IR (KBr): 3331, 3105, 2926, 1647, 1539, and 1346 cm^{-1}.

The (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycinol triethoxyl propyl carbamate (**2**) was synthesized from the reaction of the synthesized **1** (0.95 g, 2.87 mmol) and 3-(triethoxysilyl)propyl isocyanate (0.77 ml, 3.0 mmol) in benzene (50 ml) in the presence of triethylamine (0.30 mmol). The yield of **2** was 1.25 g (75.5%). mp 151–153 °C; ^{1}H NMR (C^2Hd_3): δ 0.60–0.65 (t, 2H), 1.17–1.22 (m, 9H), 1.67–1.69 (m, 2H), 3.25–3.35 (m, 2H), 3.76–3.84 (m, 6H), 4.18–4.24 (q, 1H), 4.63–4.70 (q, 1H), 5.22–5.23 (m, 1H), 5.33–5.35 (t, 1H), 7.33–7.37 (m, 5H), 8.69–8.70 (d, 1H), 9.04–9.05 (d, 2H), 9.15–9.16 (t, 1H); IR (KBr): 3331, 3105, 2926, 1647, 1539, and 1346 cm^{-1}.

The mCSP-1 was synthesized from the prepared **2** (1.15 g, 1.99 mmol) and 5 μm spherisorb silica gel (3.7 g) in toluene (50 ml), and then the column was prepared by the slurry method. The analytical data of the mCSP-1 is described in Table 1.

2.2.2. Preparation of pCSP-2

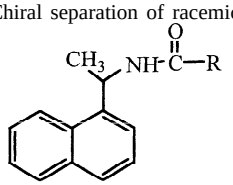
Fig. 3 shows the introduction of the γ-MAPS onto 5-μm spherisorb silica gel. To a suspended solution of 5 μm silica gel (2.5 g) in toluene (40 ml), the γ-MAPS (2.5 g, 10.0 mmol) was added under nitrogen at 0 °C. After the mixture was stirred at 80 °C for 3 days, the silica gel was collected by filtration, and washed with MeOH. The yield of the silica gel with the vinyl group was 1.10 g (22.1%).

Table 1
FT-IR, FT-Raman and elemental analysis data of the mCSP-1, pCSP-2, mCSP-3, and pCSP-4

FT-IR	mCSP-1	pCSP-2	mCSP-3	pCSP-4
	1702 cm^{-1} (C=O), 1549 cm^{-1} (NO_2), 1352 cm^{-1} (NO_2), 955 and 804 cm^{-1} (ring ''breathing'')	1725 cm^{-1} (C=O), 1638 cm^{-1} (C=C), 941–804 cm^{-1} (C–H).	1635 cm^{-1} (C=O), 1550 cm^{-1} (NO_2), 802 cm^{-1} (ring ''breathing'').	1725 cm^{-1} (C=O),1638 cm^{-1} (C=C), 1539 cm^{-1} (NO_2), 750–820 cm^{-1} (ring vibration)
FT-Raman	3068 cm^{-1} (C–H), 2932 cm^{-1} (=CH_2), 1595 cm^{-1} (C=C), 1551 cm^{-1} (C=C), 1360 cm^{-1} (NO_2), 1001 cm^{-1} (ring ''breathing'')	3000–2900 cm^{-1} (C–H), 1725–1700 cm^{-1} (C=O), 1639 cm^{-1} (C=C), 1408 cm^{-1} (CO_2^-), 1375 cm^{-1} (NO_2)	3070 cm^{-1} (C–H), 2903 cm^{-1} (=CH_2), 1592 cm^{-1} (C=C), 1552 cm^{-1} (C=C), 1358 cm^{-1} (NO_2), 1002 cm^{-1} (ring ''breathing'')	3000–2900 cm^{-1} (C–H), 1725–1700 cm^{-1} (symmetric C=O), 1639 cm^{-1} (C=C), 1408 and 804 cm^{-1} (CO_2), 1375 cm^{-1} (symmetric NO_2)
EA analysis	calc.: 0.15 mmol/g (based on C), 0.14 mmol/g (based on N)	Anal found: C, 17.7; H,2.6; N 0.2. calc.: 0.144 mmol/g (based on N)	–[a]	Anal found: C, 17.7; H, 2.6; N, 0.2. calc.: 0.144 mmol/g (based on N)

[a] Not determined.

Table 2
Chiral separation of racemic amide derivatives using mCSP-1 and pCSP-2[a]



–R	mCSP-1		pCSP-2[b]	
	k_1	α	k_1	α
$-CH_2CH_3$	5.40	1.44	1.09	1.53
$-(CH_2)_2CH_3$	3.41	1.29	0.97	1.04
$-(CH_2)_6CH_3$	2.78	1.13	1.15	1.00
$-(CH_2)_8CH_3$	2.51	1.06	1.13	1.00
$-(CH_2)_{10}CH_3$	2.41	1.00	1.13	1.00
$-(CH_2)_{11}CH_3$	2.27	1.00	1.10	1.00

[a] Column: 25×0.46 cm I.D.; eluent, hexane–2-propanol (80:20, v/v); flow rate, 0.5 mL. Absolute configuration of the first eluted enantiomer was R.
[b] Column: 10×0.46 cm I.D. was used.

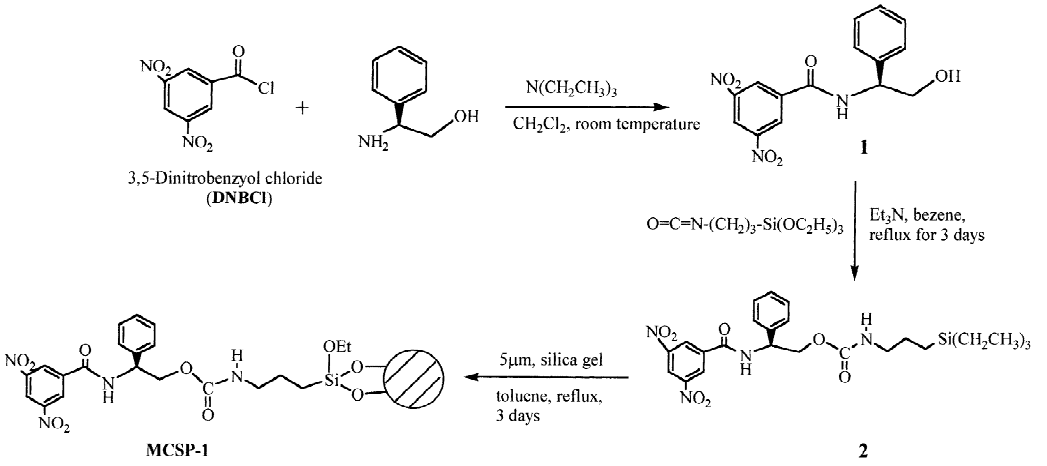


Fig. 2. Preparation procedure of mCSP-1.

FT-IR: 1736 (CO) cm^{-1}; FT-Raman: 1640 (C=C) cm^{-1}; Anal. found ($C_{10}H_{20}O_5Si$): C, 3.47%.

Fig. 4 shows the preparation procedure of chiral acryl-type monomer (**4**). (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycinol (**3**) was prepared with the same method described in Fig. 2. The chiral acryl-type monomer (**4**) (3.6 g) was prepared by the reaction of the **3** and 2-isocyanatoethyl methacrylate in benzene

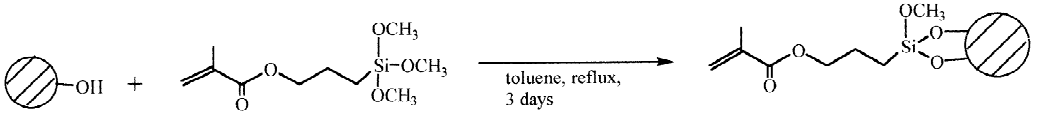


Fig. 3. Introduction of γ-MAPs onto silica gel (5 μm).

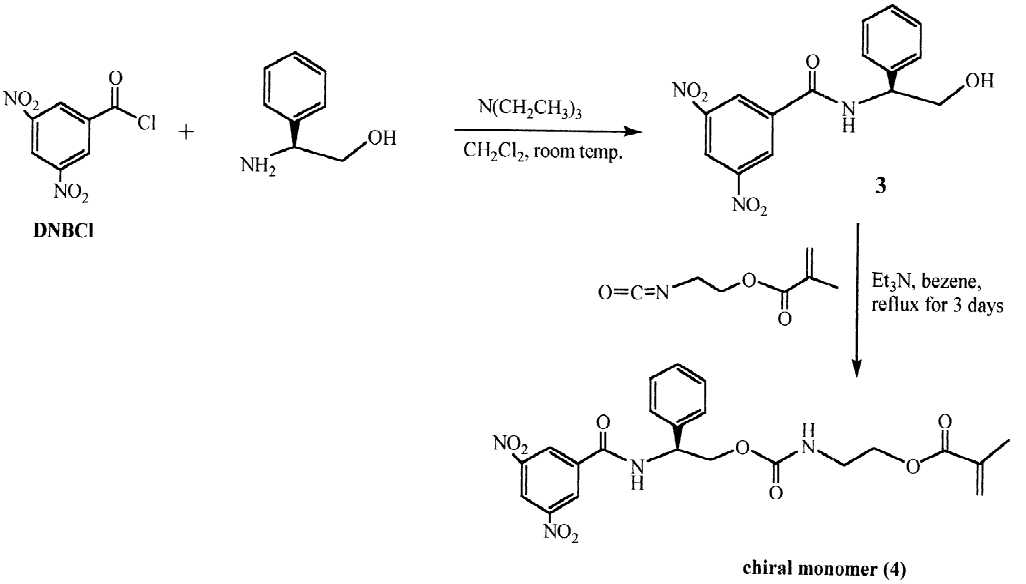


Fig. 4. Preparation of the chiral monomer **(4)** with vinyl group.

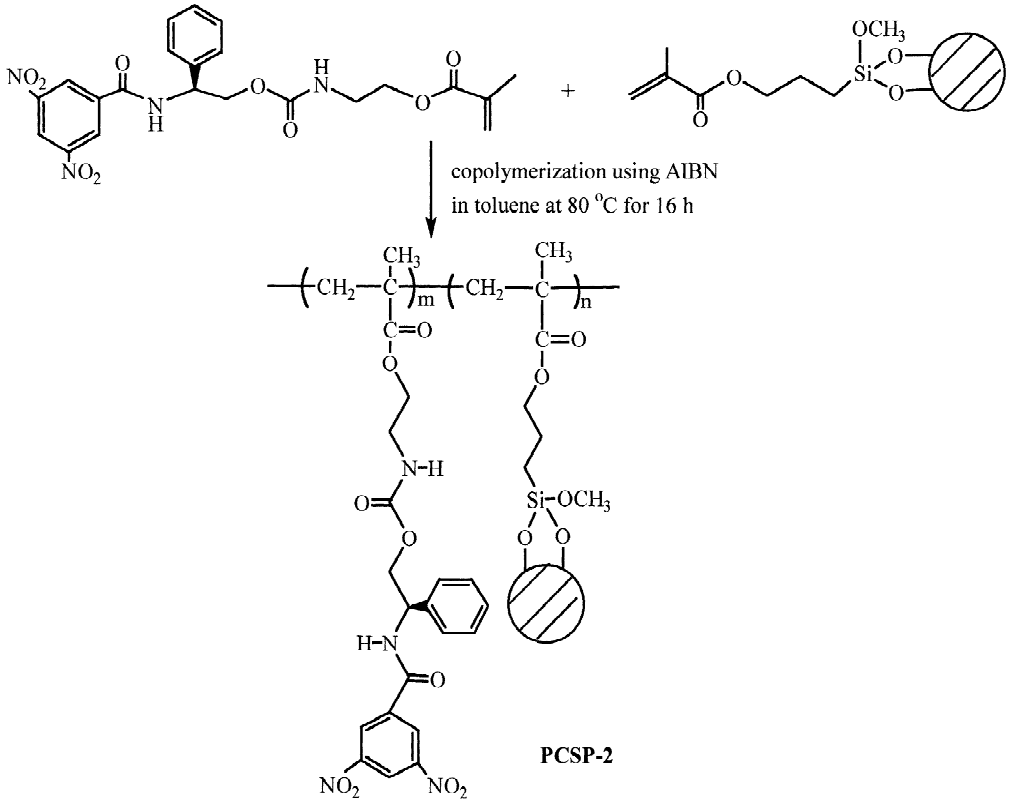


Fig. 5. Preparation of pCSP-2.

in the presence of triethylamine for 3 days. The yield of **4** was 5.1 g (98.6%). ^{1}H NMR (C^2Hd_3): δ 1.93 (s, 3H), 3.29–3.31 (m, 2H), 4.40–4.42 (t, 2H), 4.60–4.62 (d, 2H), 5.47–5.49 (m, 1H), 5.56–5.59 (d, 1H), 6.20–6.21 (d, 1H), 7.20–7.20 (m, 10H), 9.20–9.30 (m, 6H); IR(KBr): 1716 (CO), 1688 (CO), 1655 (CO), 1541 (NO_2), 1449 (NO_2), and 1262 (C–O) cm^{-1}.

Fig. 5 shows the preparation procedure of the polymeric chiral stationary phase (pCSP-2). The chiral acryl-type monomer was copolymerized with AIBN in toluene at 80 °C for 16 h in the presence of γ-MAPS-modified silica gel. The polymer was precipitated in a large amount of methanol, separated by centrifugation, and dried in vacuo at 50 °C for 3 h. The yield of pCSP-2 was 4.6 g (99.8%). The spectroscopy data of pCSP-2 is described in Table 1.

2.2.3. Structure of mCSP-3 and preparation of pCSP-4

Fig. 1 shows structures of the chiral acryl-type monomer and mCSP-3.

Fig. 6 shows the preparation procedure of polymeric stationary phase (pCSP-4). The chiral acryl-type monomer (**5** in Fig. 1) was copolymerized with AIBN in toluene at 80 °C for 16 h in the presence of γ-MAPS-modified silica gel. The polymer was precipitated in a large amount of methanol, separated by centrifugation, and dried in a vacuum oven at 50 °C for 3 h. The yield of pCSP-4 was 4.6 g (99.8%). The spectroscopy data of pCSP-4 are described in Table 1.

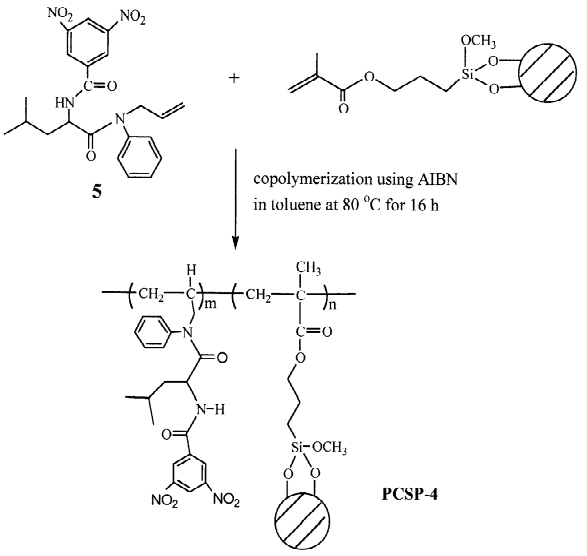


Fig. 6. Preparation procedure of the pCSP-4.

2.3. Chiral separation and characterizations

The HPLC system consisted of a Shimadzu LC-10AD HPLC pump, a Rheodyne Model 7125 injector, and a Young-In 720 Absorbance detector (Young-In, South Korea). The near infrared (NIR) Fourier transform (FT) Raman spectra were recorded with a Bruker FT-106 Raman module equipped with a Ge detector cooled by liquid nitrogen and connected to a Brucker FT-IR 66 interferometer. In order to excite the Raman signal, a continuous wave diode-pumped Nd:YAG laser with a radiation wavelength of 1064 nm (9398.4 cm^{-1}) was used. In all cases, the laser power was 300 mW and the spectral resolution 2 cm^{-1}. FT-IR spectra of the inclusion complex in the solid state were obtained using Nujol mulls with a Perkin-Elmer Model 983 infrared spectrophotometer. For SEM, a sample of 0.5×0.5 cm^2 size coated with a gold–palladium alloy prior to the measurement. The sputtered sample was then scanned by the electron beam in a scanning electron microscope (JSM-840A, Jeol, Japan).

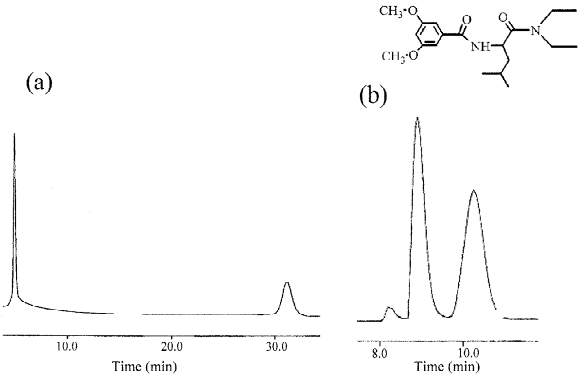


Fig. 7. Chiral separation of racemic *N*-(1-naphthylene-1-methylethyl) propanamide using mCSP-1(a) and pCSP-2 (b).

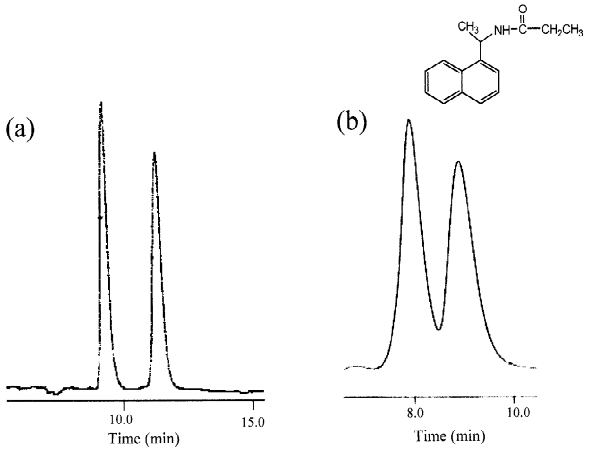


Fig. 8. Chiral separation of racemic amino acid derivatives on mCSP-3 (a) and pCSP-4 (b).

3. Results and discussion

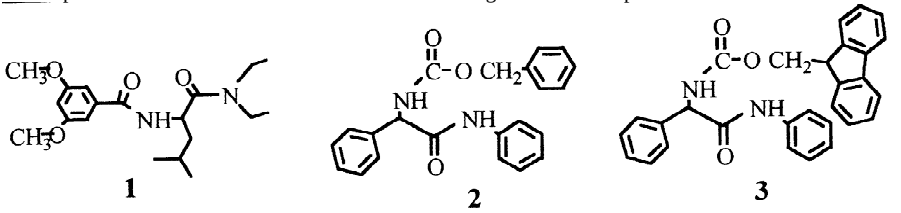
Table 1 shows the FT-IR, FT-Raman and elemental analysis data of mCSP-1, pCSP-2, mCSP-3, and pCSP-4. From the analysis data, the mCSP-1, pCSP-2, and pCSP-4 were successfully prepared.

Fig. 7 shows the chromatogram of chiral resolution of *N*-(1-naphthylene-1-methylethyl)-propanamide using mCSP-1 (a) and pCSP-2 (b) by HPLC. In Fig. 7a and b, the t_0 values were measured using 1,3,5-tri-*tert.*-buthylbenzene and were 3.72 and 3.08 min, respectively. In Fig. 7a, the capacity factor (k_1), which are defined as $(t_1 - t_0)/t_0$ was, respectively. In Fig. 7a, the separation factor (α) and k_1 was to be 1.44 and 5.40, respectively. In Fig. 7b, the α and k_1 values were 1.09 and 1.53, respectively.

Table 2 shows the k_1 and α values of the racemic amide derivatives using mCSP-1 and pCSP-2 as a function of the spacer of alkyl chains. In mCSP-1, the k_1 and α values were decreased by increasing spacer of the acyl group. Pirkle et al. [14] proposed the chiral recognition mechanism. In the proposed mechanism, the naphthyl group of the analyte interacts with the dinitrobenzyl group of the mCSP-1 to form face-to-edge, which is called π–π interaction [1,15]. The carbamate group of racemic analyte was interacted with the carbamate group of mCSP-1. The acyl group of the analytes was stretched to silica gel which has a connecting arm of chiral stationary phase. Therefore, it was considered that the steric hindrance increased by increasing the spacer of the acyl group in analyte was increased. In pCSP-2, k_1 and α were also decreased by increasing spacer of acyl group.

Fig. 8 shows the chromatogram of chiral HPLC separation of the amino acid derivatives using mCSP-3 and pCSP-4. The enantiomers were eluted at t_1 and t_2 and completely separated. In Fig. 8a, k_1 and α ($\alpha = k_2/k_1$) were 2.90 and 10.18, respectively.

Table 3
Chiral separation of racemic amino acid derivatives using mCSP-3 and pCSP-4[a]



Analytes	mCSP-3		pCSP-4[b]	
	k_1	α	k_1	α
1	2.90	10.18	0.46	2.74
2	3.40	2.83	0.44	1.45
3	3.76	3.67	0.48	1.52

[a] Column, 25×0.46 cm I.D; eluent, hexane–2-propanol 80:20, v/v); flow rate, 2.0 mL.
[b] Column, 10×0.46 cm I.D.; flow rate, 0.5 mL.

In Fig. 8b, the k_1 and the α values were 0.46 and 2.74, respectively.

Table 3 shows k_1 and α values of the amino acid derivatives using mCSP-3 and pCSP-4. The k_1 and α values in mCSP-3 were higher those that in pCSP-4.

4. Conclusions

The two-type pCSPs were prepared by surface grafting of the chiral-type acryl monomer onto a silica gel surface with γ-MAPS and monomeric chiral stationary phase was also prepared. The racemic analytes were separated using the prepared mCSP and pCSP. From the results, the conclusions were as follows:

(1) The pCSPs were successfully prepared and characterized by FT-IR, FT-Raman, SEM, and elemental analysis

(2) In analyte of the racemic amide derivatives, the α and k_1 values of the mCSP-1 were higher than that of the pCSP-2

(3) In analyte of the racemic amino acid derivatives, the α and k_1 values of the mCSP-3 were higher than those of the pCSP-4.

Acknowledgements

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