Colloid Polym Sci 274:1013–1019 (1996)
© Steinkopff Verlag 1996

ORIGINAL CONTRIBUTION

J.S. Hong
T. Nakahara
H. Maeda
Y. Kikunaga
A. Kishida
M. Akashi

Cloud points and phase separation of aqueous poly(*N*-vinylacetamide) solutions in the presence of salts

Received: 15 February 1996
Accepted: 21 March 1996

J.S. Hong · T. Nakahara
Dr. H. Maeda (✉)
Department of Chemistry
Faculty of Science
Kyushu University
Fukuoka 812, Japan

Y. Kikunaga · A. Kishida · M. Akashi
Department of Applied Chemistry
and Chemical Engineering
Faculty of Engineering
Kagoshima University
Kagoshima 890, Japan

Abstract Aqueous poly(*N*-vinylacetamide) (PNVA) solution was found to exhibit the cloud point in the presence of salt. This cloud point was shown to correspond to a liquid–liquid phase separation, as confirmed when the PNVA-salt solutions were maintained at a temperature above the cloud point. The upper layer had a higher polymer concentration and a lower salt concentration than those in the lower layer. Thus interaction between PNVA and salts are repulsive. The lower critical solution temperatures were estimated to be 18 ± 1 °C for 1.25 molal $(NH_4)_2SO_4$ and 25 ± 1 °C for 0.76 molal Na_2SO_4. Divalent anions such as SO_4^{2-}, SO_3^{2-}, HPO_4^{2-} and CO_3^{2-} were effective in causing turbidity when examined at 25 °C. Dependence of the effect on the cationic species was similar to but significantly different from that for acetyltetraglycine ethylester. The cloud points of PNVA decreased linearly with the increase of the polymer concentration at a fixed salt concentration or with the increase of the salt concentration at a fixed polymer concentration. A parameter analogous to the salting-out constant was empirically derived from the dependencies of the cloud points on the concentrations of polymer and salt.

Key words Poly(*N*-vinylacetamide) – cloud point – phase separation – salting out – LCST(lower critical solution temperature)

Introduction

Although many biopolymers including proteins, nucleic acids and polysaccharides are polyelectrolyte, short-range interactions other than the long-range electrostatic interaction are important to understand the function and stability of the structure of these biopolymers. Thermodynamic study on solutions of nonionic water-soluble polymers is hoped to contribute to our understanding on various interactions in aqueous polymer solutions.

Phase behavior of polyethylene glycol in water has been known to complex [1–3]. Several models have been proposed for describing the phenomena on the basis of either water structure [4, 5], hydrogen bond [6], or conformational effects [7]. On addition of salts, cloud points were observed on polyvinylpyrrolidone [8], poly(vinyl alcohol-acetate) [9, 10], dextran [11], polymers of *N*-alkylacrylamides [12] and of *N*-alkoxylacrylamide [13], and nonionic surfactants [14–16]. Phase separation of the solutions containing two polymer components has received much attention in relation to the separation of biomaterial [11, 17–19]. Solution properties of nonionic polymers in aqueous media have been studied [20, 21]. Salt effects on the aqueous solution of nonionic solutes have been discussed on the basis of the Hofmeister series or lyotropic series [22, 23]. The studies included nonpolar molecules [24–26], amides and peptides [27–30].

Interaction of nonionic polymer with salts were examined also by chromatography [31]. The effects involve, however, 1) internal pressure change on the addition of salt, 2) the medium effect due to the perturbation of the hydrogen-bonded water structure or the effective removal of water molecules by hydration of ions and 3) the specific binding effect of salt to the solute.

Poly(*N*-vinylacetamide) (PNVA) [32–34], an important precursor to poly(vinylamine) [35], is one of the simplest water-soluble polymer and stable at neutral pH. The polymer is of interest for understanding the interaction of nonionic polymer with water due to an acetamide group in the side chain. The amide group is possibly both a donor and an acceptor for the hydrogen bond with water molecules, and the methyl group is involved in the hydrophobic interaction in aqueous media. The results will be compared with those of the salt effects on simple amides or peptides.

Experimental

Poly(*N*-vinylacetamide) was prepared by free radical polymerization of *N*-vinylacetamide which was obtained by the pyrolysis of *N*-(α-methoxyethyl) acetamide [32]. Sample C1 was a high molecular weight fraction obtained from sample C. Fractionation of sample C was carried out as follows. Acetone (total 62 ml) was successively added to 5wt% PNVA (100 ml) solution and the mixture was kept for 2 h at 25 °C. A liquid–liquid phase separation occurred and the two phases were separated with a separatory funnel. Acetone in the supernatant was removed under reduced pressure, and the remaining aqueous solution was lyophilized. The precipitates were collected and washed with acetone and dialyzed against distilled water followed by freeze-drying. Number-average molecular weights (Mn) of the two fractions, designated PNVA-C1 and C2, were $(3.2 \pm 0.05) \times 10^5$ and $(1.3 \pm 0.05) \times 10^5$, respectively. Unfractionated samples B and C had $Mn = (1.4 \pm 0.05) \times 10^5$ and $(1.3 \pm 0.05) \times 10^5$, respectively. Two samples Lot No B and C1 were used in the present study.

Inorganic salts were of reagent grade and used without further purification. Water used throughout the experiments was distilled twice over permanganate in a glass still. The polymer concentration was expressed either in c_p (gl^{-1}) for the osmotic pressure measurements, or w_p (wt%), or m_p (monomolal: residue mole/kgH$_2$O). Salt concentrations were expressed in molality m_s (mole/kgH$_2$O).

The cloud point of PNVA solution was spectrophotometrically determined at 500 nm using a Hitachi 101 spectrophotometer (Tokyo, Japan) with a temperature control cell housing. The temperature of sample was monitored with a thermoelectric thermometer. A Hitachi 056–3001 recorder was used to record simultaneously the absorbance and temperature. The rate of heating was about 0.7 °C/min.

The PNVA solutions containing salt were sealed in glass tubes (10 cm × 7 mm ID) and were allowed to reach equilibrum for at least 96 h at the temperatures higher than the cloud point. After the separation of upper and lower layers, the concentrations of the polymer and the salt were determined using HPLC. The HPLC system consisted of a Tosoh CCPM pump (Tokyo, Japan), a Rheodyne 7125 injector with a 100 μl sample loop, the tandem columns of 30.0 cm × 7.5 mm ID Tosoh TSK gel G4000PW and G5000PW protected by a TSK guard column, and a Tosoh RI-8012 detector. The analytical and guard columns were maintained at 35 °C. The 0.01 M sodium phosphate buffer (pH 7.0) was used as a mobile phase at the flow rate of 1.0 ml/min.

Results

Effect of salts on the cloud points of PNVA

The polymer solutions became turbid on heating when an inorganic salt was added. One example is shown in Fig. 1 for a solution of 1 wt% polymer in the presence of Na$_2$SO$_4$ at a heating rate of about 0.7 °C min^{-1}. In Fig. 1 the maximum ordinate values for different m_s are normalized to unity. At high polymer concentrations, the absorbance changed quite sharply and the cloud points cloud be determined umambiguously. The cloud points at low polymer concentrations were determined as a temperature corresponding to the begining of absorbance rise. The cloud point decreases sharply with the salt concentration m_s. In the example shown in Fig. 1, the cloud point T_c was 85 °C at 0.71 molal of Na$_2$SO$_4$ while it was 34 °C at 0.82 molal. Cloud points of sample C1 at different salt concentrations m_s are shown as functions of the polymer concentration m_p for four kinds of salt [Na$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, Li$_2$SO$_4$ and CsCl] in Figs. 2–5. As shown in these figures the cloud points decrease linearly with m_p. Similar results were obtained on another sample (Sample B) in the case of (NH$_4$)$_2$SO$_4$ (not shown). The linear relation is simply written as Eq. (1).

$$T_c = T_c^* - Am_p \qquad (1)$$

The slope A depended on m_s as shown in Fig. 6(A). The intercept T_c^* was extremely sensitive to a change in m_s as shown in Fig. 6(B). In the case of Na$_2$SO$_4$, values of the slope A (10 K monomolal^{-1}) were 37.4, 42.4 63.9, 57.3, 88.9 and 76.8 for m_s of 0.71, 0.74, 0.76, 0.78, 0.80 and

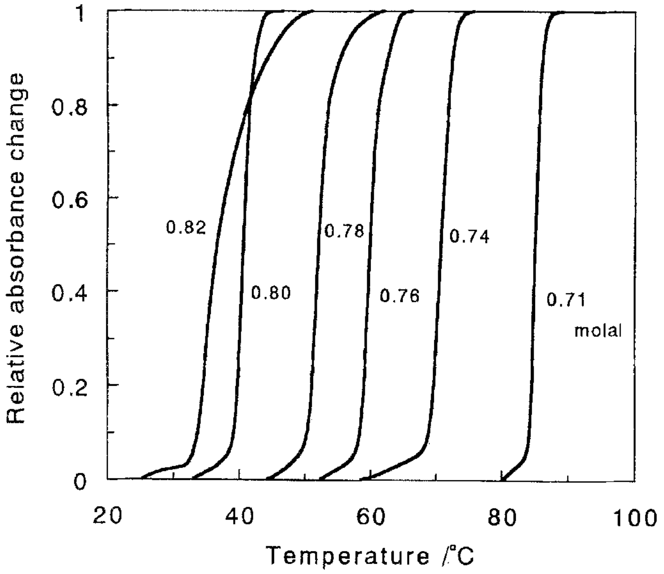

Fig. 1 Determination of the cloud points of PNVA solution at varying concentrations of Na_2SO_4. The 1% PNVA solution was heated at a heat rate of 0.7 °C/min. Sample C1

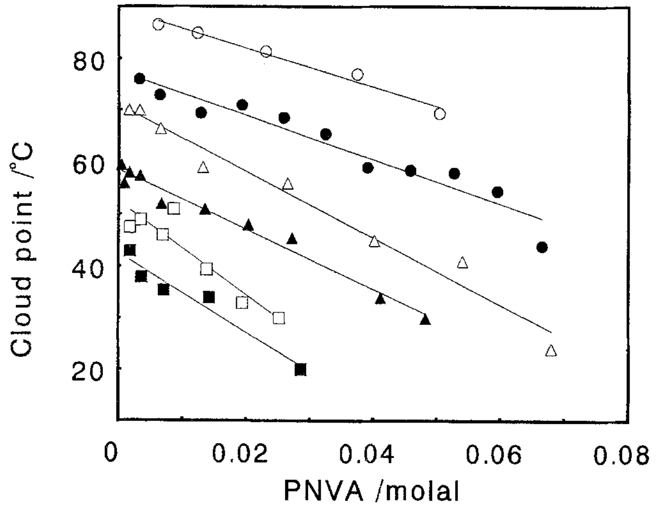

Fig. 2 Effect of Na_2SO_4 concentration (m_s) on the cloud point of PNVA. m_s (molal); 0.71 (○); 0.74 (●); 0.76 (△); 0.78 (▲); 0.80 (□); 0.82 (■)

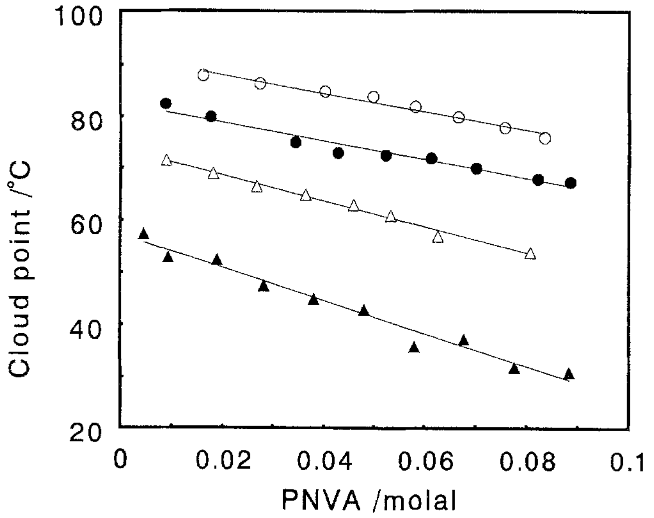

Fig. 3 Effect of $(NH_4)_2SO_4$ concentration (m_s) on the cloud point of PNVA. m_s (molal); 1.09 (○); 1.20 (●); 1.25 (△); 1.29 (▲)

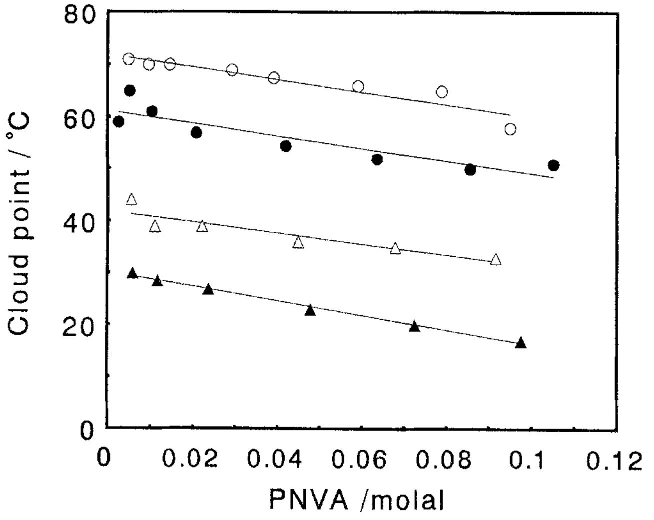

Fig. 4 Effect of Li_2SO_4 concentration (m_s) on the cloud point of PNVA. m_s (molal); 1.45 (○); 1.56 (●); 1.67 (△); 1.78 (▲)

0.82 molal, respectively. The following approximate relation was found experimentally for the slope A.

$$\log A = K_s m_s + B \;, \tag{2}$$

where $B = 0.4$, common to the four salts examined. In Fig. 6(A), the data are plotted according to Eq. (2). Different concentration scales for C_sCl and other salts should be noted. In this way the effects of a given kind of salts can be represented with its characteristic constant K_s. Values of K_s (molal^{-1}) were in the following order:

$$Na_2SO_4(3.0) > (NH_4)_2SO_4(1.8) > Li_2SO_4(1.0)$$
$$> C_sCl(0.17) \quad \text{(Series 1)}$$

Equation 1 gives the relation of the solubility at a temperature T as follows:

$$S = (T_c^* - T)/A \;. \tag{3}$$

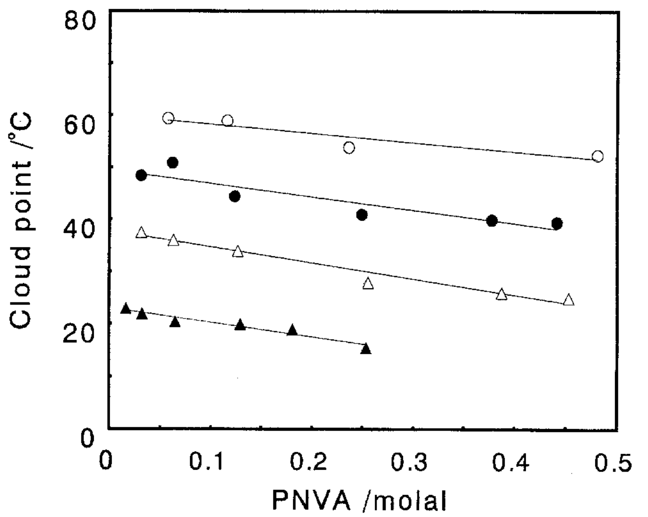

Fig. 5 Effect of CsCl concentration (m_s) on the cloud point of PNVA. m_s (molal); 5.76 (○); 6.08 (●); 6.24 (△); 6.41 (▲)

Combination of Eqs. (2) and (3) yields

$$\log S = [\log(T_c^* - T) - B] - K_s m_s$$
$$= \text{constant} - K_s m_s \,. \qquad (4)$$

If the constant term of Eq. (4) coincides with the solubility in water, Eq. (4) would be the Setschenow equation. For polymeric solutes, the Setschenow equation rarely holds. We take the parameter K_s to represent the salting-out power of a given salt. The constant term in Eq. (4) showed a weak m_s dependence which can be described with $\log m_s$.

We have examined the effectiveness of various salts to induce the turbidity at 25 °C, other than the four salts examined above, instead of determining the cloud point by heating the solutions.

The results will be summarized as follows. For the effectiveness of anions,

$SO_4^{2-}, HPO_4^{2-}, SO_3^{2-} \geq CO_3^{2-}$ (Series 2: sodium salts).

The result is in line with the Hofmeister series but for the lower members in the series starting from F^-, no phase separation occurred in the concentration ranges up to their solubility limits. The salts of divalent anions were more effective for producing the phase separation of PNVA than the chlorides. For Na_2SO_3, Na_2HPO_4, Na_2CO_3, $MgSO_4$ and $ZnSO_4$, the turbidites of 2 wt% polymer solutions at 25 °C were observed at the salt concentrations higher than 0.8, 0.8, 0.9, 1.2 and 1.4 molal, respectively. For the effectiveness of cations,

$Cs^+ > Fe^{3+} > Ca^{2+} > Rb^+ > K^+, Na^+, Li^+, Ba^{2+}$

(Series 3: chlorides)

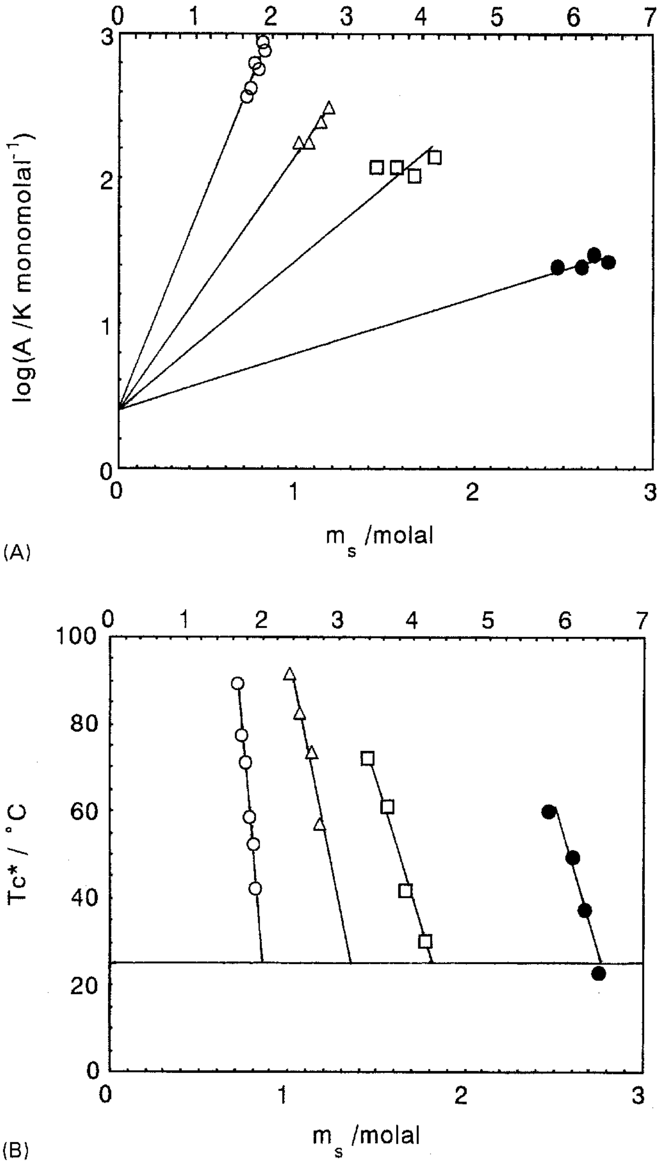

Fig. 6(A) Plots of $\log A$ of Eq. (1) as a function of salt concentration, m_s, for four salts. Na_2SO_4 (○); $(NH_4)_2SO_4$ (△); Li_2SO_4 (□); CsCl (●). The abscissa scale for CsCl is given on the upper side (**B**) Plots of intercept of Eq. (1), T_c^*, as a function of salt concentration, m_s, for four salts. Na_2SO_4 (○); $(NH_4)_2SO_4$ (△); Li_2SO_4 (□); CsCl (●). The abscissa scale for CsCl is given on the upper side

$Na^+ > Mg^{2+} \geq NH_4^+ \geq Li^+ \approx Zn^{2+}$ (Series 4: sulfates).

For the lower members in the lyotropic series starting from K^+, no phase separation occurred until the solubility limit was reached. Only $FeCl_3$ was effective for the turbidity of PNVA among the chlorides of transition metal ions examined in the present study; $MgCl_2$, $SrCl_2$, $BaCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$ and $AlCl_3$ were not effective at 25 °C to induce the turbidity of the solution of 2 wt% up to the solubility limit of each salt. Similar results were obtained for NaCl, KCl, NaF and $NaIO_3$. Also ineffective were NaBr, $NaNO_3$ and $NaClO_3$ up to 5 molal and for LiCl and $ZnCl_2$ up to 10 molal. Tetramethylammonium chloride induced the phase separation of PNVA, but its higher homologs, tetraethyl- and tetrabutylammonium chlorides were not effective.

Phase equilibria

As described above, the solutions became turbid when they were heated in the presence of a salt. The turbid portion migrated upward and the turbid top phase developed. However, the turbid part gradually turned to be transparent and liquid–liquid phase separation was attained when kept at a constant temperature for 4 days. From the observation, the top phase was judged to be polymer-rich. This apparently unusual result was reasonably understood in terms of uneven salt distributions shown below.

We have determined the concentrations of the polymer and the salt of the two separated solutions by means of HPLC. Separation of peaks PNVA and Na_2SO_4 on HPLC was complete in all the cases examined. The retention times of PNVA (C1 fraction) and Na_2SO_4 were about 13 and 23 min, respectively. Calibration curves for PNVA and Na_2SO_4 were made on the basis of the absolute amount (mole) of PNVA or Na_2SO_4 in a constant volume of solutions (25 μl). The linear relationship between the peak area and the amount of PNVA was achieved within the concentration ranges of less than 5wt%, and the linearity of peak area versus Na_2SO_4 amount was confirmed up to 2×10^{-5} mole. The separated phases were diluted, if necessary, so that the polymer and Na_2SO_4 concentrations were determined from the linear relation between peak area and their respective amounts.

In Fig. 7, the "phase diagram" at 0.76 molal Na_2SO_4 is shown together with the cloud points data. The cloud point line is not consistent with the low polymer concentration side of the coexistence curve. The latter is very close to the cloud point line obtained at 0.82 molal. This inconsistent result is caused not by the polydispersity of the sample but by the uneven distribution of the salt between the two phases. Data for the phase separation of PNVA in 0.76 molal Na_2SO_4 are summarized in Table 1. The 5wt% PNVA solutions were maintained at 30°, 35°, 40° or 45 °C in the presence of 0.76 molal initial concentration of Na_2SO_4 for 96 h. The upper phase contained higher concentrations of PNVA than those in the lower phase.

As shown in Table 1, the salt Na_2SO_4 was significantly concentrated in the lower phase. This is the reason why the polymer-rich phase was lighter than the other. The salt concentration of the lower phase is in the range of 0.86–0.90 molal, while that of the upper layer is about 0.56 molal.

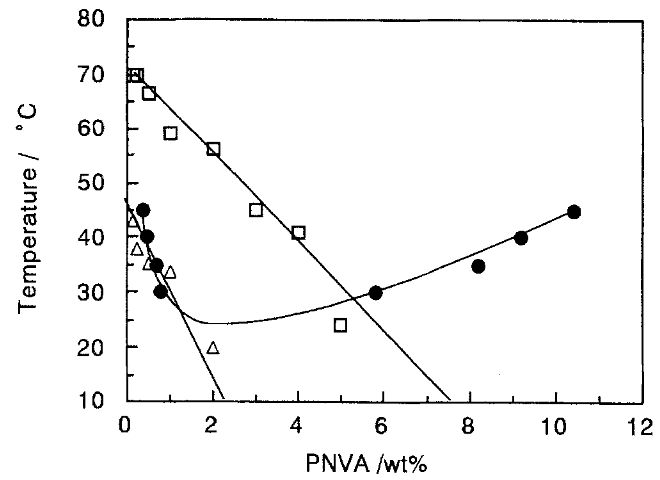

Fig. 7 Phase diagram for PNVA at 0.76 molal Na_2SO_4. Polymer concentrations of the two solutions in equilibrium are given by filled circles. Cloud point curves at 0.82 molal (△) and 0.76 molal (□) are also shown

Table 1 Phase separation of PNVA in the presence of 0.76 molal Na_2SO_4

Temperature /°C	PNVA/M		Na_2SO_4/M	
	Upper phase	Lower phase	Upper phase	Lower phase
30	0.61	0.074	0.56	0.86
35	0.90	0.071	0.58	0.90
40	1.02	0.044	0.54	0.90
45	1.15	0.036	0.56	0.87

Hence, it is not allowed to regard the salt solution as a pseudo "solvent". We should treat the phase separation of three component systems and the phase diagram should be expressed in a three-dimensional way. As shown in Table 1, the salt concentrations of both upper and lower solutions scarcely depend on the temperature and remain constant. On the other hand, the polymer concentration of the concentrated phase (the upper solution) increased with the temperature while that of the lower solution decreased corresponding to the binodal curve characteristic to LCST type. The lower critical solution temperature was 25 ± 1°C in Fig. 7.

In Fig. 8 the obtained "phase diagram" is shown for the case of $(NH_4)_2SO_4$ at two salt concentrations. The lower cirtical solution temperature T_c^* and the critical polymer concentration m_p^* were determined to be 18 ± 1 °C and 4.5 ± 0.5 wt% at 1.25 molal $(NH_4)_2SO_4$. At 1.29 molal $(NH_4)_2SO_4$, LCST was expected to be around 1 °C.

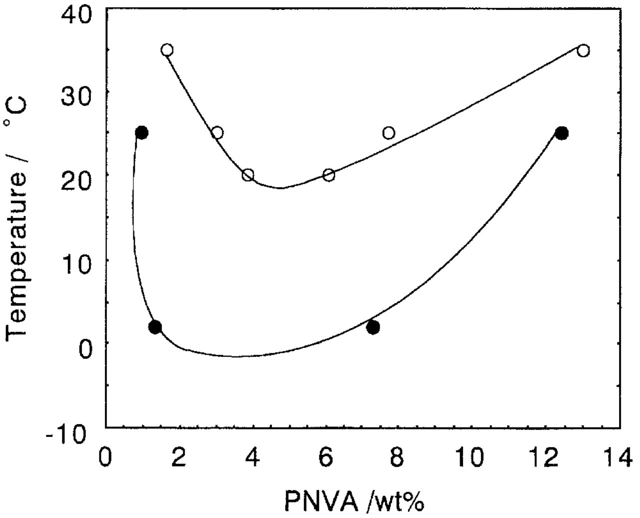

Fig. 8 Phase diagram for PNVA at 1.25 (○) or 1.29 (●) molal $(NH_4)_2SO_4$

Discussion

There are two possible ways of interpreting the phase separation or LCST found in the present study: the high-temperature side of the hourglass type as found for polyethylene glycol (PEG) in polar organic solvents or the low-temperature side of the closed loop type as found for PEG in water. If we expect the hydrogen bond between water and amide to play a central role, the latter picture will be more likely. The same mechanism proposed for PEG [6] can be applied to the present polymer. At low temperature, the hydrogen bond ethalpy contribution is significant and value of the effective χ parameter is negative and miscible. On raising temperature, the hydrogen bond becomes weak. As a result of the decrease of the attractive interaction with solvent a phase separation occurs (LCST). On further raising temperature, usual mixing entropy becomes dominant and the mixture is expected to become again miscible (UCST). This picture is in line with Hirshfelder–Stevenson–Eyring (HSE) mechanism [36].

It is to be noted however, that no LCST was observed in the absence of a salt. We expect that the LCST in water will be higher than 100 °C. The enhancement of the phase separation in the presence of several kinds of salt is known to be the salting-out effect, mainly exerted by divalent anions. The following three mechanisms are considered: a) the effect of salt on methyl groups or on aliphatic group of the polymer skeleton, b) the effect of the salt on amide, and c) the effect on the hydrogen bonding ability of water. An extensive study [27] on the effects of salts on the solubility of acetyltetraglycine ethyl ester (ATGEE) concluded that the effect (c) was insignificant.

Effects of various salts on the cloud points of poly (vinyl alcohol-acetate) copolymer were extensively studied [10]. The anions played an essential role and the results were in line with the Hofmeister series. It was thus suggested that the salts affected acetyl groups rather than hydroxyl groups of the copolymer. The effectiveness to induce the phase separation of aqueous polyethylenge glycol solutions was in the order $MgSO_4 > (NH_4)SO_4 \approx K_2HPO_4^{2-}$ [11]. Polyvinylpyrrolidone (PVP) was precipitated by the addition of $(NH_4)_2SO_4$ [8]. Uneven salt distribution was observed also on the phase separation of dextran solutions [11].

In the present study, the effect of anion species was not extensively examined, but the result is essentially in line with the Hofmeister series [22].

In the following we will discuss the effect of cations. As to cations, contrary to anions, the lyotropic series giving a similar characteristic rank ordering of cations for different kinds of measurements has not been established: alterations in the rank ordering are found for different measurements. The following orders for cations are proposed from the flocculation of inorganic colloid particles [23] (series 5) or from the precipitation of proteins [22] (series 6).

$Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+$ (Series 5)

$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} >$ guanidinium

(Series 6)

In the case of sulfates, we have $Na^+ > NH_4^+ > Li^+$ (series 1 or 4), which is inconsistent with the above series 5 and 6 and also with that found for acetyltetraglycine ethyl ester (ATGEE) [27]: $Li^+ > Na^+ > NH_4^+$. It is to be noted that this order is just the reverse of series 6 for proteins, although it is consistent with the order for the salting-out of euglobulin [27].

In the case of chlorides, the result for the present polymer,

$Cs^+ > Fe^{3+} > Ca^{2+} > Rb^+ > K^+, Na^+, Li^+, Ba^{2+}$

(Series 3)

is consistent with the cationic lyotropic series (series 5) but differs from series 6 and also from those for benzene [24]: $Ba^{2+} > Na^+ > K^+ > Rb^+ > NH_4^+ > Cs^+$ (series 7) and for nonpolar group of several amides [30]: $Ca^{2+} > Na^+, K^+ > Li^+ > Cs^+$ (series 8). Series 3 is consistent with the result for ATGEE [27]:

$Cs^+ > Na^+, K^+ > NH_4^+ > Li^+ > Ba^{2+}$, (Series 9)

which indicates that the salt effect on amide group is dominant compared with that on nonpolar groups in the case of the present polymer. However, both series 3 and 9 are inconsistent with the result for amide group [29]: $\mathrm{Li^+ > K^+, Cs^+ > Na^+ > Ca^{2+}}$.

The present polymer has more similarity to oligopeptides than to globular proteins with respect to the interaction with salts.

The effect of salt on amides is superposition of the internal pressure effect determined mainly by the volume of amides and the direct interaction between them. The difference between series 3 or 9 and series 7 or 8 will be attributed to the direct interaction. Binding tendency of cations to acrylamide gel column is given as follows [31]:

$$\mathrm{Ba^{2+}, Ca^{2+} > Mg^{2+} \gg Cs^+ > Rb^+ > Na^+ > K^+ > Li^+}.$$

The salting-out effect of a cation due to the incompressible volume of amides is cancelled, to some extent, by the salting-in effect on amide group if it binds the cation.

Acknowledgment The authors thank Ms Rie Kakehashi for the measurement of the osmotic pressure.

References

1. Malcolm GN, Rowlinson JS (1957) Trans Faraday Soc 53:921
2. Saeki S, Kuwahara N, Nakata M, Kaneko M (1976) Polymer 17:685
3. Malmsten M, Linse P, Zhang KW (1993) Macromolecules 26:2905
4. Kjellander R, Florin E (1981) J Chem Soc, Faraday Trans 1, 77:2053
5. Kjellander R (1982) J Chem Soc, Faraday Trans 2, 78:2025
6. Goldstein RE (1984) J Chem Phys 80:5340
7. Karlström GJ (1985) J Phys Chem 89:4962
8. Jingensons B (1951) J Polym Sci 8:519
9. Nord FF, Bier M, Timasheff N (1951) J Am Chem Soc 73:289
10. Saito S (1969) J Polymer Sci, Part 1, 7:789
11. Albertsson PÅ (1986) In: Partition of Cell Particles and Macromolecules 3rd Ed. John Wiley & Sons, New York
12. Ito S, Fujishige S (1991) Bull Res Inst Polym Text Jpn 167:5
13. Ito S, Fujishige S (1991) Bull Res Inst Polym Text Jpn 167:13
14. Schott H, Han SK (1975) J Pharm Sci 64:658
15. Schott H, Royce AE (1984) J Pharm Sci 73:793
16. Schott H, Royce AE, Han SK (1984) J Colloid Interface Sci 98:196
17. Haynes CA, Beynon RA, King RS, Blanch HW, Prausnitz JM (1989) J Phys Chem 93:5612
18. Linse P, Björling M (1991) Macromolecules 24:6700
19. Guan Y, Lilley TH, Treffry TH (1993) Macrmolecules 26:3971
20. Munk P, Aminabhavi TM, Williams P, Hoffman DE, Chmelin M (1980) Macromolecules 13:871
21. Polik WF, Burchard W (1983) Macromolecules 16:978
22. Creighton ThE (1993) In: Proteins 2nd Ed. WH Freeman Co, NY, p 156
23. Hiementz PC (1977) In: Principles of Colloid and Surface Chemistry. Marcel Dekker, New York, p 435
24. McDevit WF, Long FA (1952) J Am Chem Soc 74:1773
25. Long FA, McDevit WF (1952) Chem Rev 51:119
26. Deno NC, Spink CH (1963) J Phys Chem 67:1347
27. Robinson DR, Jencks WP (1965) J Am Chem Soc 87:2470
28. Schrier EE, Schrier EB (1967) J Phys Chem 71:1851
29. Nandi PK, Robinson DR (1972) J Am Chem Soc 94:1299
30. Nandi PK, Robinson DR (1972) J Am Chem Soc 94:1308
31. von Hippel PH, Peticolas V, Schack L, Karlson L (1973) Biochemistry 12:1256
32. Akashi M, Yashima E, Yamashita T, Miyauchi N (1990) J Polym Sci A, Polym Chem 28:3487
33. Ben-Ishai D, Giger R (1965) Tetrahedron Lett 50:4523
34. Dawson DJ, Gless RD, Wingard RE Jr (1976) J Am Chem Soc 98:5996
35. Stackman RW, Summerville RH (1985) Ind Eng Chem Prod Res Dev 24:242
36. Hirschfelder J, Stevenson D, Eyring H (1937) J Chem Phys 5:896