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Stereoselective Partitioning of Organic Substrates by Thermoresponsive Polymers in Aqueous Phases

Toru Uemukai, Masashi Torisaki, Takahiro Hamanaka, Manabu Ishifune
Department of Applied Chemistry, Graduate School of Science and Engineering, Kinki University, Osaka 577-8502, Japan
Correspondence to: M. Ishifune (E-mail: ishifune@apch.kindai.ac.jp)

ABSTRACT: Partitioning of organic substrates by thermoresponsive polymer having *N*-acryloylaminoalcohol moieties in aqueous phase has been studied. Thermoresponsive polymers, such as poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(NIPAAm-*co*-*N*-acryloyl-(±)-alaninol) (poly(NIPAAm-*co*-HIPAAm)), were found to concentrate several organic substrates into the hydrophobic field generated during their phase transition. The amount of the substrates recoverd from the polymer phase mainly depended on the hydrophobicity of the substrates. Aqueous solutions of PNIPAAm (lower critical solution temperature, LCST = 33°C) and poly (NIPAAm-*co*-HIPAAm) (LSCT = 41°C) containing 1-phenylethanol showed LCSTs at 22°C and 33°C, respectively. The changes of LCSTs indicate that specific interactions such as hydrogen bonding between the side chain functionalities of the polymers and the substrates influence the phase transition behavior. Moreover, new optically active polymers having chiral aminoalcohol moieties have been synthesized by copolymerizations of NIPAAm with *N*-acryloylaminoalcohols such as *N*-acryloyl-(*S*)-alaninol and *N*-acryloyl-(*S*)-prolinol. The (*R*)/(*S*) ratio of 1-phenylethanol recovered from poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol) and poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol) were determined to be 75/25 and 68/32, respectively. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 3458–3464, 2013

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INTRODUCTION

Thermoresponsive polymer materials have attracted growing interest in many biomedical fields, and widely applied to drug delivery systems,[1–5] chromatography for the separation of natural products,[6–8] ion recognition materials,[9–12] and as chiral recognition materials.[13–15] Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most representative thermoresponsive polymers, and exhibits a distinct phase transition at a lower critical solution temperature (LCST) near 32°C.[16–18] PNIPAAm is soluble in water below its LCST but becomes hydrophobic above the LCST due to a conformational changes from a random coil to a globule,[19] and then aggregates to precipitate. Thermoresponsive-type coacervation has been also reported, for instance, in a solution of the copolymers of NIPAAm with *N*-(2-hydroxyisopropyl)acrylamide (HIPAAm).[20] Phase transition behavior can be affected by introducing hydrophilic or hydrophobic comonomer units into the thermoresponsive polymers. That is, the introduction of hydrophilic units into a thermoresponsive polymer increases its LCST, while hydrophobic units decrease the LCST.[15,20] Moreover, the introduction of various functional groups onto thermoresponsive polymer chains gives additional unique properties to the corresponding thermoresponsive polymers. For instance, thermoresponsive chiral recognition materials can be made by copolymerization of chiral recognition monomers with the monomers that construct thermoresponsive polymer chains. Sugiyama et al. have reported thermoresponsive chiral recognition polymers prepared by copolymerization of *N*-methacryloyl-(*S*)-phenylalanine methyl ester with *N*-(2-hydroxypropyl)methacrylamide.[14] Aoki et al. copolymerized *N*-isopropylacrylamide (NIPAAm) with *N*-(*S*)-*sec*-butylacrylamide, and found that the LCST of the resulting copolymer in an aqueous solution of L-tryptophan was higher than that in an aqueous solution of D-tryptophan.[15] The observed shifts in LCSTs are probably due to the specific interaction of the chiral thermoresponsive polymers with the added chiral substrates during the phase transition of the polymers. These interaction are possibly usable for selective concentration of organic substrates and furthermore for optical resolution of racemic mixture. However, few studies have been reported on the isolation and analysis of the substrates obtained from the polymer phase by using the phase transition behavior of the thermoresponsive polymers.

Additional Supporting Information may be found in the online version of this article.

Recently, we have reported that acrylamide (AAm) polymerized in a solution of PNIPAAm in water around its LCST without any initiators.[21] Moreover, we have also synthesized new thermoresponsive PNIPAAm-based polymer having pyrrolidine groups as side chains and found that the pyrrolidine side chains of the polymer promoted the aldol reaction between cyclohexanone and *p*-nitrobenzaldehyde in water at their LCST.[22] Thus, the thermoresponsive polymer chains in water aggregate to form a rather hydrophobic reaction field and the substrates are probably concentrated and promoted the polymerization and the aldol reaction in the resulting hydrophobic field. In this article, we have investigated the partitioning of various organic substrates by PNIPAAm-based thermoresponsive polymer phase in aqueous phase. Moreover, we have synthesized new optically active thermoresponsive polymers having chiral aminoalcohol moieties and also demonstrated stereoselective partitioning of 1-phenylethanol by the resulting optically active thermoresponsive polymers in aqueous phases.

EXPERIMENTAL

General

IR spectra were obtained on a JASCO FT/IR-470 Plus spectrometer. ^{1}H-NMR spectra were measured on a Varian OXFORD NMR300 (300 MHz) or JEOL JNM-AL 400 (400 MHz) spectrometer, and the chemical shift values (δ) were expressed in ppm downfield from the internal TMS standard. The molecular weights of the polymers were determined by using a gel permeation chromatography (GPC). The GPC analyses were carried out on a Hitachi L-6000 high performance liquid chromatograph, L-3350 RI detector, and Shodex KD-804 or KF-804L column. Dimethylformamide or tetrahydrofuran was used as the eluent for KD-804 or KF-804L column, respectively, at 1.0 mL/min of flow rate, and the molecular weights were relative to the poly(methyl methacrylate) standards (Shodex STANDARD M-75) or the polystyrene standards (Shodex STANDARD SM-105). Optical rotation measurement was performed at 589 nm on a JASCO DIP-1000 digital polarimeter. LCST was determined by measuring the turbidity of the polymer aqueous solutions at various temperatures. The transmittance of the polymer aqueous solutions was monitored at 500 nm between 20°C and 50°C by a JASCO V-630 spectrophotometer equipped with a thermostatted cell folder (JASCO ETC717). LCST was defined as the temperature at 50% transmittance.

Materials

L-Proline and 1-phenylethanol were purchased from Sigma-Aldrich, Inc. Acryloyl chloride was supplied from Tokyo Chemical Industries, Co., Ltd. (Tokyo, Japan). Other reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). NIPAAm was purified by recrystallization from benzene-hexane (95 : 5). 2,2′-Azobisisobutyronitrile (AIBN) as a radical initiator was recrystallized from methanol below 40°C. Tetrahydrofuran (THF) was distilled from Na-benzophenoneketyl under an argon atmosphere. *N*,*N*-Dimethylformamide (DMF) (77°C/50 mmHg) and acryloyl chloride (74°C) were distilled under an argon atmosphere before use. Pure water was prepared by purification of distilled water using the Simplicity Personal Ultrapure Water System SIMS 700 0J (Merck Ltd., Tokyo, Japan). Other reagents and solvents were used as received. DL-Alaninol, L-alaninol, D-alaninol, L-leucinol, L-phenylalaninol, L-prolinol, and D-prolinol were prepared by reducing the corresponding commercially available aminoacids with lithium aluminum hydride, respectively.[23]

Preparation of *N*-Acryloyl-(*S*)-prolinol (Typical Procedure to Prepare *N*-Acryloylaminoalcohols)

Into a solution of L-prolinol (55 mmol) and triethylamine (83 mmol) in chloroform (100 mL) was added dropwise acryloyl chloride (83 mmol) for 30 min at −5°C under an argon atmosphere, and the reaction mixture was stirred for 2 h at this temperature. The solution was concentrated under reduced pressure, and into the resulting viscous oil was added 100 mL of ethyl acetate to precipitate triethylamine hydrochloride. After suction filtration of the salts the filtrate was concentrated and purified by column chromatography with ethyl acetate as an eluent to afford *N*-acryloyl-(*S*)-prolinol ((*S*)-**3d**) as a viscous liquid in 44% yield. IR (neat) 3391, 2879, 1642, 1585 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.60–1.68 (m, 1H, CH—CH*H*—CH_2), 1.83–2.06 (m, 3H, CH—CH*H*—CH_2), 3.52–3.69 (m, 4H, N—CH_2 and CH_2—OH), 4.24–4.34 (m, 1H, C*H*), 5.15 (br s, 1H, O*H*), 5.74 (dd, J = 9.0, 3.0 Hz, 1H, C*H*=CH_2), 6.36–6.45 (m, 2H, CH=CH_2); $[\alpha]_D^{20} = -26.0°$ (c = 1 in benzene).

HIPAAm (*rac*-**2a**), *N*-acryloyl-(*S*)-alaninol ((*S*)-**2a**), *N*-acryloyl-(*R*)-alaninol ((*R*)-**2a**),[20] *N*-acryloyl-(*S*)-leucinol ((*S*)-**2b**), *N*-acryloyl-(*S*)-phenylalaninol ((*S*)-**2c**), and *N*-acryloyl-(*R*)-prolinol ((*R*)-**2d**) were prepared in the same manner using the corresponding aminoalcohols as starting materials.

***N*-Acryloyl-(*S*)-leucinol**: IR (neat) 3280, 3075, 2956, 2870, 1659 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.87 (m, 6H, CH(CH_3)$_2$), 1.28–1.44 (m, 2H, CH_2), 1.54–1.63 (m, 1H, C*H*(CH_3)$_2$), 3.56 (m, 2H, CH_2), 4.02–4.10 (m, 1H, CH), 5.58 (m, 1H, CH_2=CH), 6.09 (m, 1H, C*H*=CH_2), 6.21 (m, 1H, CH_2=CH), 6.37 (bs, 1H, N*H*); $[\alpha]_D^{20} = -29.3°$ (c = 1 in ethanol).

***N*-Acryloyl-(*S*)-phenylalaninol**: IR (KBr) 3294, 3087, 2958, 2871, 1656, 1630, 733, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.74 (bs, 1H, O*H*), 2.92 (m, 2H, CH_2—C_6H_5), 3.60–3.74 (m, 2H, CH_2—OH), 4.21–4.27 (m, 1H, C*H*—NH_2), 5.64 (dd, 1H, CH_2=CH, J = 1.5, 10.2 Hz), 5.66 (bs, 1H, NH), 6.05 (dd, 1H, C*H*=CH_2, J = 10.2, 17.0 Hz), 6.26 (dd, 1H, CH_2=CH, J = 1.5, 17.0 Hz), 7.20–7.34 (m, 5H, C_6H_5); $[\alpha]_D^{20} = -12.9°$ (c = 9 in ethanol).

Synthesis of poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol) (Typical Procedure to Synthesize Poly(NIPAAm-*co*-*N*-acryloylaminoalcohol)s)

NIPAAm (53 mmol) and AIBN (1.0 mmol) were placed in a glass ampoule under an argon atmosphere, and was added dry DMF (50 mL) containing *N*-acryloyl-(*S*)-prolinol (35 mmol). The resulting solution was degassed repeatedly by freeze–evacuation–thaw cycles, and sealed. The polymerization was carried out under light exclusion condition at 60°C for 20 h. After cooling the ampoule in an ice-water bath, the polymer solution was poured into a large amount of diethyl ether. The resulting precipitate was filtered and dried to constant weight under

reduced pressure at room temperature. The crude polymer was purified by reprecipitation from methanol into diethyl ether.

Poly(*N*-acryloyl-(*S*)-prolinol): IR (KBr) 3400, 1626, 1546 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.35–2.25 (m, 7H), 3.20–3.97 (m, 5H), 3.97–4.30 (m, 1H).

Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol): IR (KBr) 3314, 1655, 1543 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.00–1.25 (m, $CH(CH_3)_2$), 1.42–2.24 (m, CH_2 in main chains, 3H of pyrrolidine ring in *N*-acryloylprolinol unit), 2.24–2.47 (m, C*H* in main chains), 2.50–2.80 (br, N*H*), 3.20–3.86 (m, N—CH_2 and CH_2OH), 3.88–4.09 (m, $CH(CH_3)_2$), 4.09–4.30 (m, N—C*H* in *N*-acryloylprolinol unit), 4.80–5.30 (br, O*H*).

HIPAAm (*rac*-**2a**), *N*-acryloyl-(*S*)-alaninol ((*S*)-**2a**), *N*-acryloyl-(*R*)-alaninol ((*R*)-**2a**), *N*-acryloyl-(*S*)-leucinol ((*S*)-**2b**), *N*-acryloyl-(*S*)-phenylalaninol ((*S*)-**2c**), and *N*-acryloyl-(*R*)-prolinol ((*R*)-**2d**) were polymerized with NIPAAm in the same manner by using DMF (**2a–b, 2d**) or THF (**2c**) as a polymerization solvent.

Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol): IR (KBr) 3439, 2972, 2877, 1650 cm^{-1}; ^{1}H-NMR (D_2O) δ 0.98−1.16 (m, $CH(CH_3)_2$, CH_3 in *N*-acryloyl-alaninol unit), 1.24–1.80 (m, CH_2 in main chains), 1.80–2.25 (m, C*H* in main chains), 3.33–3.71 (m, CH_2—OH), 3.75–4.03 (m, C*H*—NH).

Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-leucinol): IR (KBr) 3313, 2958, 1652, 1542 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.82–1.03 (m, $CH(CH_3)_2$ in *N*-acryloyl-(*S*)-leucinol unit), 1.03–1.26 (m, $CH(CH_3)_2$ in NIPAAm unit), 1.50–1.72 (m, CH_2 in main chains, $CH(CH_3)$—CH_2), 1.75–1.95 (m, C*H* in main chains, $CH(CH_3)_2$ in *N*-acryloyl-leucinol unit), 3.62–3.78 (m, CH_2—OH), 3.80–4.18 (m, C*H*—NH).

Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-phenylalaninol): IR (KBr) 3304, 3066, 2971, 2875, 1651, 747, 701 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.98–1.20 (m, $CH(CH_3)_2$), 1.25–2.20 (m, CH_2—C*H* in main chains), 2.60–3.05 (m, CH_2—C_6H_5), 3.40–3.72 (m, CH_2—OH), 3.85–4.09 (m, C*H*—NH), 7.10–7.35 (m, C_6H_5).

Partitioning of Organic Substrates by the Thermoresponsive Polymers in Aqueous Phases (Typical Procedure)

Into a solution of 0.25 g of poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol) (NIPAAm unit : *N*-acryloyl-(*S*)-alaninol = 70 : 30, M_n = 20,300, M_w/M_n = 1.19) in 10 mL of water was added 1.0 mmol of (±)-1-phenylethanol, and the mixture was vigorously stirred at 5°C to be homogeneous. The mixture was warmed to 60°C for 5–120 min and centrifuged at 12,000 rpm. The precipitated polymer was separated by decantation and washed with a small amount of hot water. The polymer was then dissolved in 2 mL of methanol and poured into 150 mL of diethyl ether. The reprecipitated polymer was filtered and ethereal solution was evaporated. The aqueous supernatant and washing were extracted with diethyl ether (20 mL × 3). The combined ethereal layers was dried over Na_2SO_4 and concentrated. The amounts of the recovered 1-phenylethanol from the polymer phase and water phase were weighed and the enantiomeric ratio was determined by a liquid chromatography using a Shodex ORpak CDB-453 HQ for chiral separation with 7 : 3 water–acetonitrile as an eluent.

RESULTS AND DISCUSSION

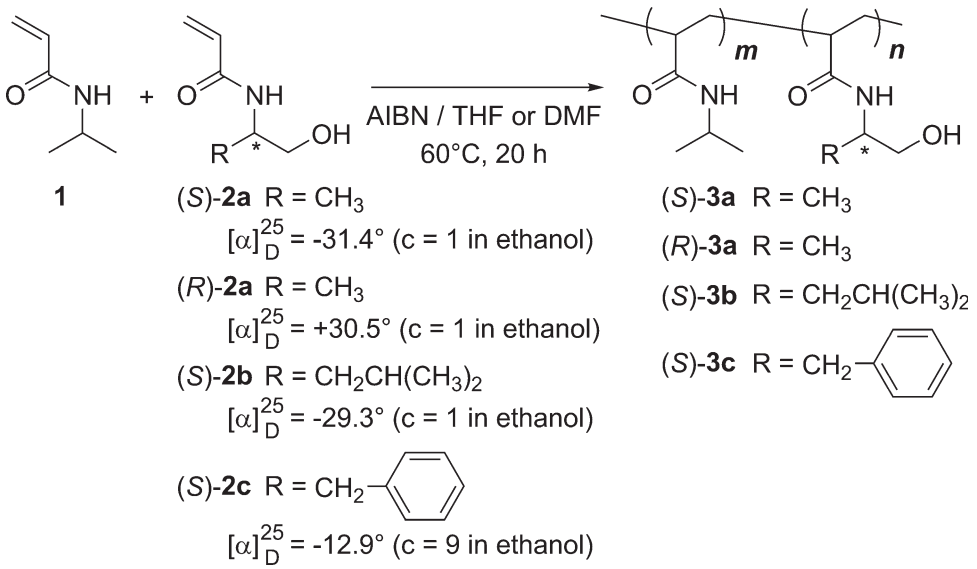
Partitioning of Organic Substrates by Thermoresponsive Polymer Phase in Aqueous Phase

Various organic substrates were dissolved or emulsified in aqueous solutions of PNIPAAm or poly(NIPAAm-*co*-HIPAAm) (NIPAAm units : HIPAAm units = 70 : 30) at 5°C. The resulting solutions or emulsions were then warmed at higher than the LCSTs of these thermoresponsive polymers, and the organic substrates were partitioned by the polymer phase in the aqueous phase (Table I). The amount of the substrates recovered from the polymer phase roughly depends on their hydrophobicity. For instance, 87% or 84% of citronellol, which was practically insoluble in water, was recovered from the PNIPAAm or poly

Table I. Partitioning of Organic Substrates by Thermoresponsive Polymers in Aqueous Phases at their LCSTs[a]

		PNIPAAm[b]			Poly(NIPAAm-*co*-HIPAAm)[c]		
		Recovered substrates[d] (%)		Observed	Recovered substrates[d] (%)		Observed
Entry	Substrates	From polymer	From water	LCST (°C)	From polymer	From water	LCST (°C)
1	Citronellol	87	9	12	84	9	23
2	Limonene	64	19	32	65	23	40
3	Methyl benzoate	85	14	27	63	20	37
4	Phenol	53	45	12	22	54	17
5	benzyl alcohol	24	44	26	13	87	36
6	1-phenylethanol	27	51	22	12	67	33
7	cycloheptanone	20	70	28	14	73	37
8	4-fluorobenzaldehyde	34	40	17	38	38	30
9	2,5-hexanedione	7	64	32	10	60	41

[a] Conditions: Solvent, distilled water; Total volume, 10 mL; Concentrations, [polymer] = 2.5 wt/vol %, [substrate] = 0.1 mol/L.
[b] M_n = 41,400, M_w/M_n = 2.58, LCST = 33°C.
[c] NIPAAm units : HIPAAm units = 70 : 30, M_n = 32,300, M_w/M_n = 3.55, LCST = 41°C.
[d] Isolated.



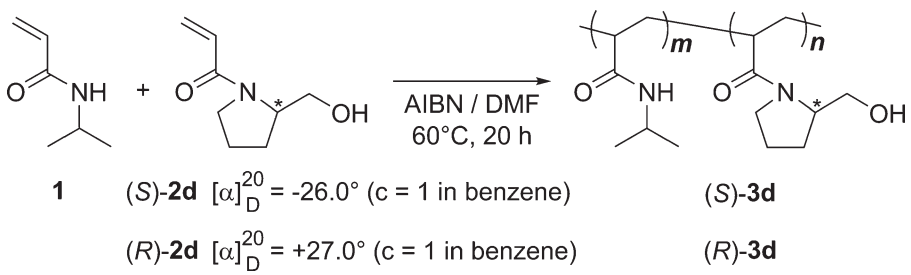
Scheme 1. Copolymerization of NIPAAm (**1**) with optically active *N*-acryloylaminoalcohols **2**.

(NIPAAm-*co*-HIPAAm) phase (Table I, Entry 1). More hydrophilic substrates such as benzyl alcohol and cycloheptanone were recovered mostly from aqueous phase (Entries 5–7). Observed LCSTs of the polymer solutions containing organic substrates were lower than those without the substrates. For instance, aqueous solutions of PNIPAAm (LCST = 33°C) and poly(NIPAAm-*co*-HIPAAm) (LSCT = 41°C) containing 1-phenylethanol (0.1 mol/L) showed LCSTs at 22°C and 33°C, respectively (Entry 6). Although the relation between temperature shifts of LCSTs and the structure of dissolved substrates has not been clarified yet, temperature shifts of LCSTs of aqueous solutions of alcoholic substrates were relatively large (Entries 1, 4–6). The changes of LCSTs indicates that specific interactions such as hydrogen bonding between the side chain functionalities of the polymers and the substrates influence the phase transition behavior of the polymer solutions. These interactions are probably affected by the stereochemistry of the polymers and substrates. In fact, poly(*N*-(*S*)-*sec*-butylacrylamide-*co*-NIPAAm) aqueous solution in the presence of L-tryptophan was reported to show higher LCST than that in the presence of D-tryptophan.[15] These results encouraged us to investigate the stereoselectivity in partitioning of racemic mixture of the organic substrates having chiral centers by the optically active thermoresponsive polymers in aqueous phases.

Synthesis of Optically Active Thermoresponsive Polymers

Optically active thermoresponsive polymers have been already reported such as poly(*N*-(*S*)-*sec*-butylacrylamide-*co*-NIPAAm),[14,24] poly(*N*-methacryloyl-(*S*)-phenylalanine methyl ester-*co*-*N*-(2-hydroxypropyl)methacrylamide),[14] poly(*N*-acryloyl-L-proline methyl ester),[25] and poly(*N*-acryloyl-4-*trans*-hydroxy-L-proline methyl ester),[26] and most of them are *N*-acryloylaminoacid methyl ester based polymers. We have also prepared in this study several optically active thermoresponsive polymers by copolymerization of NIPAAm with *N*-acryloylaminoalcohols (Schemes 1 and 2, Table II, see also Supporting Information). The resulting copolymers were confirmed to be optically active by measuring their optical rotations. The polarity of the optical rotations of the copolymers was same as that of their *N*-acryloylaminoalcohol repeat units except for poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-leucinol) (**3b**) (Entries 8–10), and the absolute values of the optical rotations increased with increasing *N*-acryloylaminoalcohol repeat unit contents.

Hydrogen bonding ability and hydrophobicity of the side chains are known to affect the phase transition behavior of thermoresponsive polyacrylamides.[14–18] Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol)s (**3a**) and their (*R*)-forms having higher than 50% NIPAAm units showed higher LCSTs than PNIPAAm (Entries 1–7). The copolymers having lower than 50% NIPAAm units



Scheme 2. Copolymerization of NIPAAm (**1**) with optically active *N*-acryloylprolinol **2d**.

Table II. Synthesis and Properties of Optically Active Thermoresponsive Polymers **3**

Entry	In feed[a] (%)			In copolymer[b] (%)		Yield (%)	M_n[c]	M_w/M_n[c]	Optical rotation[d]	LCST (°C)[e]
	1	2		*m*	*N*					
1	100	0		100	0	Quant.	7390	1.23	-	34
2	90	10	(*S*)-**3a**	89	11	55	15,300	1.64	−1.24°	36
3	70	30		70	30	59	11,700	2.33	−3.48°	44
4	50	50		52	48	58	13,900	2.05	−6.17°	53
5	90	10	(*R*)-**3a**	86	14	72	20,600	1.75	+0.43°	36
6	70	30		65	35	82	16,700	1.32	+3.56°	43
7	50	50		57	43	72	15,400	1.44	+6.79°	50
8	90	10	(*S*)-**3b**	89	11	44	2020	2.00	+0.60°	23
9	70	30		75	25	32	1620	2.24	+1.42°	17
10	50	50		58	52	57	1260	2.70	+2.28°	–[f]
11	90	10	(*S*)-**3c**	84	16	79	4790	2.10	−5.08°	19
12	70	30		68	32	87	6030	2.40	−10.13°	–[f]
13	60	40	(*S*)-**3d**	67	33	72	66,200	1.78	−26.8°	31
14	50	50		43	57	22	14,500	2.16	−35.3°	35
15	30	70		29	71	47	9840	2.25	−42.3°	40
16	0	100		0	100	44	8880	2.09	−56.6°	55
17	70	30	(*R*)-**3d**	65	35	55	12,800	2.10	+21.9°	34
18	30	70		29	71	68	9150	2.40	+45.9°	42
19	0	100		0	100	65	8720	2.26	+62.1°	55

[a] Polymerization conditions: Solvent, DMF; Total volume, 20–30 mL; Polymn. temp., 60°C; Polymn. time, 20 h; Initiator, AIBN (1 mol %).
[b] Determined by integrated intensity of ^{1}H-NMR.
[c] Estimated by GPC (based on poly(methylmethacrylate) standard).
[d] Measurement conditions: Solvent, distilled water (Entries 1–7, 13–19) or ethanol (Entries 8–12); c = 1; Temp., 20°C.
[e] Determined by transmittance measurements using UV-vis spectrometer equipped with a thermocontroller. Wavelength was fixed at 500 nm. Temperature was raised at 1.0°C/min.
[f] Insoluble in water.

were soluble in water between 5 and 70°C. Poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol)s (**3d**) and their (*R*)-forms also showed higher LCSTs than PNIPAAm (Entries 13–19), and interestingly homopolymers of *N*-acryloyl-(*S*)-prolinol and its (*R*)-form are thermoresponsive (LCST = 55°C, Entries 16, 19). On the other hand, LCSTs of less hydrophilic poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-leucinol) (**3b**) and poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-phenylalaninol) (**3c**) were observed at lower temperatures than that of PNIPAAm (Entries 8, 9, 11), and copolymers **3b** and **3c** having lower content (<70%) of NIPAAm repeat units were insoluble in water (Entries 10, 12).

Stereoselective Partitioning of 1-Phenylethanol by Optically Active Thermoresponsive Polymers in Aqueous Phases

We have performed preliminary study on enantioselective partitioning of organic substrates having asymmetric carbon centers by the prepared optically active thermoresponsive polymers in aqueous phases. Hydrophobic substrates such as citronellol and limonene are not expected to show enantioselective inclusion into the polymer phase since higher than 60% of them are recovered from the polymer phase after the phase transition of the thermoresponsive polymers (Table I, Entries 1 and 2). As a model substrate was then chosen 1-phenylethanol which was recovered in about 10% yield from the polymer phase (Table I, Entry 6). Stereoselective partitioning tests were carried out at various polymer concentrations, and the use of 2.5 wt/vol % of (*S*)-**3a** having 30% of *N*-acryloyl-(*S*)-alaninol units gave the best stereoselectivity and reproducibility for the partitioning of 0.1 mol/L aqueous solution of (±)-1-phenylethanol. The minimum detectable concentration for the recognition seems to depend on the relative amount of the chiral moieties of the thermoresponsive polymers to substrates. The net amount of *N*-acryloyl-(*S*)-alaninol units was 0.6 mmol, while about 0.1 mmol of 1-phenylethanol was collected into the polymer phase. In the poly(*N*-(*S*)-*sec*-butylacrylamide-*co*-NIPAAm) system reported by Aoki et al.,[15] the LCST changes due to the chiral recognition of L-tryptophan was observed at 0.015 of molar ratio of L-tryptophan to *N*-(*S*)-*sec*-butylacrylamide units. Therefore in our stereoselective partitioning system 0.01 mol/L of 1-phenylethanol is probably detectable by using 2.5 wt/vol % of (*S*)-**3a**. The (*R*)/(*S*) ratio of 1-phenylethanol recovered from poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol) ((*S*)-**3a**) having 30% of *N*-acryloyl-(*S*)-alaninol units was determined to be 75/25, while the use of racemic poly(NIPAAm-*co*-HIPAAm) (*rac*-**3a**) showed no enantioselectivity (Table III, Entries 1 and 2). The use of poly(NIPAAm-*co*-*N*-acryloyl-(*R*)-alaninol) ((*R*)-**3a**) gave the opposite stereoselectivity and (*S*)-isomer of 1-phenylethanol was preferencially obtained from the polymer phase (Table III, Entry

Table III. Stereoselective Partitioning of (±)-1-Phenylethanol by Optically Active Thermoresponsive Polymers in Aqueous Phases at their LCSTs[a]

		Recovered 1-phenylethanol[b] (%)		Enantiomers ratio[c] (*R*) : (*S*)	
Entry	Polymers	From polymer	From water	From polymer	From water
1	Poly(NIPAAm-*co*-HIPAAm) (*rac*-**3a**)	12	67	50 : 50	50 : 50
2	(*S*)-**3a**	9	58	75 : 25	45 : 55
	$m : n = 70 : 30$,				
	$M_n = 20{,}300$, $M_w/M_n = 1.19$				
3	(*R*)-**3a**	17	71	38 : 62	54 : 46
	$m : n = 65 : 35$,				
	$M_n = 16{,}700$, $M_w/M_n = 1.32$				
4	(*S*)-**3b**	35	65	44 : 56	61 : 39
	$m : n = 89 : 11$,				
	$M_n = 2020$, $M_w/M_n = 2.00$				
5	(*S*)-**3d**	8	58	68 : 32	53 : 47
	$m : n = 67 : 33$,				
	$M_n = 66{,}200$, $M_w/M_n = 1.78$				

[a] Conditions: Solvent, distilled water; Total volume, 10 mL; Concentrations, [polymer] = 2.5 wt/vol %, [(±)-1-phenylethanol] = 0.1 mol/L.
[b] Isolated.
[c] Determined by chiral phase HPLC analyses.

3). Stereoselective concentration behavior of 1-phenylethanol into the hydrophobic field generated during the shrinking of poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol)s having various NIPAAm contents around at their LCSTs was observed by using several temperature raising patterns. The structure of the aminoalcohol side chains of the optically active thermoresponsive polymers remarkably affected the stereoselective partitioning behavior. The (*R*)/(*S*) ratio of 1-phenylethanol recovered from poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol) ((*S*)-**3d**) having 33% of *N*-acryloyl-(*S*)-prolinol units was 68/32 (Table III, Entry 5), on the other hand, poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-leucinol) ((*S*)-**3d**) was not so effective for stereoselective partitioning and slightly higher amount of (*S*)-1-phenylethanol was recovered from the polymer phase ((*R*)/(*S*) = 44/56, Entry 4). Moreover, slow temperature raising (0.5°C/min) and maintaining the temperature at around LCST were effective procedures for stereoselective concentration of the substrate into the asymmetric polymer field, while fast temperature raising (10°C/min) decreased the (*R*)/(*S*) ratio to 56/45. Transmission electron microscope (TEM) images of aqueous solution of (*S*)-**3a** in the presence of 1-phenylethanol showed coacervate formation (ca., 100–500 nm) over LCST. These results indicate that the stereospecific interaction between the substrates and the polymers occurs during the phase transition.

CONCLUSIONS

Several organic substrates were found to be concentrated into the hydrophobic field generated during the phase transition of thermoresponsive polymers, such as PNIPAAm and poly (NIPAAm-*co*-*N*-acryloyl-(±)-alaninol), around at their LCST in water. The amount of the substrates recoverd from the polymer phase mainly depended on the hydrophobicity of the substrates, but relatively large differences were observed in the amount of alcoholic substrates recovered from polymer phase between PNIPAAm and poly(NIPAAm-*co*-*N*-acryloyl-(±)-alaninol). Moreover temperature shifts of observed LCSTs of aqueous solutions of alcoholic substrates were relatively large. These results indicate that not only hydrophobic character of the polymer field but also the specific interaction between the substrates and the functional groups on the polymers, such as hydrogen bonding, influence the substrate concentration behavior of the thermoresponsive polymers.

We have then carried out enantioselective partitioning of organic substrates having asymmetric carbon centers by the optically active thermoresponsive polymers in aqueous phases. Several new optically active thermoresponsive polymers have been successfully synthesized by copolymerizations of NIPAAm with *N*-acryloylaminoalcohols such as *N*-acryloyl-(*S*)-alaninol and *N*-acryloyl-(*S*)-prolinol. Poly(NIPAAm-*co*-*N*-acryloyl-(*R*)-alaninol), poly(NIPAAm-*co*-*N*-acryloyl-(*S*)-alaninol), and poly (NIPAAm-*co*-*N*-acryloyl-(*S*)-prolinol) were found to be effective for enantioselective partitioning of 1-phenylethanol by the polymers in aqueous phases. Stereospecific interaction between the substrates and the polymers probably occurs during the phase transition. It is reported that the chiral recognition of the thermoresponsive polymers containing chiral AAm units were attained by the hydrogen bonding and π–π or CH/π interaction between substrates and chiral side chains of the polymers.[14,15] The precise mechanism of chiral recognition in our system has not been clarified yet, but the hydrogen bonding between hydroxy groups of 1-phenylethanol and the chiral *N*-acryloylalaninol units along with the CH/π interaction between the phenyl group of the substrate and chiral alkyl side chains probably select a favorable configuration of the substrate and lead to the stereoselectivity. The information obtained in this study about the asymmetric hydrophobic polymer field generated by the

thermoresponsive polymers is preliminary but highly important to develop new seteroselective synthetic methods in aqueous media.

REFERENCES

1. Ward, M. A.; Georgiou, T. K. *Polymers* **2011**, *3*, 1215.
2. Eeckman, F.; Moës, A. J.; Amighi, K. *Int. J. Pharm.* **2002**, *241*, 113.
3. Kohori, F.; Sakai, K.; Aoyagi, T.; Yokoyama, M.; Sakurai, Y.; Okano, T. J. *Control. Release* **1998**, *55*, 87.
4. Kidchob, T.; Kimura, S.; Imanishi, Y. J. *Control. Release* **1998**, *50*, 205.
5. Okano, T.; Bae, Y. H.; Jacobs, H.; Kim, S. W. J. *Control. Release* **1990**, *11*, 255.
6. Nagase, K.; Kobayashi, J.; Kikuchi, A.; Akiyama, Y.; Annaka, M.; Kanazawa, H.; Okano, T. *Langmuir* **2008**, *24*, 10981.
7. Nagase, K.; Kumazaki, M.; Kanazawa, H.; Kobayashi, J.; Kikuci, A.; Akiyama, Y.; Annaka, M.; Okano, T. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1247.
8. Kanno, S.; Watanabe, K.; Hirano, S.; Yamagishi, I.; Gonmori, K. Minakata, K.; Suzuki, O. *Foren. Toxicol.* **2009**, *27*, 103.
9. Zhang, B.; Ju, X.-J.; Xie, R.; Liu, Z.; Pi, S.-W.; Chu, L.-Y. *J. Phys. Chem. B* **2012**, *116*, 5527.
10. Ito, T.; Oshiba, Y.; Ohashi, H.; Tamaki, T.; Yamaguchi T. J. *Membr. Sci.* **2010**, *348*, 369.
11. Shamsipur, M.; Rajabi, H. R. *Microchim. Acta* **2013**, *180*, 243.
12. Rajabi, H. R.; Shamsipur, M.; Pourmortazavi, S. M. *Mater. Sci. Eng. C* **2013**, *33*, 3374.
13. Yang, M.; Chu, L.-Y.; Wang, H.-D.; Xie, R.; Song, H.; Niu, C. H. *Adv. Funct. Mater.* **2008**, *18*, 652.
14. Sugiyama, K.; Rikimaru, S.; Okada, Y.; Shiraishi, K. J. *Appl. Polym. Sci.* **2001**, *82*, 228.
15. Aoki, T.; Nishimura, T.; Sanui, K.; Ogata, N. *React. Funct. Polym.* **1998**, *37*, 299.
16. Ono, Y.; Shikata, T. J. *Am. Chem. Soc.* **2006**, *128*, 10030.
17. Plummer, R.; Hill, D. J. T.; Whittaker, A. K. *Macromolecules* **2006**, *39*, 8379.
18. Cheng, H.; Shen, L.; Wu, C. *Macromolecules* **2006**, *39*, 2325.
19. Oku, H.; Fujimoto, J.; Ohyama, T.; Hiroki, A.; Yoshida, M.; Katakai, R. J. *Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 2671.
20. Maeda, T.; Kanda, T.; Yonekura, Y.; Yamamoto, K.; Aoyagi, T. *Biomacromolecules* **2006**, *7*, 545.
21. Ishifune, M.; Suzuki, R.; Yamane, M.; Tanabe, H.; Nakagawa, Y.; Uchida K. J. *Macromol. Sci. A Pure Appl. Chem.* **2008**, *45*, 523.
22. Uemukai, T.; Ishifune, M. *J. Appl. Polym. Sci.* **2013**, *129*, 2554.
23. Wallen, E. A.; Christiaans, J. A.; Saario, S. M.; Forsberg, M. M.; Venalainen, J. I.; Paso, H. M.; Mannisto, P. T.; Gynther J. *Bioorg. Med. Chem.* **2002**, *10*, 2199.
24. Li, P.-F.; Wang, W.; Xie, R.; Yang, M.; Ju, X.-J.; Chu, L.-Y. *Polym. Int.* **2009**, *58*, 202.
25. Oku, H.; Ohashi, H.; Fujimoto, J.; Shimizu, M.; Yoshida, M.; Katakai, R. *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 4524.
26. Mori, H.; Kato, I.; Matsuyama, M.; Endo, T. *Macromolecules* **2008**, *41*, 5604.