Preparation and Application of Chiral Recognizable Thermosensitive Polymers and Hydrogels Consisting of *N*-Methacryloyl-*s*-Phenylalanine Methyl Ester

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ABSTRACT: Amphiphilic random copolymers, poly(*R*-HPMA-co-*S*-PAM) and poly(HPMA-co-*S*-PAM), were prepared by radical copolymerization of *N*-methacryloyl-(*S*)-phenylalanine methyl ester (*S*-PAM) and *N*-[(*R*)-2-hydroxypropyl]methacrylamide (*R*-HPMA) or *N*-(2-hydroxypropyl)methacrylamide (HPMA) with various molar ratios of *R*-HPMA (or HPMA) (m) to *S*-PAM (n). Either aqueous solution of poly(*R*-HPMA-co-*S*-PAM) with the molar ratio of m : n = 0.81 : 0.19 or poly(HPMA-co-*S*-PAM) with the molar ratio of m : n = 0.79 : 0.21 exhibited the lower critical solution temperature (LCST) at 16°C. The LCST in the presence of (*S*)-(−)-phenylalanine (*S*-Phe) shifted from 16 to 20°C and 18°C for poly(*R*-HPMA-co-*S*-PAM) and poly(HPMA-co-*S*-PAM), respectively, whereas the LCST did not shift in the presence of (*R*)-(+)-phenylalanine (*R*-Phe). Thermosensitive Gel(*R*-HPMA-co-*S*-PAM) and Gel(HPMA-co-*S*-PAM) were also prepared from radical copolymerization of *S*-PAM and *R*-HPMA or HPMA in the presence of *N,N′*-ethylenebisacrylamide (EBAAm) as a crosslinker. When the gels shrunk at 40°C, the release of dansyl-(*R*)-phenylalanine (Dans-*R*-Phe) from the gel in which loaded Dans-*R*-Phe occurred was more easily done than that of Dans-*S*-Phe from the gel that loaded Dans-*S*-Phe. Thus, these thermosensitive copolymers and gels were found to exhibit chiral recognition for phenylalanine derivatives. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 228–236, 2001

Key words: thermosensitive polymer hydrogel; chiral polymer; chiral recognition; selective releasing

INTRODUCTION

There has been a growing interest in thermosensitive polymer materials. It is known that aqueous polymer solutions such as poly(*N*-isopropylacrylamide),[1] poly[*N*-(3-ethoxypropyl)acrylamide],[1,2] and poly(*N*-vinylisobutylamide)[3] exhibit phase separation at elevated temperature, which is called the lower critical solution temperature (LCST). These thermosensitive polymer materials have been widely applied to a variety of areas such as drug delivery systems,[4,5] liquid chromatography for the separation of steroids,[6,7] peptides,[6,8] protein,[6,8] dextran,[9] and chiral recognition.[10] On the other hand, Kopeček, and coworkers have concentrated on poly[*N*-(2-hydroxypropyl)methacrylamide], [poly-(HPMA)] for possible application in the biomedical field, for instance, the copolymer of HPMA being used for fusion solution,[11] and drug delivery systems (DDSs).[12] Recently, we found the phase separation of aqueous solution of the copolymer of HPMA and alkyl methacrylate (RMA, alkyl :

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methyl, butyl) with the molar ratio of RMA more than 0.08 in response to a change in temperature, although no phase transition occurred for poly(HPMA).[13] We also found the adsorption of protein on the poly(methyl methacrylate) microspheres modified with the HPMA moiety being changed in response to a change in temperature.[14]

In the course of our study on the functionalized polymers including the HPMA moiety,[13,14] our interest was directed to the thermally controlled chiral recognizable amphiphilic copolymers as well as hydrogels consisting of the poly(HPMA) segments. In the present article we describe the synthesis and characterization of copolymers and hydrogels prepared from the copolymerization of *N*-methacryloyl-(*S*)-phenylalanine methyl ester (*S*-PAM) and *N*-[(*R*)-(2-hydroxypropyl)methacrylamide (*R*-HPMA) or HPMA as a novel chiral recognizable material used for the chromatographic system.

EXPERIMENTAL

Materials

N-Methacryloyl-(*S*)-phenylalanine methyl ester (*S*-PAM) was prepared according to the method of Sanda.[15] m.p. 53–55°C (ref[15] m.p. 53–68°C), $[\alpha]_D^{25}$ −83.5° (*c* 1.0, $CHCl_3$) (ref.[15] $[\alpha]_D^{25}$ −83.4°). *N*-(2-Hydroxypropyl)methacrylamide (HPMA) was prepared by the method of Strohalm and Kopeček.[16] m.p. 67–68°C, (ref.[16] m.p. 67–68°C). Found: C, 58.37%; H, 9.03%; N, 9.58%. Calcd for $C_7H_{13}NO_2$ = 143.186: C, 58.72%; H, 9.15%; N, 9.78%. *N*-[(*R*)-(2-Hydroxypropyl)]methacrylamide (*R*-HPMA) was prepared by the reaction of methacryloyl chloride with (*R*)-1-amino-2-propanol in a similar manner as HPMA. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.24 (d, 6.3 Hz, 3H, —C$\underline{H}_3$), 1.97 (s, 3H, ═C—C$\underline{H}_3$), 3.15–3.22 (m, 1H, —C$\underline{H}_2$—), 3.48–3.51 (m, 1H, —C$\underline{H}_2$—), 3.94–3.98 (m, 1H, >C$\underline{H}$—), 5.35, 5.73 (d, 1.5 Hz, 2H, ═C$\underline{H}_2$), 6.30 (br, 1H, —N$\underline{H}$—).

^{13}C-NMR ($CDCl_3$) δ (ppm): 16.8 (—$\underline{C}H_3$), 19.0 (═C—$\underline{C}H_3$), 45.4 (>$\underline{C}$HOH), 65.0 (—$\underline{C}H_2$—), 118.4 ($\underline{C}H_2$═C<), 137.7 (CH_2═$\underline{C}$<), 167.8 (—$\underline{C}$ONH—), $[\alpha]_D^{25}$ −28.1° (*c* 1.0, C_2H_5OH). *N*-Pivaloyl-(*S*)-phenylalanine methyl ester (P-*s*-Phe) with $[\alpha]_D^{25}$ +72.1° (*c* 5.2, $CHCl_3$) was prepared by the reaction of pivaloyl chloride with (*S*)-phenylalanine methyl ester hydrochloride (*S*-Phe-OMe · HCl).

N-(*R*,*S*)-2-Methylbutyryl-(*S*)-phenylalanine methyl ester (MB-*s*-Phe) with $[\alpha]_D^{25}$ +75.5° (*c* 5.1, $CHCl_3$) was prepared by the reaction of *N*-(*R*,*S*)-2-methylbutyric acid with *S*-Phe-OMe · HCl in the presence of 2-chloro-1-methylpyridinium iodide and triethylamine in dichloromethane. *N*-(*S*)-2-Methylbutyryl-(*S*)-phenylalanine methyl ester (*s*-MB-*s*-Phe) with $[\alpha]_D^{25}$ +92.1° (*c* 5.1, $CHCl_3$) was prepared by the reaction of (*S*)-2-methylbutyroyl chloride and *S*-Phe-OMe · HCl.

N,*N*′-Ethylenebisacrylamide (EBAAm) was obtained from Fluka and used without further purification. 2,2′-Azobisisobutyronitrile (AIBN) was purified by recrystallization from methanol. *tert*-Amyl peroxy-2-ethylhexanoate (50% toluene solution: APEH) was obtained from Akuzo Chemicals and used without further purification. 5-Dimethylamino-1-naphthalene-sulfonyl-(*R*)-phenylalanine (Dans-*R*-Phe) and (*S*)-phenylalanine (Dans-*S*-Phe) were obtained from Sigma Chemical Co. and were used without further purification. Distilled and deionized water was used throughout the experiments.

Radical Polymerization

A solution of *R*-HPMA or HPMA (21.0 mmol), AIBN (0.03 mmol) and the prescribed amount of *S*-PAM (3.14–6.29 mmol) in ethanol (10 mL) in a glass tube was degassed by the freeze–thaw technique using a liquid nitrogen bath, and then sealed under reduced pressure. After being heated at 60°C for 8 h, the reaction mixture of the tube were poured into a large amount of ether to precipitate the polymer. The polymer was purified by reprecipitation from ethanol solution with ether. The obtained copolymers are abbreviated to poly(*R*-HPMA-co-*S*-PAM) and poly(HPMA-co-*S*-PAM), respectively, as shown in Figure 1. For seeing the effect of the molar ratio of HPMA to *S*-PAM on the specific rotation $[\alpha]_D^{25}$ of poly(HPMA-co-*S*-PAM), the continuous variation method was applied to the radical copolymerization of HPMA and *S*-PAM, by changing the molar ratio of HPMA to *S*-PAM from 0.20 : 0.80 to 0.90 : 0.10 in the feed. Homopolymers of *R*-HPMA and *S*-PAM, poly(*R*-HPMA) and poly(*S*-PAM), were also prepared by radical polymerization of *R*-HPMA and *S*-PAM in ethanol and chloroform in a similar manner mentioned above, respectively. The number-average molecular weight (M_n) of polymer was calculated from the gel permeation chromatography (GPC). GPC analysis was conducted in dimethylformamide solution with TSK gel (column: TOSOH HHR 6000, 5000, 4000, 3000, 2000), using a

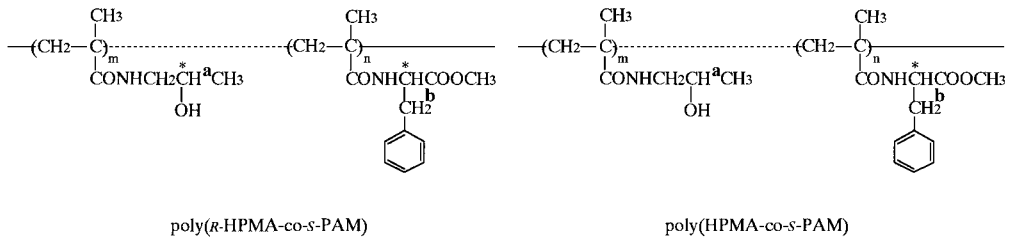

Figure 1 Structure of copolymers used.

TOSOH LC-8020 GPC apparatus. The molar ratio of HPMA and *S*-PAM in the copolymer was determined from the peak areas due to the methine proton (δ 3.67 ppm) of HPMA and methylene protons (δ 3.49 ppm) of *S*-PAM using a JEOL EX-400 NMR spectrometer.

Preparation of Gels

Ten milliliters of ethanol solution containing 5.0 g (35.0 mmol) of *R*-HPMA (or HPMA), 1.73 g (6.98 mmol) of *S*-PAM, 0.18 g (1.05 mmol) of EBAAm as a crosslinker and 181 μL (0.35 mmol) of APEH as an initiator was bubbled with dried nitrogen for 10 min and injected between two glass plates separated by a Teflon gasket (d = 2 mm), and then the glass plate were fastened with two clasps. The solution was heated to polymerize at 60°C for 24 h. The sheets of gels, Gel(*R*-HPMA-co-*S*-PAM) and Gel(HPMA-co-*S*-PAM) were separated from the glass plates and cut into disks (ϕ = 11 mm) using a cork borer, followed by immersing in ethanol for 5 days to remove unreacted materials. The swollen disks dried ambiently for 2 days and under reduced pressure for 1 day at room temperature.

Measurements of the Turbidity

Optical transmittance of the 0.5 wt % of aqueous copolymer solutions at various temperatures was monitored at 600 nm using a Shimadzu UV-160A spectrophotometer. The quartz cell was kept in a thermostat maintained at a definite temperature (10−40°C) with a circular water jacket equipped with a temperature controller. The LCST was defined as the temperature at the beginning of turbid.

Swelling Measurements

Dried gel disks were immersed into water at a fixed temperature (20 and 40°C) for 15 min, re-

Table I Preparation and Characterization of Various Copolymers[a]

Copolymers		*S*-PAM mmol	Yield %	Molar Ratio[c] in Copolymer m : n	$M_n \times 10^{-4}$[d]	M_w/M_n[d]	$[\alpha]_D^{25}$[e] Degree
Poly(HPMA-*co*-*S*-PAM)	−I	3.14	64.1	0.89 : 0.11	6.9	2.34	−5.8
	−II	4.19	64.0	0.86 : 0.14	4.5	2.20	−6.9
	−III	5.24	73.5	0.81 : 0.19	8.6	1.99	−7.6
	−IV	6.29	86.7	0.79 : 0.21	8.1	2.02	−8.2
Poly(*R*-HPMA-*co*-*S*-PAM)	−I	3.14	62.9	0.84 : 0.16	10.6	2.23	−44.3
	−II	4.19	64.6	0.81 : 0.19	9.1	2.54	−45.6
	−III	5.24	72.9	0.80 : 0.20	8.1	2.78	−41.8
Poly(*R*-HPMA)		0	70.0	1.00 : 0.00	4.9	2.40	−49.7
Poly(*S*-PAM)[b]		21.0	94.0	0.00 : 1.00	2.3	2.93	+42.2[f]

[a] Reaction Condition: HPMA or L-HPMA: 21.0 mmol, AIBN: 0.03 mmol, Ethanol: 10 mL, 60°C, 8 h.
[b] Reaction Condition: *S*-PAM: 21.0 mmol, AIBN: 0.03 mmol, $CHCl_3$: 10 mL, 60°C, 8 h.
[c] Calculated from ^{1}H-NMR spectoroscopy.
[d] Calculated from GPC by use of polystyrene standard.
[e] Measured by polarimeter at 25°C (c 1.00, ethanol).
[f] Measured by polarimeter at 25°C (c 1.00, $CHCl_3$).

moved from water, and weighed after being tapped with filter paper to remove an excess of water on the surface. The swelling ratio (S) is defined as

$$S = \frac{W_s - W_d}{W_d}$$

where W_s and W_d represent the weights of swelling gel and dried one, respectively.

Loading and Releasing of Amino Acid Derivatives

Dried gel disks were equilibrated for 3 days in 10 mL of 20% aqueous ethanol containing 20 mg of Dans-*S*-Phe (or Dans-*R*-Phe) at 20°C. These swollen disks (loading 100 nmol/g of dansyl derivatives per disks) were washed with ethanol and freeze dried under reduced pressure for 1 day at room temperature. Gel pellets loaded Dans-*S*-Phe (or Dans-*R*-Phe) were immersed into 10 mL of 2% aqueous ethanol at 20 and 40°C. The amount of released Dans-*S*-Phe (or Dans-*R*-Phe) was calculated from the absorbance at 335 nm by UV spectrophotometer using a Shimadzu UV-160A.

Other Measurements

Circular dichroism (CD) was measured by means of a JASCO J-720W spectropolarimeter in the range of 210–260 nm, equipped with a quartz cell with a 0.1-cm path length. Specific rotation $[\alpha]_D^{25}$ was measured with a Horiba Seisakusho SEPA-200 autopolarimeter.

RESULTS AND DISCUSSION

Thermoresponsible Amphiphilic Copolymers Containing the PAM Moiety

A series of copolymers as denoted by poly(*R*-HPMA-*co*-*S*-PAM)-I–III with $M_n = 8.1 \times 10^4$–10.6×10^4 were obtained by radical copolymerization of *R*-HPMA and *S*-PAM with AIBN as an initiator in ethanol, by changing the molar ratio of *R*-HPMA to *S*-PAM in feed. The molar ratio of *R*-HPMA (m) to *S*-PAM (n) in copolymer was found to be m : n = 0.80 : 0.20–0.84 : 0.16 determined by ^{1}H-NMR spectroscopy, measuring the areas of 3.67 and 3.49 ppm assigned to H^a in *R*-HPMA and H^b in *S*-PAM (see H^a and H^b marked in Fig. 1, respectively).

The copolymers as written by poly(HPMA-*co*-*S*-PAM)-I–IV with $M_n = 6.9 \times 10^4$–8.6×10^4 and m : n = 0.79 : 0.21–0.89 : 0.11 were also prepared in a similar manner as mentioned above. Results of polymerization are summarized in Table I, together with data for homopolymers of *R*-HPMA and *S*-PAM, poly(*R*-HPMA) and poly(*S*-PAM). To obtain basic information for chiral recognition, the specific rotation $[\alpha]_D^{25}$ of the copolymers was first measured.

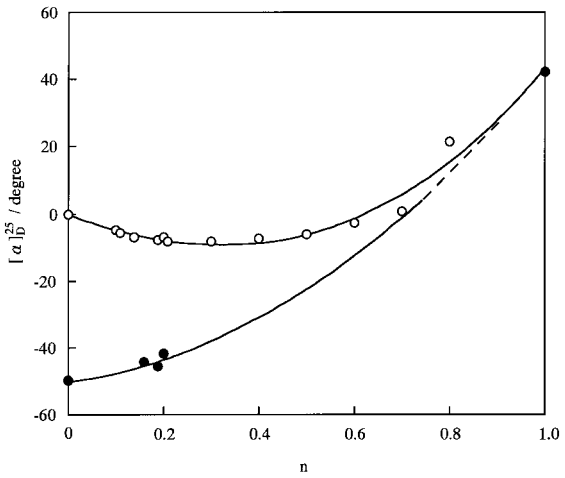

Figure 2 Effect of the molar ratio of *R*-PAM (n) on $[\alpha]_D^{25}$ of copolymers. ●; poly(*R*-HPMA-*co*-*S*-PAM), ○; poly(HPMA-*co*-*S*-PAM).

$[\alpha]_D^{25}$ showed from −44.3° to −41.8° for poly(*R*-HPMA-*co*-*S*-PAM)-I–III but $[\alpha]_D^{25}$ was varied from −5.8° to −8.2° for poly(HPMA-*co*-*S*-PAM)-I–VI with increasing the molar ratio of *S*-PAM in both copolymers. For the purpose of seeing the effect of the *S*-PAM on the $[\alpha]_D^{25}$ of the copolymer, the poly(HPMA-*co*-*S*-PAM)s with different molar ratios of HPMA to *S*-PAM from 0.9 : 0.1 to 0.2 : 0.8 were separately prepared, applying the continuous variation method for each monomers in 90% or above yield. Poly(HPMA-*co*-*S*-PAM)s showed $[\alpha]_D^{25}$ −4.6° to +21.5°. $[\alpha]_D^{25}$ was changed with changing the *S*-PAM content as shown in Figure 2, together with $[\alpha]_D^{25}$ for poly(HPMA-*co*-*S*-PAM)-I–IV in Table I. $[\alpha]_D^{25}$ of poly(HPMA-*co*-*S*-PAM) exhibits a negative within the molar ratio of *S*-PAM (n) from $n = 0.1$ to $n = 0.6$, although the HPMA segment has achiral property and poly(*S*-PAM) with $M_n = 2.3 \times 10^4$ has $[\alpha]_D^{25}$ +42.2° in $CHCl_3$. $[\alpha]_D^{25}$ of poly(*R*-HPMA-*co*-*S*-PAM) was varied from −49.7° to +42.2°, depending on the molar ratio of *S*-PAM from 0 to 1, as also shown in Figure 2, where poly(*R*-HPMA) with $M_n = 4.9 \times 10^4$, *R*-HPMA, and *S*-PAM were found to be $[\alpha]_D^{25}$ −49.7° (*c* 1.0 C_2H_5OH), $[\alpha]_D^{25}$ −28.1° (*c* 1.0 C_2H_5OH), and +83.5° (*c* 1.0 $CHCl_3$), respectively.

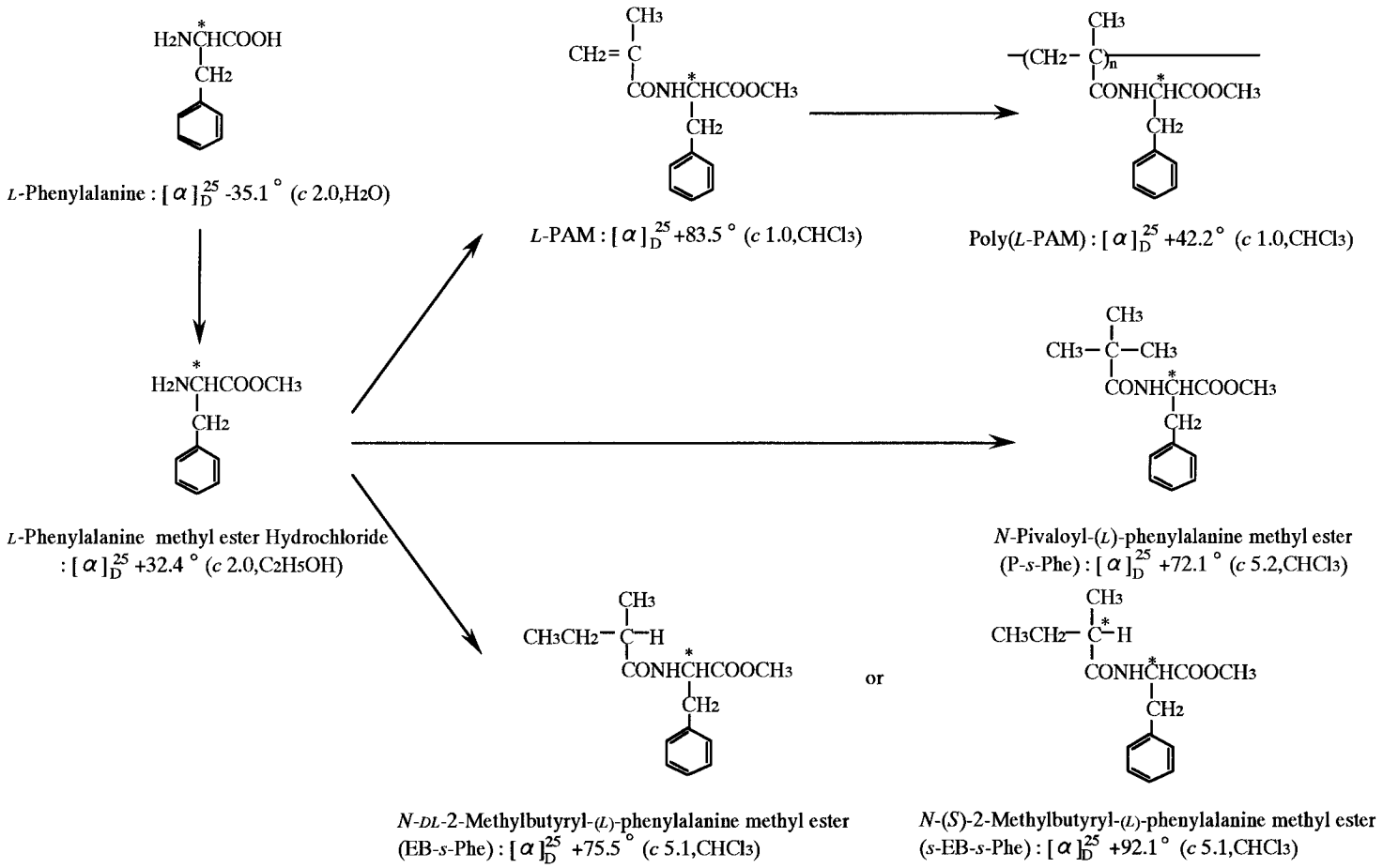

Figure 3 Preparation of model compounds.

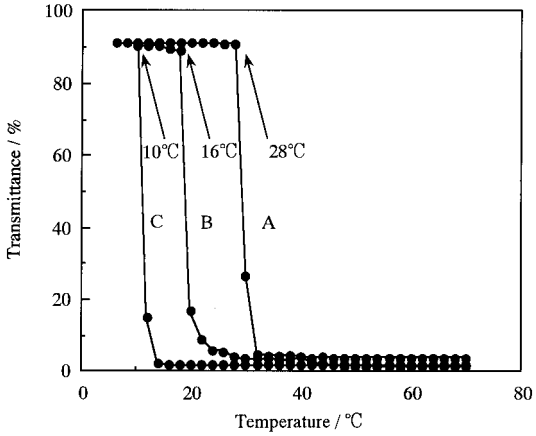

Figure 4 Transmittance of 0.5 wt % aqueous poly(*R*-HPMA-*co*-*S*-PAM)-I–III solution. The molar ratio of *S*-PAM (n) in copolymers: (A) $n = 0.16$, (B) $n = 0.19$, (C) $n = 0.20$.

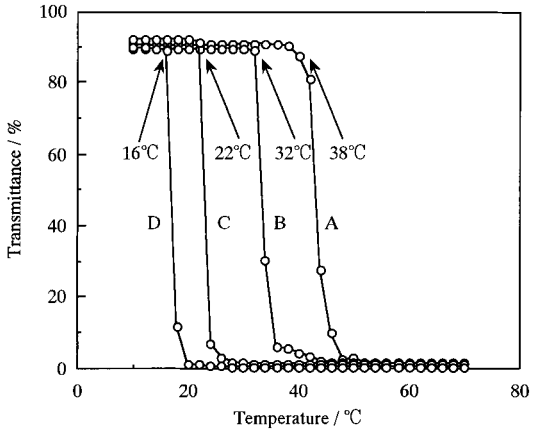

Figure 5 Transmittance of 0.5 wt % aqueous poly(H-PMA-*co*-*S*-PAM)-I–IV solution. The molar ratio of *S*-PAM (n) in copolymers: (A) $n = 0.11$, (B) $n = 0.14$, (C) $n = 0.19$, (D) $n = 0.21$.

To clarify this phenomena, P-*S*-Phe, MB-*S*-Phe, and *S*-MB-*S*-Phe were prepared as model compounds for poly(HPMA-*co*-*S*-PAM) (Fig. 3). Both P-*S*-Phe and MB-*S*-Phe are a model for poly(H-PMA-*co*-*S*-PAM) with a chiral center to adjacent phenethyl group in the side chain. *S*-MB-*S*-Phe are also a model of poly(HPMA-*co*-*S*-PAM) introduced two chiral centers in the side chain as well as in the main chain.

$[\alpha]_D^{25}$ of P-*S*-Phe, MB-*S*-Phe, and *S*-MB-*S*-Phe were +72.1° (c 5.2, $CHCl_3$), +75.5° (c 5.1, $CHCl_3$), and +92.1° (c 5.1, $CHCl_3$), respectively. No significant difference in terms of $[\alpha]_D^{25}$ between P-*S*-Phe or MB-*S*-Phe and *S*-MB-*S*-Phe was observed. It can be explained not by the asymmetric induction in the main chain, but the attribution to asymmetric perturbation caused by the *S*-PAM moiety.[17]

LCST of Amphiphilic Copolymers

The transmittance of aqueous solution of poly(*R*-HPMA-*co*-*S*-PAM)-I–III and poly(HPMA-*co*-*S*-PAM)-I–IV ($c = 0.5$ wt %) was measured for each 1°C rise in temperature from 10 to 70°C, as shown in Figures 4 and 5. The aqueous solution of poly-(HPMA-*co*-*S*-PAM)-IV with m : n = 0.79 : 0.21 shows, for instance, transparent at below 16°C and became sharply opaque in 20°C when the temperature was raised to 20°C. The effect of the molar ratio of the *S*-PAM moiety to the LCST was shown in Figure 6. An increase of the molar ratio of *S*-PAM results in a lowering of the LCST as that of the copolymers of HPMA and alkyl methacrylates.[13] The hydrophobicity of the copolymer chains affected the dehydration behavior, changing the phase transition temperatures.[1–3] It is interesting to say that poly(*R*-HPMA-*co*-*S*-PAM) exhibits a lower LCST than poly(HPMA-*co*-*S*-PAM), that is, the *R*-HPMA moiety influences effectively lower the LCST. This is explained by attribution to a peculiar conformation of the *S*-PAM segment considering the CD spectrum of poly(*R*-HPMA) as shown in Figure 7 in addition to the attribution to the asymmetric perturbation described above.

Addition Effect of Amino Acid on LCST

The effect of the addition of *S*-Phe and *R*-Phe on the LCST of aqueous solution of poly(*R*-HPMA-

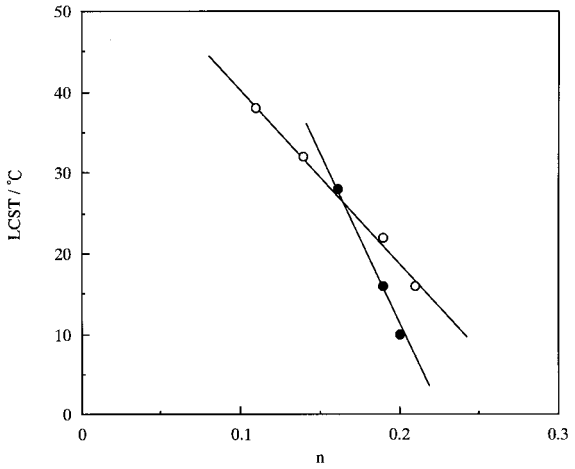

Figure 6 Effect of the molar ratio of *S*-PAM (n) on the LCST. ●; poly(*R*-HPMA-*co*-*S*-PAM); ○; poly(H-PMA-*co*-*S*-PAM).

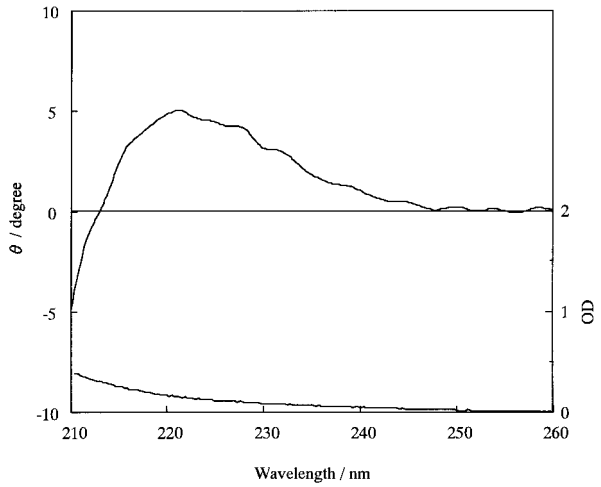

Figure 7 CD spectrum of poly(*R*-HPMA) in ethanol solution. [poly(*R*-HPMA)] = 0.03 wt %.

co-*S*-PAM)-II with $n = 0.19$, $[\alpha]_D^{25}$ −45.6° and poly(HPMA-*co*-*S*-PAM)-IV with $n = 0.21$, $[\alpha]_D^{25}$ −8.2° was shown in Figures 8 and 9, respectively. It was found that the LCSTs shifted from 16 to 20°C and from 16 to 18°C for poly(*R*-HPMA-*co*-*S*-PAM)-II and poly(HPMA-*co*-*S*-PAM)-IV, respectively, in the presence of *S*-Phe, whereas no shift in LCST occurred in the presence of *R*-Phe. The LCST of the copolymers were apparently influenced in response to *R*-Phe and *S*-Phe. It is considered that the *S*-Phe moiety in the copolymer interacts stereospecifically not with the *R*-Phe molecule but with the *S*-Phe molecule, as described below.

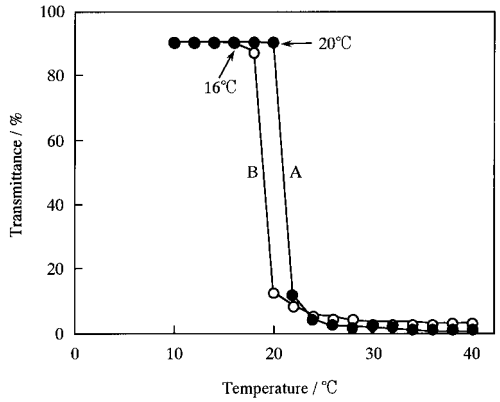

Figure 8 Transmittance of 0.5 wt % of aqueous solution of poly(*R*-HPMA-*co*-*S*-PAM)-II with $n = 0.21$ and $[\alpha]_D^{25}$ −45.6° in the presence of (*S*)-phenylalanine (*S*-Phe, (A) and in the presence of (*R*)-Phenylalanine (*R*-Phe, B).

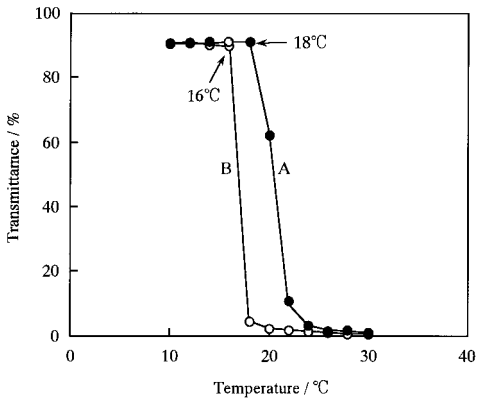

Figure 9 Transmittance of 0.5 wt % of aqueous solution of poly(HPMA-*co*-*S*-PAM)-IV with $n = 0.21$ and $[\alpha]_D^{25}$ −8.2° in the presence of (*S*)-phenylalanine (*S*-Phe, A) and in the presence of (*R*)-phenylalanine (*R*-Phe, B).

Thermoresponsible Hydrogels Containing the PAM Moiety

Figure 10 depicts the behavior of Gel(HPMA-*co*-*S*-PAM) and Gel(*R*-HPMA-*co*-*S*-PAM) undergoing pulses in temperature. Pulsatile swelling change of the gel occurred across the LCST and Gel(*R*-HPMA-*co*-*S*-PAM) showed a larger swelling ratio than Gel(HPMA-*co*-*S*-PAM) due to a peculiar conformation of the *R*-HPMA segment considered from Figure 7. As a result of the elevating temperature, the network began to collapse due to the hydrophobic interaction of the polymer chains and the gels were returned to their original environment when the temperature decreased. To apply the gels to a temperature-responsive chromatographic system, Gel(*R*-HPMA-*co*-*S*-PAM)

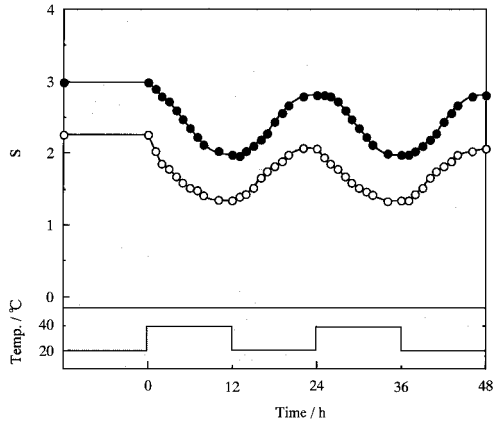

Figure 10 Pulsatile swelling change of gel in response to stepwise temperature change at 20 and 40°C. ●; Gel(*R*-HPMA-*co*-*S*-PAM), ○; Gel(HPMA-*co*-*S*-PAM).

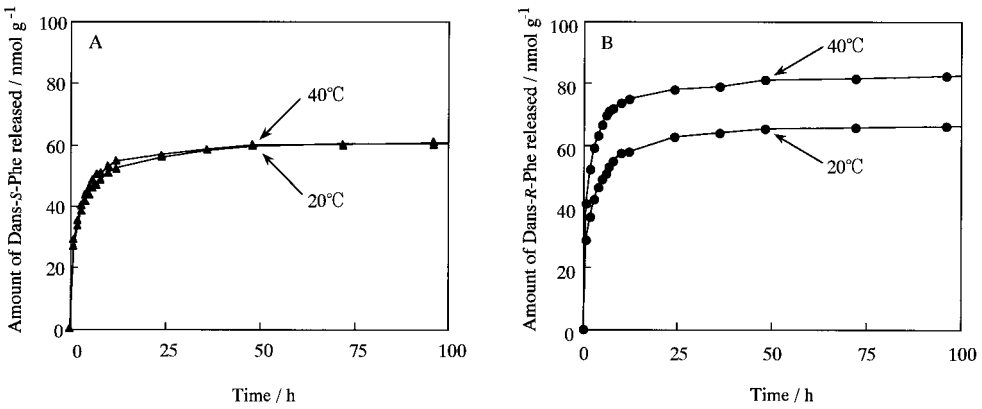

Figure 11 The amount of Dans-*R*-Phe or Dans-*S*-Phe released from Gel(*R*-HPMA-*co*-*S*-PAM) in the mixed solvent of water and ethanol (98 : 2 vol/vol %). (A) Release of Dans-*S*-Phe; (B) release of Dans-*R*-Phe.

and Gel(HPMA-*co*-*S*-PAM), which loaded Dans-*S*-Phe or Dans-*R*-Phe were prepared, where dansyl derivatives were used for facility in UV measurements. The time course of the amounts of Dans-*S*-Phe and Dans-*R*-Phe released from Gel(*R*-HPMA-*co*-*S*-PAM) in 2% aqueous ethanol was shown in Figure 11. At 20°C, Dans-*S*-Phe and Dans-*R*-Phe were released from the swollen gel by a diffusion mechanism. When a shrinkage of the hydrogel occurred at 40°C, the amount of Dans-*R*-Phe released from the hydrogel was increased as time passes, whereas no excess release of Dans-*S*-Phe occurred from the gel. A similar releasing behavior in response to Dans-*S*-Phe or Dans-*R*-Phe was observed in the case of Gel(HPMA-*co*-*S*-PAM), as shown in Figure 12. The amount of the released Dans-*R*-Phe from Gel(*R*-HPMA-*co*-*S*-PAM) was much larger than that from Gel(HPMA-*co*-*S*-PAM) by attribution to the large swelling ratio of the hydrogel.

Stereospecific Interaction the *s*-Phe Moiety

The effects of *S*-Phe on LCST of aqueous copolymer solutions and the releasing behavior of Dans-*R*-Phe from the hydrogel should be explained by considering the specific interaction of the hydrophobic and optically active *S*-Phe moiety with *S*-form isomer of phenylalanine derivatives. To clarify the mechanism for the chiral recognition, the interaction between *S*-PheOMe and Dans-*S*-Phe or Dans-*R*-Phe was spectrophotometrically studied: an optical density (OD) of λ_{max} 251.5 nm of *S*-PheOMe was measured by applying the continuous variation method to the mixture of *S*-

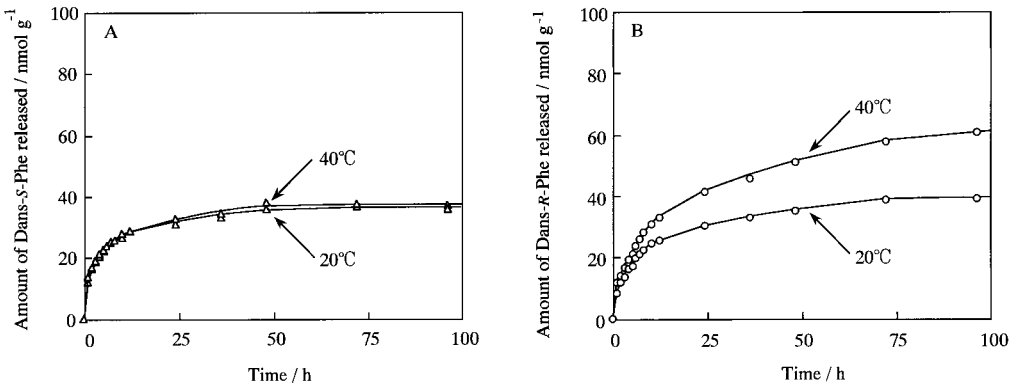

Figure 12 The amount of Dans-*R*-Phe or Dans-*S*-Phe released from Gel(HPMA-*co*-*S*-PAM) in the mixed solvent of water and ethanol (98 : 2 vol/vol %). (A) Release of Dans-*S*-Phe; (B) release of Dans-*R*-Phe.

PheOMe and dansyl derivatives in water, as shown in Figure 13. The OD increased linearly with increasing the molar ratio of Dans-*R*-Phe in the mixture of *S*-Phe-OMe and Dans-*R*-Phe, whereas in the case of the mixture of *S*-PheOMe and Dans-*S*-Phe the OD exhibited the strongest hyperchromic effect at the molar ratio of 0.5 : 0.5 attributed to π–π interaction of phenyl rings. Taking into account this hyperchromic effect, the interaction of the *S*-Phe moiety in copolymers and phenylalanine molecule is suggested, as shown in Figure 14. In the case of the *S*-Phe-OMe moiety in the copolymer and *S*-Phe derivatives added, there are two hydrogen bondings between the *S*-Phe moiety in the copolymer and *S*-Phe derivatives in addition to the π–π interaction of phenyl rings. Consequently, as shown in Figures 8 and 9, the LCST of aqueous copolymer solution shifted to higher temperature due to the relatively strong interaction between the *S*-Phe moiety as the pendant group of the copolymer and free *S*-Phe. On the other hand, although the amount of released Dans-*S*-Phe was depressed due to the similar interaction between the *S*-Phe moiety in the gels and Dans-*S*-Phe, Dans-*R*-Phe was easily released when the gel shrank because a little interaction was expected with the *S*-Phe moiety in the gels and Dans-*R*-Phe.

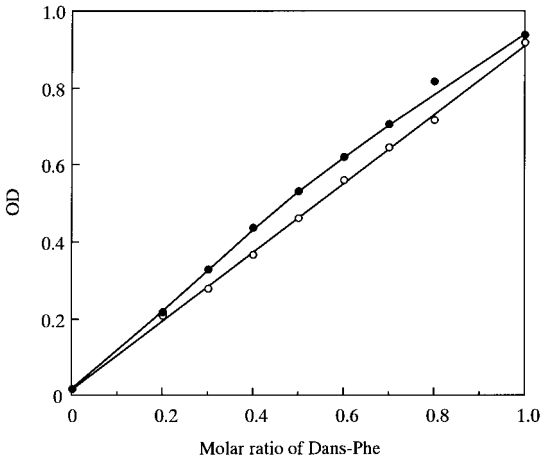

Figure 13 Hyperchromic effect for the mixture of *S*-PheOMe and Dans-*S*-Phe in ethanol. ●; mixture of *S*-PheOMe and Dans-*S*-Phe, ○; mixture of *S*-PheOMe and Dans-*R*-Phe.

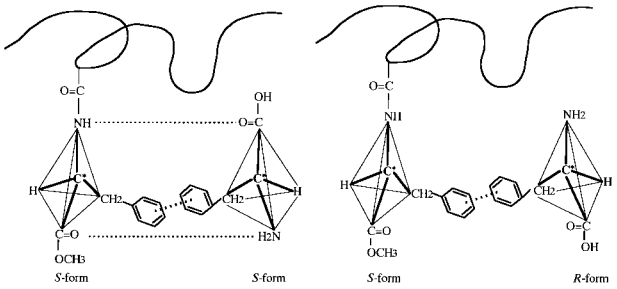

Figure 14 Schematic representation for chiral recognition to the enantiomers.

REFERENCES

1. Heshins, M.; Guillet, J. E. J Macromol Sci Chem 1968, A2, 1441.
2. Terada, T.; Inaba, T.; Kitano, H.; Maeda, Y.; Tsukida, N. Macromol Chem Phy 1994, 195, 3261.
3. Akashi, M.; Nakano, S.; Kishida, A. J Polym Sci Part A Polym Chem 1996, 34, 301.
4. Chung, J. E.; Yokoyama, M.; Aoyagi, T.; Sakurai, Y.; Okano, T. J Control Rel 1998, 53, 119.
5. Okuyama, Y.; Yoshida, R.; Sakurai, K.; Okano, T.; Sakurai, Y. J Biomater Sci Polym Ed 1993, 4, 545.
6. Kanazawa, H.; Kashiwase, Y.; Yamamoto, K.; Matsushima, Y.; Kikuchi, A.; Sakurai, Y.; Okano, T. Anal Chem 1997, 69, 823.
7. Kanazawa, H.; Yamamoto, K.; Matsushima, Y.; Takai, N.; Kikuchi, A.; Sakurai, Y.; Okano, T. Anal Chem 1996, 68, 100.
8. Kanazawa, H.; Yamamoto, K.; Kashiwase, Y.; Matsushima, Y.; Takai, N.; Kikuchi, A.; Sakurai, Y.; Okano, T. J Pharm Biomed Anal 1997, 15, 1545.
9. Gewehr, M.; Nakamura, K.; Ise, N.; Kitano, H. Makromol Chem 1992, 193, 249.
10. Aoki, T.; Nishimura, T.; Sanui, K.; Ogata, N. React Funct Polym 1998, 37, 299.
11. Sprincl, L.; Exmer, J.; Sterba, O.; Kopeček, J. J Biomed Mater Res 1976, 10, 953.
12. Kopeček, J.; Kopečeková, P.; Konák, C. JMS Pure Appl Chem 1997, A34, 2103.
13. Sugiyama, K.; Mitsuno, S.; Yasufuku, Y.; Shiraishi, K. Chem Lett 1997, 219.
14. Sugiyama, K.; Mituno, S.; Shiraishi, K. J Polym Sci Part A Polym Chem 1997, 35, 3349.
15. Sanda, F.; Abe, T.; Endo, T. J Polym Sci Part A Polym Chem 1997, 35, 2619.
16. Stohalm, J.; Kopeček, J. Angew Makromol Chem 1978, 70, 109.
17. Oishi, T.; Nagata, H.; Tsutsumi, H. Polymer 1998, 39, 4135.