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Multi-step precipitation separation system using mixture of thermosensitive polymers

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Summary

Multi-step precipitation separation system was developed by using aqueous mixtures of some thermosensitive polymers. The following three polymers were used here; poly(*N*-*n*-propylacrylamide), poly(*N*-isopropylacrylamide), and poly(*N*-isopropylmethacrylamide). A mixture of the three polymers showed three endothermic peaks, and the peak top temperatures were almost consistent with that of the each polymer solution. The polymers were purified by thermal precipitation to obtain fractions which can respond in narrow temperature ranges prior to use. In the case of the precipitation separation of two polymers mixtures, purities of the obtained precipitate and supernatant fractions became high comparing with the case in which the unpurified polymers were used. Parts of the polymers which were not the precipitation targets were also precipitated by the separation procedures. This was caused not only by insolubilization of the non-targeted polymers due to their phase transitions but also by their non-specific entanglement with the targeted polymers. The purities of the fractions also improved when the difference of the phase transition temperature between two polymers was large enough to avoid the coprecipitation. In the case of the precipitation separation of mixtures of the three polymers, purities of each fraction also improved when the purified polymers were used.

Introduction

The thermosensitive polymers which become insoluble to be separated out from aqueous solvent when heated have been applied to batch-type precipitation separation systems [1-15]. For example, affinity ligands-immobilized polymers were applied to separations of target molecules such as metal ions [1], organic molecules [2], proteins [3-7], and nucleic acids [8-11]. Catalytic molecules-pendent polymers were useful when separating the catalysts and products containing solutions after catalytic reactions [12-15]. A principal advantage of the separation systems using the soluble polymers as supports over those using conventional solid supports is that a capturing of targeted molecules and a catalytic reaction can be conducted in a homogeneous solution so that the rates of these reactions become faster [16, 17].

The other advantage of the separation system utilizing the thermosensitive polymer support is the variety of the thermosensitive polymers and their transition temperatures. Many thermosensitive polymers with various transition temperatures between 0 and 100 °C in aqueous media have been reported so far [18-25]. However, the previously reported precipitation separation systems used only one kind of thermosensitive polymer and thus separation was conducted in one step. If we extend the system to the mixture of some thermosensitive polymers, the resulting system can separate some kinds of target molecules in one batch by stepwise change of the solution temperature.

Therefore, we studied here the properties of thermosensitive polymers mixtures and their applicability to a multi-step precipitation separation system shown in Figure 1. In the system, first, polymer **1** which has the lowest phase transition temperature (T_{c1}) among the three polymers is selectively phase separated and precipitated by heating the solution. Next, polymer **2** is precipitated out from the resulting solution to leave polymer **3** in the supernatant by further heating of the solution. Thus, the stepwise separation of polymers will be realized by only raising the solution temperature.

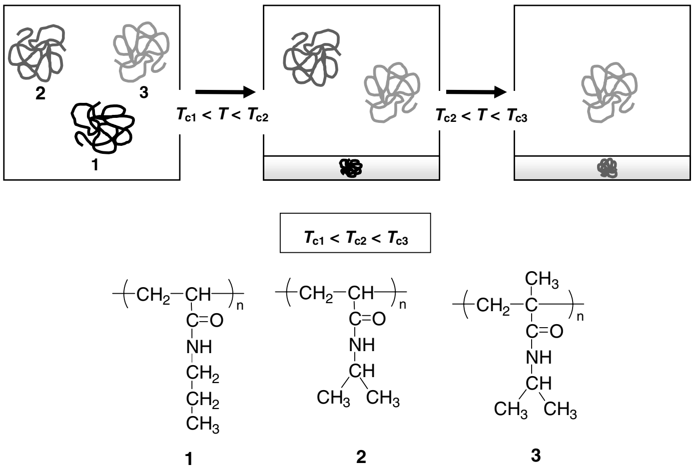

Figure 1. Multi-step thermal precipitation separation of mixture of three polymers (**1**-**3**) with different transition temperatures (T_{c1}, T_{c2}, and T_{c3}).

Experimental

Materials

N-*n*-propylacrylamide was synthesized according to the reported procedures.[18] *N*-isopropylacrylamide (TCI) and *N*-isopropylmethacrylamide (Aldrich) were recrystalized from benzene / hexane mixture. 2,2'-Azobisisobutyronitrile (AIBN) (Kanto Kagaku) was recrystalized from methanol. Other reagents were used as received.

Syntheses of polymers

The three monomers were polymerized at 70 °C in a thermostatic bath, with AIBN as a free radical initiator, as follows. A DMF solution containing the monomer (1.1 M) and the initiator (2.1 mM) was placed in a round bottom flask. The solution was cooled in a cooling bath and degassed at high vacuum. After 3 times cooling-degassing cycle, the solution was sealed and polymerization was conducted for 6 h. The obtained polymer was precipitated three times into an excess amount of diethyl ether and dried at reduced pressure. The yields of the polymers were 65, 68, and 13 % for polymers **1**, **2**, and **3**, respectively. M_w (M_w/M_n) of the polymers determined by GPC analysis were $4.1\text{x}10^5$(1.8) for **1**, $8.3\text{x}10^4$(2.1) for **2**, and $5.7\text{x}10^4$(1.9) for **3**, respectively. The obtained polymers were further purified by thermal precipitation to sort out the polymer fractions which can respond in narrow temperature ranges. The precipitation purification was carried out as follows. The aqueous polymer solutions (10 g/L) were incubated and centrifuged at temperatures which were ca. 5 °C higher than those of their cloud points. The precipitated polymers were dissolved in deionized water and lyophilized. The yields of the polymers after the purification procedures were 84, 91, and 78 % for polymers **1**, **2**, and **3**, respectively. M_w (M_w/M_n) of the purified polymers were $4.6\text{x}10^5$ (1.8) for **1**, $9.2\text{x}10^4$ (1.5) for **2**, and $8.6\text{x}10^4$ (2.0) for **3**, respectively.

Cloud point measurements

The optical transmittance curve of the aqueous polymer solution was recorded with a spectrophotometer (Hitachi U-3210) equipped with a temperature controller (Shimadzu SPR-10), by monitoring the transmittance at 500 nm at a heating rate of 1.0 °C/min. The cloud point of the polymer solution is defined as the temperature at which the transmittance begins to decrease.

Precipitation separation

The precipitation separation procedures of polymers **1** / **2** / **3** mixture are described here as a typical example and the flow chart of the procedures is shown in Figure 2. 1.0 mL of polymer solution containing an equal amount of the three polymers (total polymers concentration = 10 g/L) were prepared in 1.5 mL of test tube (the total polymer concentration was also the same in the separation of two kinds of polymers mixtures). The solution was heated to 25 °C for 3 h for phase separation of **1**. The resulting turbid mixture was centrifuged at 26±2 °C for 3 min (17 000 g) by using a centrifuge (TOMY MRX-150) and the supernatant was collected. The supernatant fraction was applied to the next precipitation procedures. The incubation and centrifugation temperatures were 35 and 37±3 °C, respectively. The resulting two precipitate fractions were dissolved in deionized water and the obtained two solutions and the supernatant fraction were lyophilized. The amounts of the polymers included in these fractions were determined gravimetrically. The polymers' compositions of these fractions were determined by ^{1}H NMR.

Other measurements

^{1}H NMR spectra were recorded on a JEOL-GX400 spectrometer using d_6-DMSO as a solvent. Differential scanning calorimetry (DSC) measurement was performed using Seiko Instruments Inc. EXSTAR6000 DSC. 60 μL of polymer solutions (10 g/L) were sealed in Ag sample pans. DSC thermograms were scanned from 10 to 70 °C at a heating rate of 1.0 °C/min, and distilled water was used as the reference. Gel permeation chromatography was performed on a Tosoh SC-8020 equipped with columns of Tosoh G4000H_{HR}, G3000H_{HR}, and G2000H_{HR} at 45 °C. 10 mM LiCl/DMF (elution rate: 1.0 ml/min) and polystyrenes were used as an eluent and standards, respectively.

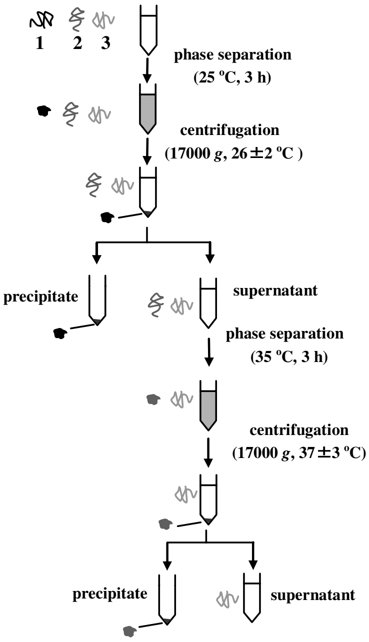

Figure 2. Procedures of precipitation separation of mixture of three polymers.

Results and Discussion

Precipitation ability of each polymer

We selected the three polymers of Figure 1 for the construction of multi-step separation system. Polymer **2** is well-know thermosensitive polymer [26] and has been applied to precipitation separation systems because of its mild transition temperature [1-15]. The phase transition temperature of the polymer is ca. 32 °C. The other two polymers **1** and **3** were chosen to give enough difference of transition temperature with polymer **2** (ca. 10 °C) because in reality the phase transitions of the thermosensitive polymers do not occur in narrow temperature ranges.

First, the precipitation ability of the three polymers was investigated. The cloud points of the polymer solutions were determined from temperature-transmittance curves (Figure 3, open symbols) and the values were summarized in Table 1. The polymer solutions (10 g/L) were incubated at temperatures which were ca. 5 °C higher than their cloud points, and the resulting turbid solutions were centrifuged at almost the same temperatures to precipitate the polymers. The amounts of the precipitate and supernatant fractions were gravimetrically determined. As shown in Table 2 ("unpurified" columns), precipitation percentages were not so high, especially in polymers **1** and **3**. This indicates that the phase transition of the polymers occurred in a wide temperature range. It is known that the phase transition temperature of these thermosensitive polymers depends on their molecular weight [27], molecular weight distribution [27, 28], and tacticity [29].

Therefore, thermal precipitation purification was conducted for the three polymers to sort out polymer fractions which can respond in narrow temperature ranges. As shown in Table 2 ("purified" columns), the precipitation percentages of the polymers were improved after purification. This indicates that a polymer fraction with a higher transition temperature was removed by the purification procedures. However, the phase transition temperatures determined from transmittance curves were almost the same with those of unpurified ones (Figure 3). This means that the transition temperature of the polymer is governed by fractions with lower transition temperatures in the total polymer fractions when a transmittance curve is used for determination of a transition temperature. As described above, M_w values of the purified polymers were somewhat larger than those of the unpurified ones. Molecular weight dependence of transition temperature of poly(*N*-isopropylacrylamide) was reported by two groups [27, 28], though these dependences were contradictory with each other. Our GPC results show that the polymer with lower molecular weight has higher phase transition temperature. In Table 2, ca. 95 % of the polymers precipitated out from the solutions when the purified polymers were used. It seems that ca. 5 % of the polymers remained in supernatants as suspended aggregates because the supernatants were slightly turbid. The suspended aggregates could not be precipitated by centrifugation probably due to their small size.

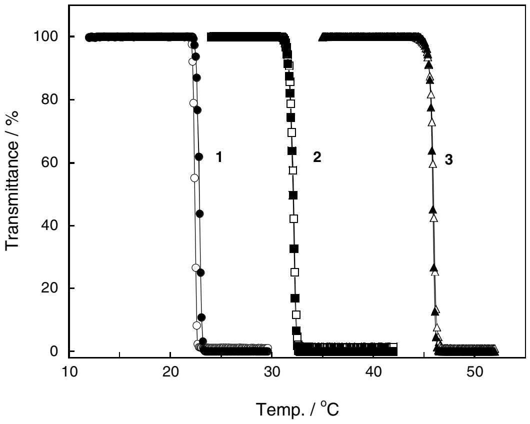

Figure 3. Transmittance curves of 10 g/L aqueous polymer solutions before (open symbols) and after (closed symbols) purification. Heating rate = 1.0 °C/min.

Table 1. Phase transition behavior of solutions of each polymer and three polymer mixture.

polymer	cloud point (°C)	endothermic peak temperature (°C) singular	endothermic peak temperature (°C) mixture
1	22.3	22.0	23.9
2	31.7	32.5	32.5
3	45.8	45.9	46.0

Table 2. Gravimetrically determined percentages of polymers found in precipitate (p) and supernatant (s) fractions obtained by precipitation of each polymer solution.

polymer	unpurified		purified	
	p (wt%)	s (wt%)	p (wt%)	s (wt%)
1	83.3	16.7	96.6	3.4
2	93.5	6.5	93.2	6.8
3	83.1	16.9	94.6	5.4

Phase transition behavior of polymers mixture

Figure 4 shows the DSC thermograms of the solutions of each purified polymer and their mixture. The endothermic peak temperature of each polymer solution was almost consistent with its cloud point (Table 1). The transition heats of the three polymers were independently observed in the mixture. The endothermic peak temperatures of the mixture hardly changed from those of the each polymer solution (Table 1). Theses results indicate that the phase transition of the polymers was not perturbed by the coexisting other polymers. The same conclusion has been reached for a polymer **2** / **3** mixture by using turbidimetry [30].

The temperature ranges of the phase transitions of these three polymers were about 10 °C. The difference in the transition temperatures between polymers **1** and **2** (ca. 10 °C) was narrower than that between **2** and **3** (ca. 13 °C). Thus, there was an overlap region of the transition temperature ranges of the former two polymers (ca. 3 °C) as estimated from the thermograms. This property affected the separation efficiency of the polymers as described in the following section.

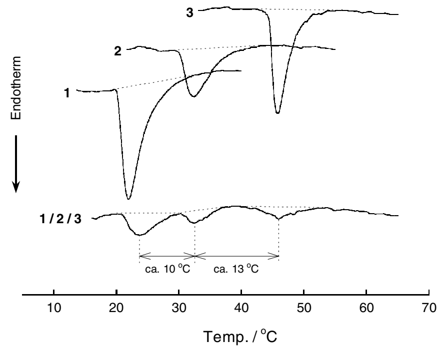

Figure 4. DSC thermograms of each polymer solution and their mixture containing an equal concentration of the polymers (total polymer concentration = 10 g/L, heating rate = 1.0 °C/min).

Precipitation separation of mixtures of two polymers

Precipitation separations were conducted for mixtures of the two polymers. The polymer with a lower phase transition temperature was precipitated and the other polymer was left in the supernatant. As shown in Table 3, the percentages of the precipitated polymers were less than ca. 90 % when the unpurified polymers were used. This indicates that a part of the polymer of a precipitation target remained in the supernatant. On the other hand, in the purified polymers, precipitation percentages exceeded 100 % for **1** / **2** and **1** / **3** mixtures. This result indicated that the polymers which were not the precipitation target were also precipitated.

Then, we determined the compositions of the polymers both in the precipitate and supernatant fractions by ^{1}H NMR. The determination procedures were explained below using **1** / **2** mixture as an example. Figure 5 shows the ^{1}H NMR spectra of the three polymers together with those of the supernatant and the precipitate fractions of **1** / **2** mixture. The compositions of these fractions were determined by using areas of *d* of **1** (2.6-3.2 ppm) and *d'* of **2** (3.6-4.2 ppm). The compositions of the fractions obtained from **1** / **3** mixture were determined similarly, and those from **2** / **3** mixture were determined from areas of *d'* and *d''* (3.6-4.2 ppm) and sum of the other protons

Table 3. Gravimetrically determined percentages of polymers found in precipitate (p) and supernatant (s) fractions obtained by precipitation separation of mixtures of two polymers.

mixture	unpurified		purified	
	p (wt%)	s (wt%)	p (wt%)	s (wt%)
1 / 2	86	114	112	88
1 / 3	91	109	108	92
2 / 3	80	120	96	104

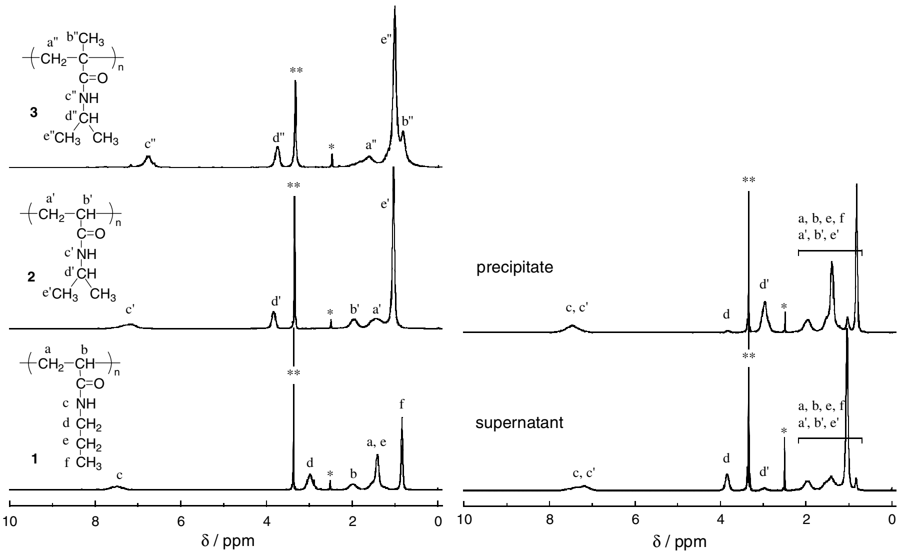

Figure 5. ^{1}H NMR spectra of three polymer solutions and precipitation and supernatant fractions obtained from **1** / **2** mixture. Asterisk and double asterisk indicate the solvent (DMSO) and H_2O signals, respectively.

(*a, b, e, f, a', b', e'*: 0.1-2.4 ppm) except for amide (*c, c'*). The determined compositions were summarized in Figure 6. In the case of the unpurified polymers, the purities of the precipitation fractions (denoted as "p") were more than 90 %, while those of the supernatants (denoted as "s") were relatively low because of the insufficient precipitation of the targeted polymers. In the purified polymers, though the purities of the precipitates were somewhat lower than the unpurified ones, those of the supernatant fractions were much improved. The best results were obtained in the **1** / **3** mixture (purified), in which the transition temperature ranges of the polymers clearly separated in the thermograms (Figure 4). Thus, the precipitation of polymer **3** should result from non-specific entanglement with the targeted polymer **1**. This entanglement would be suppressed when lower polymer concentration is adopted. However, the lower polymer concentration leads a longer time for intermolecular aggregation of the polymer to form a precipitable aggregate. In the **1** / **2** mixture, the content of the non-targeted polymer **2** in the precipitate (ca. 18 %) was much larger than the other two mixtures. This should be resulted from the phase transition of the part of polymer **2** coincided with that of **1**, because there was an overlap region between the transition temperature ranges of **1** and **2** as described above.

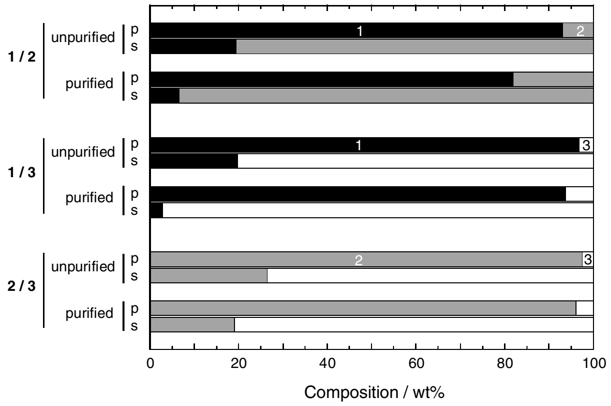

Figure 6. Compositions of precipitation (p) and supernatant (s) fractions obtained by separation of mixtures of two polymers determined by ^{1}H NMR (concentration of each polymer = 5.0 g/L, total polymer concentration = 10 g/L).

Precipitation separation of mixtures of three polymers

Table 4 summarizes the results of the precipitation separation of the mixtures of the three polymers. In the case of the unpurified polymers, the amounts of the precipitated polymers obtained in the first precipitation step were smaller than those of the purified ones. This should be caused by the inadequate precipitation of polymer **1**. The fraction compositions were determined by ^{1}H NMR similarly as described above and the results are shown in Figure 7. The purity of the first precipitate fraction was high (93.0 %) compared with the purified ones. However, a missed part of polymer **1** contaminated the second precipitate. Similarly, the supernatant fraction was contaminated by a missed part of polymer **2** in the second precipitation step. Thus, the purities of the fractions decreased with the progress of the precipitation steps.

On the other hand, in the case of the purified polymers, the amounts of both the first and the second precipitate fractions were about 100 % (Table 4). The first precipitate fraction was composed of polymers **1** (85.6 %), **2** (8.7 %), and **3** (5.7 %), respectively. In the two non-targeted polymers, polymer **2** was more precipitated than **3**. This was due to the coprecipitation phenomenon observed in the **1** / **2** mixture. The second precipitate and the supernatant fractions kept the purities of more than 70 %. The values were lower than those of the two polymers mixtures (Figure 6), but higher than the results of the unpurified three polymers mixture.

Table 4. Gravimetrically determined percentages of polymers found in fractions obtained by precipitation separation of mixtures of three polymers.

polymers	1st precipitate (wt%)	2nd precipitate (wt%)	supernatant (wt%)
unpurified	77	109	113
purified	106	92	102

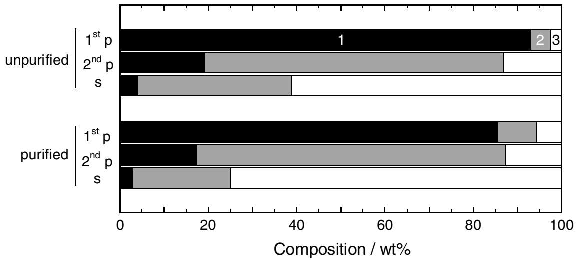

Figure 7. Compositions of fractions obtained by separation of three polymers mixtures determined by ^{1}H NMR (concentration of each polymer = 3.3 g/L, total polymer concentration = 10 g/L).

Conclusion

A three polymers mixture showed independent three endothermic peaks. The endothermic peak temperatures were almost consistent with those of each polymer solution. The polymers were purified by thermal precipitation to obtain the polymers with a sharp thermal responsiveness. In the precipitation separation of both two and three polymers mixtures, the purities of the obtained precipitate and supernatant fractions became high when the purified polymers were used. The purities of the fractions also improved when the difference of the phase transition temperatures between two polymers became larger. Thus, it was concluded that important two things to raise the purity of the fractions are sharpness of the polymer's transition and enough difference of transition temperatures between two polymers to avoid coprecipitation. The multi-step precipitation separation system proposed here will be able to independently separate some kinds of target molecules in one batch by only raising the solution temperature when we attach the corresponding affinity ligands to the polymers. In addition, if different dyes were introduced to each polymer, the polymers mixture will be applicable to a thermometer based on color changes of the solution. These studies are now in progress in our laboratory.

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