Polymer Vol. 37 No. 4, pp. 681–685, 1996

0032-3861/96/$15.00+0.00

Preparation of polyacrylamide derivatives showing thermo-reversible coacervate formation and their potential application to two-phase separation processes

Hiroaki Miyazaki and Kazunori Kataoka*
Department of Materials Science, and Research Institute for Biosciences, Science University of Tokyo, 2641 Yamazaki, Noda-shi, Chiba 278, Japan
(Received 15 June 1995; revised 3 July 1995)

A synthetic polymer forming a thermo-reversible coacervate in aqueous milieu was prepared in this study by radical copolymerization of *N,N*-dimethylacrylamide (DMAA) with *N*-phenylacrylamide (PA). Increased content of PA in the copolymer (DPA) led to an abrupt decrease in its cloud point (lower critical solution temperature, *LCST*) owing to an increase in hydrophobicity. The heat of transition (ΔH) for a 1.0 wt% aqueous solution of the copolymer at the *LCST* was determined to be in the range of 3–4 cal g^{-1} by differential scanning calorimetry, considerably smaller than that obtained for a neutral amphiphilic polymer, such as poly(*N*-isopropylacrylamide), undergoing a steep dehydration at the *LCST*. This small value of ΔH suggests the incomplete dehydration of the polymer chain at the *LCST*. Indeed, microscopic observation revealed the formation of coacervate droplets in the aqueous solution of the copolymer, which is clear evidence of liquid–liquid phase separation. The concentration of the copolymer in the coacervate phase formed from 1.0 wt% aqueous solution of the copolymer at 37°C is approximately 20 wt%. It is of interest that the formation and disappearance of the coacervate are completely thermo-reversible. To estimate the feasibility of applying this thermo-sensitive coacervate system to thermo-modulated aqueous two-phase separation, partitioning of model solutes from aqueous milieu to the coacervate was carried out. By increasing the temperature, preferential partitioning of Trypan Blue (3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid) tetrasodium salt) from aqueous phase to coacervate phase was observed, whereas no partitioning of vitamin B_{12} to coacervate phase took place. Consequently, the separation of Trypan Blue from the mixed solution of vitamin B_{12} and Trypan Blue was achieved solely through the change in the environmental temperature. It may be feasible to apply such copolymers showing liquid–liquid phase separation that responds to temperature as the stationary phase in thermo-regulated liquid chromatography for the separation of water-soluble drugs and dyestuffs.

(Keywords: *N,N*-dimethylacrylamide; thermo-reversible coacervates; thermo-modulated separation)

INTRODUCTION

A mixed aqueous solution of positively and negatively charged weak polyelectrolytes is known to undergo liquid–liquid phase separation to form a 'complex coacervate', a droplet composed of a condensed solution of a pair of the oppositely charged polyelectrolytes[1–5]. The driving force of complex coacervation is the electrostatic interaction between oppositely charged polyelectrolytes, such as acidic gelatin/gum arabic and DNA/histone. The other type of coacervation is known as 'simple coacervation', and is a liquid–liquid phase separation to form a condensed droplet of a single polymer species. This is caused by a change in the polymer–solvent (polymer) interactions with certain variables such as temperature and solvent composition. For example, the addition of alcohol to an aqueous solution of gelatin causes dehydration of the gelatin, leading to the formation of a simple coacervate[6]. Other than gelatin, simple coacervation is observed for the solution of several kinds of proteins. Interestingly, an aqueous solution of α-elastin, which is obtained by hot oxalic acid cleavage of purified aortic elastin fibres, undergoes a liquid–liquid phase separation above the lower critical solution temperature (*LCST*) to form a simple coacervate[7]. This type of material showing coacervation in aqueous milieu that responds to temperature may find wide applications, particularly in the area of separation technology, such as aqueous two-phase partitioning[8–10] and temperature-regulated chromatography. In spite of these potential uses there seems to have been few reports, as far as we know, that directly pursue the preparation of synthetic polymers showing thermo-reversible coacervate formation. This is in sharp contrast with a great trend in research towards polymer systems, including poly(*N*-isopropylacrylamide), showing a sharp liquid–solid phase transition over the *LCST*. This study is devoted to the synthesis of polyacrylamide derivatives showing coacervation at regulated tempera-

* To whom correspondence should be addressed

ture, and is aimed at their application in thermo-regulated partitioning systems. To reach this goal, we introduced a regulated amount of hydrophobic comonomer, *N*-phenylacrylamide, into the water-soluble polyacrylamide derivative, poly(*N*,*N*-dimethylacrylamide) (PDMAA). The aqueous solution of PDMAA has no *LCST* at least in the region below the boiling point (100°C) of water at 1 atm. Nevertheless, it was reported to have a negative heat of dilution[11], suggesting the possibility of exhibiting *LCST* by introducing a small amount of hydrophobic component into the chain. In this study, *N*-phenylacrylamide (PA) was selected as the hydrophobic comonomer because π–π interaction, as well as hydrophobic interaction, is expected to contribute to the induction of phase separation. Further, thermo-regulated partitioning of model dyestuffs (Trypan Blue and vitamin B_{12}) between coacervate phase and aqueous phase was carried out to demonstrate the feasibility of these copolymers as media for use in thermo-regulated separation systems.

EXPERIMENTAL

Materials

Ethanol was distilled over sodium, and was stored under an argon atmosphere. 2,2′-Azobis(2,4-dimethylvaleronitrile) (V-65; Wako Pure Chem. Ind. Ltd) was recrystallized from ethanol and dried *in vacuo* at room temperature. *N*,*N*-Dimethylacrylamide (DMAA; Kohjin Co. Ltd) was purified by distillation *in vacuo* (b.p. 37°C at 2 mmHg). *N*-Phenylacrylamide (PA; Polyscience Inc.) was used as received.

Copolymerization of N,N-*dimethylacrylamide with* N-*phenylacrylamide*

Radical copolymerization of *N*,*N*-dimethylacrylamide (DMAA) with *N*-phenylacrylamide (PA) was carried out in a sealed glass ampoule at 45°C using ethanol and 2,2′-azobis(2,4-dimethylvaleronitrile) as solvent and initiator, respectively. Detailed conditions are summarized in *Table 1*. After 0.5 h, the reaction mixture was poured into an excess amount of diethyl ether to precipitate the polymer. The precipitated polymer was filtered off, and thoroughly dried under vacuum to give a white powder. The composition of the copolymer was determined through 400 MHz ^{1}H n.m.r. (JNM-EX400, JEOL) measurement (in deuterated dimethyl sulfoxide (DMSO-d_6; Isotec Inc.); room temperature; polymer concentration, 7 mg ml^{-1}). The analysis was carried out for proton signals at 2.0–0.8 ppm for $-CH_2-$ groups in DMMA and PA units, at 3.0–2.1 ppm for $-N(CH_3)_2$ groups in DMAA units, and at 7.8–6.8 ppm for phenyl groups in PA units. The copolymers are abbreviated as DPA-*X*, where *X* stands for the PA content in the copolymer (mol%).

G.p.c. measurement

The molecular weights were characterized using gel permeation chromatography (system controller, SC-8010, Tosoh; column, oven, CO-8010, Tosoh; RI detector, RI-8012, Tosoh; pump, CCPD, Tosoh; guard column, H_{HR}-H, Tosoh; column, GMHHR-M ×2, Tosoh). Dimethylformamide (DMF) was used as carrier solvent. Calibration was performed with commercial polystyrene standards.

Determination of lower critical solution temperature

Turbidity measurement. The *LCST* was determined from the turbidity of the polymer solution. The concentration of the polymer was varied in the range from 0.05 to 5 wt%. The sample was placed in a u.v.-vis. spectrophotometer (Ubest 50, Japan Spectroscopic Co.) equipped with Perche-type thermo-controlling system. The temperature was raised from 3 to 80°C in 1°C increments every 5 min. The *LCST* was defined as the onset temperature of turbidity at 500 nm.

D.s.c. measurements. The *LCST* of the polymer solutions was also determined from differential scanning calorimetry (d.s.c.). Calorimetric measurements were performed on an ultrasensitive scanning calorimeter MC-2 (Microcal Inc., USA) with a cell volume of 0.986 ml at 0.5 kgf cm^{-2}. The heating rate was 90 K h^{-1}. Before the sample measurement, sample solutions were vacuumed with stirring for 5 min to eliminate air in the solution.

Measurement of polymer concentration in the coacervate phase

First, 5 ml of 1 wt% aqueous solution of PA-18.3 was incubated at 37°C, a temperature above the *LCST*, for 1 h. Then, the turbid solution was centrifuged at 2000 rev min^{-1} for 5 min to separate the coacervate phase. The absorption of the upper aqueous solution at 243 nm was measured to determine the concentration of the polymer remaining in the upper phase. Concomitantly, the volume of the upper solution was determined. The amounts of polymer in the upper aqueous phase and the coacervate phase were then calculated, respectively, based on these determined values.

Observation of coacervate

First, 1 wt% aqueous solution of PA-18.3 was placed into a flat-bottomed multi-well plate (Sumilon, MS-8096f, Sumitomo Bakelite Co. Ltd, Japan), adjusting the temperature to 37°C. After 1 h, a photograph of the coacervate droplets was taken under a microscope (Photo-TMD, Nikon) equipped with a single-lens reflex camera (F-301, Nikon).

Solute partitioning between coacervate phase and aqueous phase

A definite amount of Trypan Blue (3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid) tetrasodium salt; Direct Skyblue 5B, Nippon Kayaku Co.) or vitamin B_{12} (cyanocobalamin, Wako Pure Chem. Ind. Ltd) was dissolved in 1 wt% aqueous solution of PA-18.3 at room temperature, and the solution was incubated at 37°C for 3 h. Then, the solution was centrifuged at 2000 rev min^{-1} for 5 min to separate the two phases. The absorption of the upper aqueous phase was measured to determine the concentration of solute remaining in the aqueous phase. Wavelengths of 550 and 650 nm, respectively, were used to determine the concentration of Trypan Blue and vitamin B_{12}. The amount of solute partitioned into the coacervate phase was then calculated from the amount remaining in the aqueous phase.

Table 1 Copolymerization of *N,N*-dimethylacrylamide (DMAA)[a] with *N*-phenylacrylamide (PA)

Code	Feed composition DMAA/PA (mole ratio)	Yield (%)	M_w^b ($\times 10^4$)	PA content[c] in copolymer (mol%)
DPA-14.5	90.0/10.0	22.1	3.5	14.5
DPA-18.3	87.0/13.0	25.2	4.1	18.3
DPA-21.2	84.0/16.0	35.4	6.5	21.2
DPA-26.7	80.0/20.0	22.0	5.9	26.7
DPA-37.3	70.0/30.0	19.7	5.7	37.3

[a] Solvent, EtOH; initiator, V-65; monomer conc., 0.5 mol l^{-1}; initiator conc., 2.5 mmol l^{-1}; react. time, 0.5 h; react. temp., 45°C
[b] Determined by g.p.c. using polystyrene standards
[c] Determined by ^{1}H n.m.r.

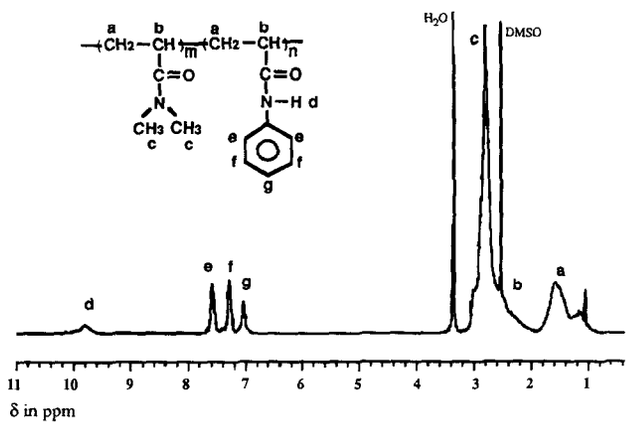

Figure 1 The 400 MHz ^{1}H n.m.r. spectrum (7 mg ml^{-1} in DMSO) of DPA-18.3 at 25°C

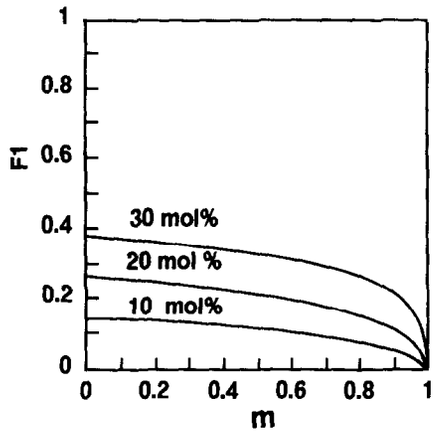

Figure 2 Instantaneous copolymer composition (F_1) as a function of conversion (m) in each feed composition of *N*-phenylacrylamide

Two-phase separation of Trypan Blue and vitamin B_{12}

Definite amounts of Trypan Blue and vitamin B_{12} were dissolved in 1 wt% aqueous solution of PA-18.3 containing 8.0 gl^{-1} of NaCl at 37°C, and the mixed solution was incubated at 37°C for 3 h. Then, the solution was centrifuged at 2000 rev min^{-1} for 5 min. The absorption of the upper aqueous phase at 550 nm was measured to determine the concentration of remaining Trypan Blue. For vitamin B_{12}, absorption at 650 nm was used in quantitative analysis.

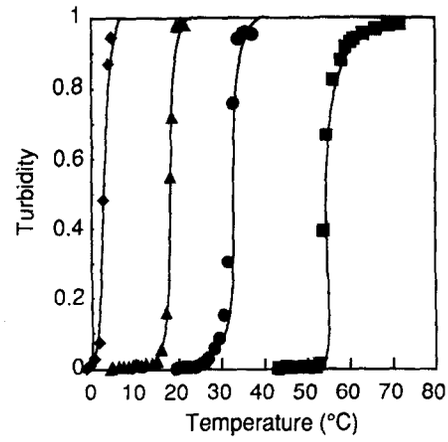

Figure 3 Temperature dependence of the turbidity of the aqueous solution of DPA copolymers with varying composition: (■) DPA-14.5, (●) DPA-18.3, (▲) DPA-21.2, (◆) DPA-26.7. Turbidity = 1-transmittance/ 100; polymer conc., 1.0 wt%

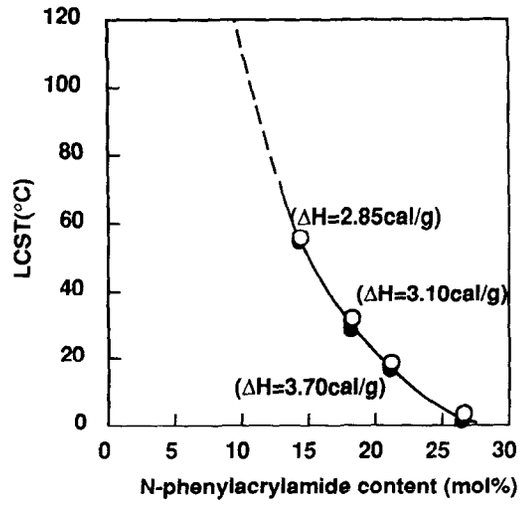

Figure 4 Relationship between *LCST* and *N*-phenylacrylamide content in the copolymer: (●) determined from turbidity; (○) determined from d.s.c. Polymer conc., 1 wt% aqueous solution

RESULTS AND DISCUSSION

Copolymerization of N,N-*dimethylacrylamide with* n-*phenylacrylamide*

Radical copolymerization of DMAA (M_1) with PA (M_2) was carried out in ethanol at 45°C using 2,2′-azobis(2,4-dimethylvaleronitrile) (V-65) as initiator. Copolymers with varying composition were prepared to determine the monomer reactivity ratio, because the distribution of monomer units in the polymer chain should crucially affect the *LCST* behaviour. Results of the copolymerization are summarized in *Table 1*. All the copolymers were readily precipitated into diethyl ether from the reaction mixture as a white powder. Further purification was done by reprecipitating the sample into diethyl ether from ethanol solution. A typical ^{1}H n.m.r. spectrum of the copolymer (DPA-18.3) is shown in *Figure 1*. From the area ratio of phenyl protons (e,f,g) to other protons, the copolymer composition was determined.

Monomer reactivity ratios were then calculated by the Kelen–Tüdös method, and were determined as $r_1 = 1.1$ and $r_2 = 0.63$, respectively, indicating that the sequence

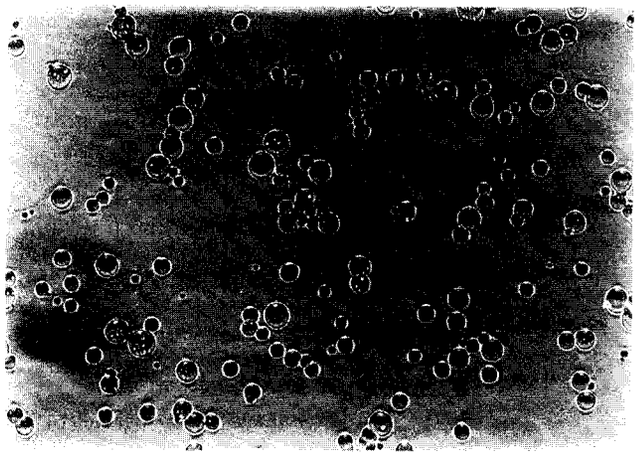

Figure 5 Photograph of coacervate formed from aqueous solution of DPA-16.8 (1.0 wt%) in aqueous milieu at 37°C

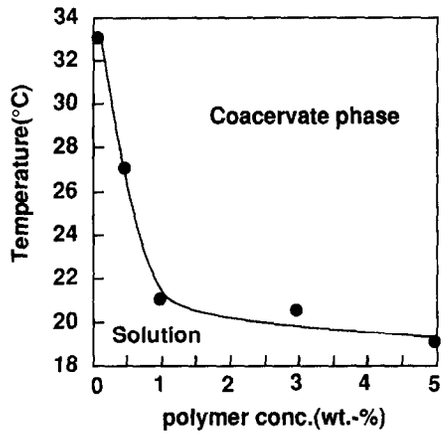

Figure 6 Phase diagram for aqueous solution of DPA-18.3

distribution in the copolymer is considerably random[12,13]. In order to achieve a sharp phase transition in the copolymer system, it is important to minimize the compositional deviation between each polymer chain. This deviation can be estimated by calculating the instantaneous composition at a given conversion from the values of monomer reactivity ratio. The results of the calculation are shown in *Figure 2*. In this study, all the samples used in *LCST* determination have yields below 35%, suggesting a fairly narrow compositional deviation between polymer chains.

Measurement of lower critical solution temperature

The *LCST* of the copolymer with varying PA content in aqueous milieu was determined from turbidity at 500 nm. *LCST* was defined as the inflection point of turbidity with temperature. As shown in *Figure 3*, decrease in *LCST* was observed by increasing PA content in the copolymers. Sharp change in turbidity at the *LCST* is consistent with the considerably homogeneous composition of copolymers assumed from the result of copolymerization. Change in *LCST* determined from turbidity with PA content in the copolymer was plotted as full circles in *Figure 4*. Of interest, a drastic decrease in *LCST* is obvious by introducing a small amount of PA into poly(DMAA). The *LCST* determined from turbidity is in good correlation with that

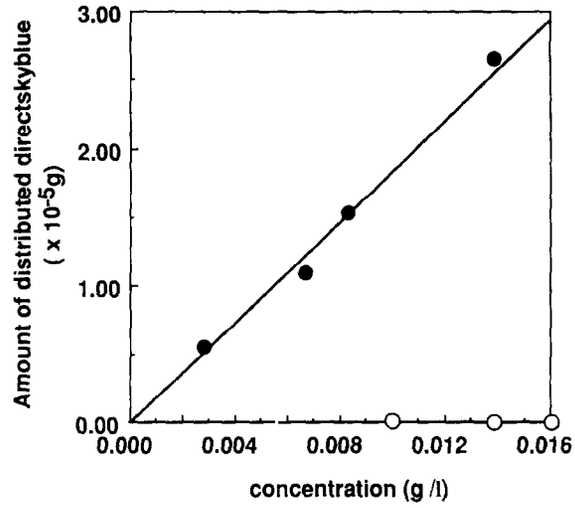

Figure 7 Solute partitioning to coacervate phase of DPA-18.3 at 37°C: (●) Trypan Blue, (○) vitamin B_{12}. Initial polymer conc., 1 wt%; temp., 37°C

determined from d.s.c. measurement, as shown by open circles in *Figure 4*. From the d.s.c. thermogram, the heat of transition (ΔH) can also be calculated, which is shown in parentheses in *Figure 4*. ΔH showed a slight increase with PA content in the copolymer, yet the values were significantly smaller than the reported value of ΔH (16.7 cal g^{-1}) for poly(*N*-isopropylacrylamide), a typical polymer showing a sharp transition due to abrupt dehydration at *LCST*[14]. The rather small ΔH at the transition suggests that the dehydration of PA at *LCST* should be incomplete, and PA may undergo a liquid–liquid phase separation over *LCST*. In fact, as shown in *Figure 5*, the micrograph of PA-18.3 solution clearly indicates coacervate formation over the *LCST*. It should be noted that coacervation has a thermo-reversible nature, disappearing promptly with decreasing temperature. Then, a partial phase diagram was prepared for PA-18.3, and is summarized in *Figure 6*. The *LCST* took a relatively constant value for the solution with concentration higher than 1.0 wt%, and then increased steeply with a further decrease in polymer concentration. At 33°C, the concentration of the polymer in the solution phase is below 0.01%, and virtually all the polymer should be transferred into the coacervate phase. It is of interest to determine the polymer concentration in the coacervate phase to get a definite characteristic of the coacervate. Thus, the upper aqueous solution was separated from the coacervate phase by centrifugation at a temperature above *LCST*, and its absorbance at 246 nm, a characteristic band for phenyl groups in PA, was measured. In this way, the polymer concentration of the coacervate phase was calculated to be 21 wt% when 1 wt% polymer solution was heated to 37°C.

Partitioning of Trypan Blue and vitamin B_{12} to coacervate phase

A thermo-reversibile coacervate system might have an application in separation systems using temperature as a variable. To explore this possibility, the distribution of model solutes to the coacervate phase was evaluated. First, partitioning of a single solute (Trypan Blue or vitamin B_{12}) between coacervate and aqueous phase was

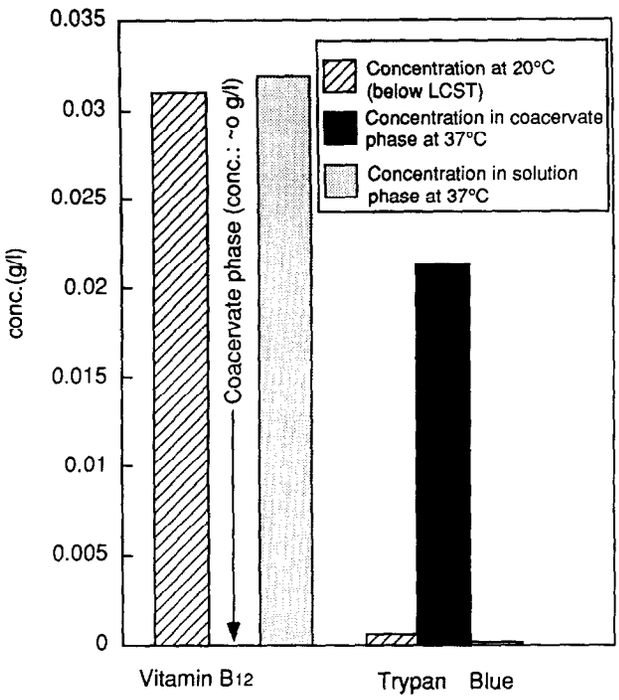

Figure 8 Aqueous two-phase separation of vitamin B_{12} and Trypan Blue in DPA-18.3 system at 37°C. Initial polymer conc., 1 wt%

estimated at the varying solute concentration to determine the partition coefficient at 37°C. DPA-18.3 was used in this study. As shown in *Figure 7*, partitioning of Trypan Blue to coacervate phase increased linearly with Trypan Blue concentration. On the other hand, vitamin B_{12} showed no partitioning to the coacervate phase under these conditions, as shown in *Figure 7*. Then, selective extraction of Trypan Blue and vitamin B_{12} was carried out. Obviously, as shown in *Figure 8*, significant enrichment of Trypan Blue into the coacervate phase was achieved by increasing the temperature from 20 to 37°C. Approximately 92% of dye molecules in the initial solution were transferred to the coacervate by this single partitioning procedure. In contrast, vitamin B_{12} was not detected in the coacervate phase.

Trypan Blue is classified in the group of direct dyes. Thus, it may have a considerably strong interaction with DPA polymer chain in the coacervate phase. On the other hand, vitamin B_{12} is highly water-soluble, and is insoluble in most organic solvents except alcohols. This high compatibility with water may contribute to vitamin B_{12} molecules remaining in the aqueous phase even after the formation of the coacervate phase. Although further systematic evaluation for many kinds of solutes with different structures is required, the solubility parameter of solutes is assumed to be an important determining factor in separation based on thermo-sensitive coacervate formation using a neutral *LCST* polymer such as DPA. These results of thermo-regulated partitioning suggest the feasibility of using this type of copolymer in a separation process including thermo-regulated aqueous two-phase partitioning and liquid chromatography.

REFERENCES

1 Bungenberg de Jong, H. G. 'Colloid Science' (Ed. H. R. Kruyt), Vol. 1, Elsevier, New York, 1949, and references therein
2 Dubin, D. L., Ross, D. T., Sharma, I. and Yagerlehner, B. E. in 'Ordered Media in Chemical Separations' (Eds. L. W. Hinze and W. D. Armstrong), *ACS Symp. Ser.* 1987, **342**, 162, and references therein.
3 Lenk, T. and Thies, C. in 'Coulombic Interaction in Macromolecular Systems' (Eds. A. Eisenberg and F. E. Bailey), *ACS Symp. Ser.* 1986, **302**, 240
4 Nakajima, A. and Sato, H. *Biopolymers* 1972, **11**, 1345
5 Tsuchida, E. and Abe, K. *Adv. Polym. Sci.* 1982, **45**, 1
6 Evreinova, T. N. 'Concentration of Matter and Action of Enzyme in Coacervates', Nauka, Moscow, 1966
7 Cox, A. B., Starcher, C. B. and Urry, W. D. *Biochim. Biophys. Acta* 1973, **317**, 209
8 Walter, H., Brooks, E. D. and Fisher, D. (Eds.) 'Partitioning in Aqueous Two-Phase Systems', Academic Press, London, 1985, and references therein
9 Abbott, N. L., Blankschtein, D. and Hatton, T. A. in 'Colloid–Polymer Interactions' (Eds. P. Dubin and P. Tong), *ACS Symp. Ser.* 1993, **532**, 53
10 Ganger, O. G. 'Microcapsulation Process and Applications', Plenum Press, New York, 1973, p. 1
11 Day, C. J. and Robb, D. I. *Polymer* 1981, **22**, 1530
12 Coleman, B. D. and Fox, T. G. *J. Polym. Sci. (A)* 1963, **1**, 3183
13 Harwood, H. J. and Ritchey, W. M. *J. Polym. Sci. (B)* 1964, **2**, 601
14 Heskins, M. and Guillet, E. J. *J. Macromol. Sci. Chem. (A)* 1968, **2**(8), 1441