Cloud-Point Measurements for (Water + Poly(ethylene glycol) + Salt) Ternary Mixtures by Refractometry Method

Mohsen Mohsen-Nia,*,† Hossein Rasa,† and Hamid Modarress‡

Thermodynamic Research Laboratory, Kashan University, Kashan, Iran, and Department of Chemical Engineering, Amir-Kabir University of Technology, Tehran, Iran

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The cloud-point temperatures and phase separation of (water + poly(ethylene glycol) + salt) ternary systems were studied by the refractometry method. The salts were K_3PO_4, K_2HPO_4, Na_2HPO_4, or Na_2CO_3. The phase separation was also observed by visual inspection. Differences between the measured cloud point using the refractometry method, and visual inspection was up to ±1 °C. The measured cloud-point temperatures are compared with those obtained by viscometry and laser beam scattering methods. These comparisons indicate complete agreement between the different methods for cloud-point temperature measurements for the (water + poly(ethylene glycol) + salt) ternary system. The salt-included liquid−liquid separation is studied by using the Flory−Huggins polymer solution theory. A simple linear temperature composition-dependent interaction parameter (χ) was applied for correlation of the measured cloud-point temperatures. The comparison between measured and correlated cloud-point temperatures indicates that average absolute deviation is less than 0.61 °C. Therefore, the Flory−Huggins model was successfully applied to determine the phase separation conditions of the (water + poly(ethylene glycol) + salt) ternary systems.

Introduction

Continuous economical and environmental interest for developing the industrial extraction, biochemistry, and biotechnology purification processes have caused a lot of investigations on the separation techniques such as purification of proteins and enzymes extraction.[1] Aqueous two-phase systems have the widespread applications for purification of biological material.[2,3] The systems are composed of two incompatible polymers in water solution (e.g., dextran and poly(ethylene glycol) (PEG)) or an aqueous solution of one polymer (e.g., PEG) in concentrated salt (e.g., phosphate) solution.[4,5]

Aqueous two-phase systems for laboratory-scale separations are most often composed of PEG and dextran. Dextran is a relatively expensive polymer, and for large-scale purification (e.g., industrial-scale enzyme extraction), PEG−salt mixtures are more commonly used.[6] However, the PEG−salt mixtures have the disadvantage of low solubility for amphiphilic proteins, which have a high tendency to aggregate in the presence of high salt concentrations.

The use of thermoseparating polymers such as PEG in aqueous two-phase systems has been introduced.[7,8] When such polymers are heated above a critical temperature (i.e., the cloud point), the solubility of the polymer will decrease, and a system composed of water and a polymer phase is formed. This makes it possible to perform temperature-induced phase separation whereby a target protein can be separated from the polymer and recovered in a “clean” water phase.[7] Recycling of polymers in aqueous two-phase systems has been shown with thermoseparating polymers.[9] Many thermoseparating polymers contain ethylene oxide groups. PEG is also a thermoseparating polymer,[9] but its cloud point is too high (above 100 °C) for separation of labile biomolecules. The cloud point of the (water + PEG + salt) ternary system depends on the polymer and salt concentrations of the solution.[10]

The cloud point of aqueous polymer was measured by different physical techniques. The cloud-point phenomena can be detected by turbidimetry, neutron scattering, microcalorimetric study, and low-angle light scattering measurements.[11]

In (water + PEG + salt) systems, an aqueous two-phase polymer mixture is produced by adding the small amounts of inorganic salts to the aqueous homogeneous mixture. Then, the aqueous polymer mixture is separated into two immiscible water-rich phases. The phase separation is occurred at finite temperature (cloud-point temperature). The cloud-point temperature of a system was determined by heating the sample in a water bath. The cloud-point temperature was defined as the temperature at the time when the sample was visually observed to start to get cloudy. The clouding was closely followed by a macroscopic phase separation.[12]

The cloud-point temperature and partitioning can be influenced by changing polymers, polymer molecular mass, and addition of salts.[10,11] In recent years, many research groups focused on the measurements of the phase equilibrium and cloud-point data for aqueous polymer−salt systems. The cloud point can be affected by the kind of salts, the molecular mass of the polymer, and the composition of the system.[11,12]

In this work, we have studied the effect of the salt and polymer concentrations and kind of salts on the cloud point of (water + PEG + salt) ternary systems by refractometry. The results of measurements indicated that the use of the refractometry method for cloud-point measurements of a (water + PEG + salt) ternary system is quite successful. The obtained results were compared with results of other methods such as viscometry and laser beam scattering methods.[11] The comparisons indicate the good applicability of the refractometry method as the simple method for cloud-point measurements of (water + PEG + salt) ternary systems.

* Corresponding author. E-mail: m.mohsennia@kashanu.ac.ir.
† Kashan University.
‡ Amir-Kabir University of Technology.

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Experimental Section

Materials and Instruments. The salts (K_3PO_4, K_2HPO_4, Na_2HPO_4, and Na_2CO_3) and PEG ($\langle M_n \rangle = 10000$, polydispersity index is 1.1) were obtained from Merck Germany. All chemicals were used without further purification. Before use, the salts were dried in an oven at a temperature of 393.15 K for 24 h. Double-distilled water was used for making the solutions. The solutions were made by mass using a Sartorius analytical balance (model A200S) with an uncertainty of ± 0.0001 g. The refractive index measurements were done by Abbe Refractometer (Atago 1T/4T, made in Japan) equipped with a digital thermometer with an uncertainty of ± 0.05 °C. The refractometer was frequently calibrated by using double-distilled water. Water was circulated into the instrument through a thermostatically controlled bath maintained constant to ± 0.1 °C. The mixtures were directly injected into the prism assembly of the instrument using a Hamilton syringe from the stock solution stored at the working temperature to avoid evaporation. The refractive index measurements were done after the liquid mixtures attained the constant temperature of the refractometer. This procedure was repeated at least three times. The average of these readings was taken for the refractive index values. The maximum deviations from the average value were always < 0.1 %. The refractive index values are accurate to 0.0002 units.

Correlating Model. For the theoretical study of solvent−polymer systems, the well-known Flory−Huggins lattice theory provides the most useful model. This model considers the polymer solution as a lattice of sites, each of which is occupied by either a solvent molecule or a polymer segment.[13−16] Although the assumptions involved in the Flory−Huggins theory restrict its applications, it is particularly suitable for evaluation of the thermodynamic properties of binary [water (1) + PEG (2)] and ternary [water (1) + PEG (2) + salt (3)] systems.[14,15]

For a binary mixture of solvent (1) + polymer (2), the Gibbs free energy of mixing in the Flory−Huggins theory can be expressed as[13, 14]

$$\frac{\Delta G}{RT} = \phi_1 \ln \phi_1 + (\phi_2/r) \ln \phi_2 + \chi(T, \phi_2)\phi_1\phi_2 \tag{1}$$

where ϕ_i is the volume fraction of component i, r is the segment number of the polymer and is expressed as polymer to solvent molar volume ratio. $\chi(T, \phi_2)$ is the solvent−polymer interaction parameter. On the basis of this theory, the chemical potential change of the solvent (1) upon mixing is[11,13]

$$\frac{\Delta\mu_1}{RT} = \left[\frac{\partial(\Delta G/RT)}{\partial n_1}\right]_{T,P,n2} = \ln \phi_1 + (1 - 1/r)\phi_2 + \chi(T, \phi_2){\phi_2}^2 \tag{2}$$

The interaction parameter $\chi(T, \phi_2)$ has considerable importance in the exact evaluation of thermodynamic behavior of solvent−polymer mixtures. Therefore, from experimental measurements,[17] various functionality has been proposed to express the temperature and volume fraction dependency of $\chi(T, \phi_2)$ in the following form:[11,18−20]

$$\chi(T, \phi_2) = E(\phi_2)F(T) \tag{3}$$

For sufficiently small concentrations, $E(\phi_2)$ can be represented by the series expansion:[19,20]

$$E(\phi_2) = E_0 + E_1\phi_2 + ... \tag{4}$$

The various temperature functions have been presented for $F(T)$;[11,17,19] however, for a limited temperature range $F(T)$can be expressed by a linear simple function as[11]

$$F(T) = F_0 + F_1T \tag{5}$$

Using $F(T)$ and $E(\phi_2)$ in the equation $\chi(T, \phi_2)$, substituting for $\chi(T, \phi_2)$ in the Flory−Huggins equation (eq 1), and considering the phase separation condition $\partial^2\Delta G/\partial{\phi_1}^2 < 0$, the following equation will be obtained:[19]

$$\frac{1}{1-\phi_2} + \frac{1}{r\phi_2} - 2\chi(T, \phi_2) - \phi_2\frac{\partial\chi(T, \phi_2)}{\partial\phi_2} < 0 \tag{6}$$

Upon substituting eqs 4 and 5 into eq 6, one obtains

$$\frac{1}{1-\phi_2} + \frac{1}{r\phi_2} - \left[2E(\phi_2) + \phi_2\frac{\partial E(\phi_2)}{\partial\phi_2}\right]F(T) < 0 \tag{7}$$

For a binary mixture of solvent (1) and polymer (2), the above inequality indicates that the phase separations occurs if $[2E(\phi_2) + \phi_2(\partial E(\phi_2)/\partial\phi_2)]$ and $F(T)$ have the same sign. For a ternary mixture of solvent (1) + polymer (2) + salt (3), the chemical potential change of solvent upon mixing can be obtained from the extended Flory−Huggins equation for the binary mixtures as[13,22]

$$\Delta\mu_1 = RT(\ln \phi_1 + (1 - 1/r_2)\phi_2 + (1 - 1/r_3)\phi_3 + \chi_{12}\phi_2(1 - \phi_1) + \chi_{13}\phi_3(1 - \phi_1) - \chi_{23}\phi_2\phi_3/r_2) \tag{8}$$

where ϕ_1, ϕ_2, and ϕ_3 are the volume fractions of solvent, polymer, and salt, respectively. Also, χ_{12}, χ_{13}, and χ_{23} are the interaction parameters between the components in the mixture, and r_2 and r_3 are the segment numbers defined for polymer and salt components, respectively.

Equation 8 can be presented in a simple form by introducing ϕ_2^* as the sum of salt and polymer volume fractions:[22,23]

$$\phi_2^* = \phi_2 + \phi_3 \tag{9}$$

Also, the mole fraction of polymer (2) and salt (3) are defined as

$$x_2^* = \frac{x_2}{x_2 + x_3} \quad x_3^* = \frac{x_3}{x_2 + x_3} \tag{10}$$

r_2^* was introduced as

$$r_2^* = x_2^*r_2 + x_3^*r_3 \tag{11}$$

Finally, by using eqs 9 and 11, eq 8 is presented as

$$\Delta\mu_1 = RT(\ln \phi_1 + (1 - 1/r_2^*)\phi_2^* + (1 - 1/r_3)\phi_3 + (\chi_{12}\phi_2 + \chi_{13}\phi_3)(1 - \phi_1) - \chi_{23}\phi_2\phi_3/r_2) \tag{12}$$

At cloud point, the equilibrium condition of the ternary mixtures leads to the following equation:

$$\ln \phi_1 + (1 - 1/r_2^*)\phi_2^* + \chi_{12}^*(T,\phi_2^*)\phi_2^{*2} = 0 \tag{13}$$

where $\chi_{12}^*(T, \phi_2^*)$is introduced in the following form:[22]

$$\chi_{12}^*(T, \phi_2^*) = ((\chi_{12}\phi_2 + \chi_{13}\phi_3)(1 - \phi_1) - \chi_{23}\phi_2\phi_3/r_2)/(1 - \phi_1)^2 \tag{14}$$

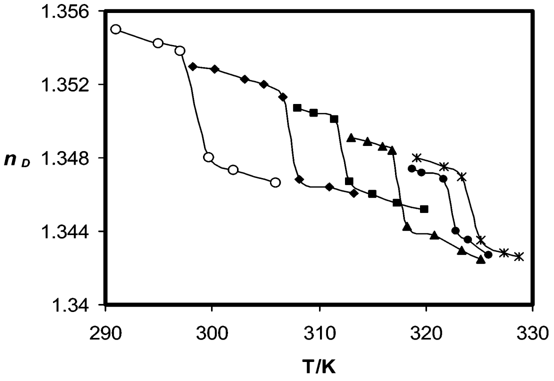

Figure 1. Effect of K_3PO_4 concentration on refractive index of {water (1) + PEG (2) + K_3PO_4 (3)} ternary systems (w_2/w_1= 0.0711). The points are experimental and lines are drawn to show the experimental trend. The points indicate the weight percent of K_3PO_4; ○♀ 0.0800; ◆, 0.0728; ■, 0.0683; ▲, 0.0639; ●, 0.0600; *, 0.0589.

$\chi^*_{12}(T, \phi^*_2)$ is an important parameter in ternary mixtures, and it can be evaluated by using the relative vapor pressure and osmotic pressure measurements.[17] Attempts have been made to express a reasonable form for $\chi^*_{12}(T, \phi^*_2)$, and a number of expressions have been presented.[11,18,19]

Similar to the expression for binary mixtures (eq 3), $\chi^*_{12}(T, \phi^*_2)$ is considered as separate functions of volume fraction of polymer and salt $E(\phi^*_2)$ and temperature $F(T)$:

$$\chi^*(T, \phi^*_2) = E(\phi^*_2)F(T) \tag{15}$$

where ϕ^*_2 is given by eq 9. The functions $E(\phi^*_2)$ and $F(T)$ are expressed by polynomial expansion of ϕ^*_2 and T, respectively, as

$$E(\phi^*_2) = E_0 + E_1\phi^*_2 + E_2\phi^{*2}_2 + E_3\phi_2^{*3} + ... \tag{16}$$

$$F(T) = F_0 + F_1T + F_2T^2 + F_3T^3 + ... \tag{17}$$

By substitution of eqs 15 to 17 in eq 13 and neglecting the higher order terms for the sake of simlification, the following equation is obtained:

$$\ln \phi_1 + (1 - 1/r^*_2)\phi^*_2 + (E_0 + E_1\phi^*_2)(F_0 + F_1T)\phi^{*2}_2 = 0 \tag{18}$$

This equation can be used for phase separation prediction of [water (1) + PEG (2) + salt (3)] ternary mixtures into two or more phases at certain temperature and compositions.

The measured cloud points (eq 18) for [water (1) + PEG (2) + salt (3)] ternary mixtures are correlated by using the nonlinear regression analysis of Nelder−Mead[24] for minimizing the average absolute deviation (AAD) in the cloud-point temperature as expressed by the following equation:

$$\mathrm{AAD} = \frac{1}{N}\sum_{i=1}^{N}|T_i^{\mathrm{exp}} - T_i^{\mathrm{cal}}| \tag{19}$$

where N is the number of data points, T_i^{exp} and T_i^{cal} are respectively the experimental and calculated cloud-point temperatures. The coefficients E_0, E_1, F_0, and F_1 are evaluated for the minimum AAD of studied mixtures. From a physical interpretation viewpoint, same as for the binary systems, the minimum AAD as well as the following relation are satisfied:

$$\frac{1}{1 - \phi^*_2} + \frac{1}{r\phi^*_2} - (2E_0 + 3E_1\phi^*_2)(F_0 + F_1T) < 0 \tag{20}$$

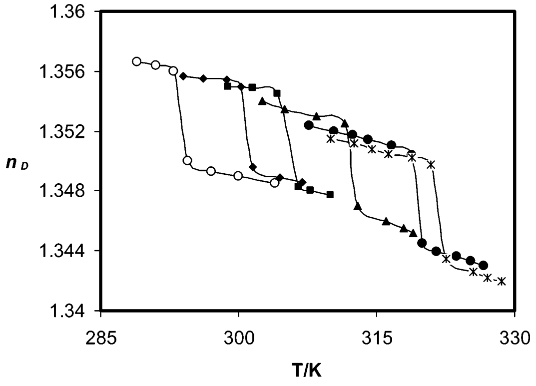

Figure 2. Effect of Na_2HPO_4 on refractive index of {water (1) + PEG (2) + Na_2HPO_4 (3)} ternary systems (w_2/w_1= 0.1114). The points are experimental and lines are drawn to show the experimental trend. The points indicate the weight percent of Na_2HPO_4; ○, 0.0600; ◆, 0.0562; ■, 0.0532; ▲, 0.049; ●, 0.0459; *, 0.0444.

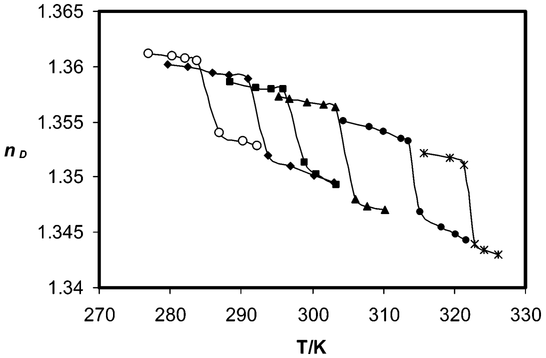

Figure 3. Effect of K_2HPO_4 on refractive index of {water (1) + PEG (2) + K_2HPO_4 (3)} ternary systems (w_2/w_1= 0.1222). The points are experimental and lines are drawn to show the experimental trend. The points indicate the weight percent of K_2HPO_4; ○, 0.0780; ◆, 0.0731; ■, 0.0696; ▲, 0.0638; ●, 0.0577; *, 0.0520.

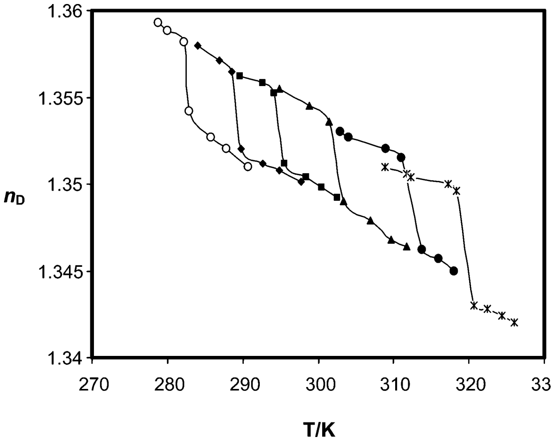

Figure 4. Effect of Na_2CO_3 on refractive index of {water (1) + PEG (2) + Na_2CO_3 (3)} ternary systems (w_2/w_1= 0.1000). The points are experimental and lines are drawn to show the experimental trend. The points indicate the weight percent of Na_2CO_3; ○, 0.0518; ◆, 0.0490; ■, 0.0465; ▲, 0.0435; ●, 0.0385; *, 0.0353.

Results and Discussion

The cloud-point temperatures of the ternary systems of [water (1) + PEG (2) + salt (3)] were measured at different concentrations of polymer and salts by the refractometry method. The variation of refractive index of the studied ternary systems versus temperature is presented in Figures 1 to 4. According to these figures, on increasing temperature, the refractive index of solutions shows a slow decreasing trend, but at the cloud point

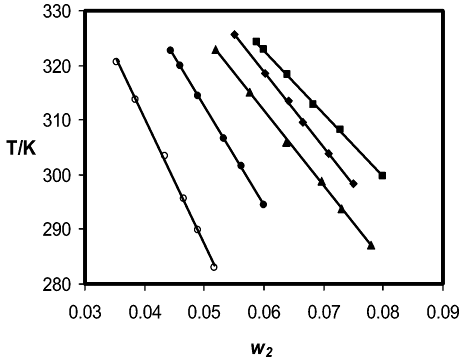

Figure 5. Effect of salt type on the cloud-point temperature of {water (1) + PEG (2) + salt (3)} ternary systems as presented in Table 1. The points are experimental and the line indicates the correlated results of this work by eqs 15 to 18. The points indicate the salt type: ○, Na_2CO_3; ●, Na_2HPO_4; ▲, K_2HPO_4 (w_2/w_1= 0.1222); ◆, K_2HPO_4 (w_2/w_1= 0.1117); ■, K_3PO_4.

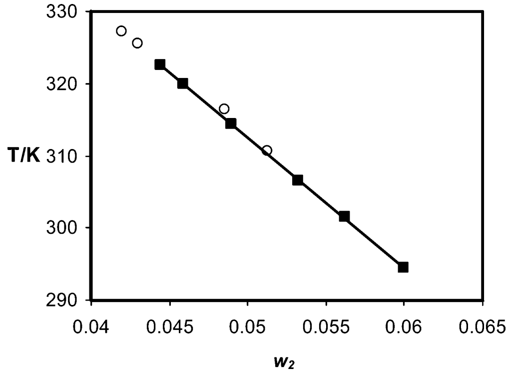

Figure 6. Comparison between measured cloud-point temperature for {water (1) + PEG (2) + Na_2HPO_4 (3)} ternary system (w_2/w_1= 0.1114) obtained by ■ this work and ○ laser beam scattering method.[11] The line indicates the correlated results of this work by eqs 15 to 18.

Table 1. Measured Cloud Point for the Studied [Water (1) + PEG (2) + Salt (3)] Ternary Systems

(Water + PEG + K_3PO_4) System, w_3/w_1 = 0.0488						
w_2	0.1503	0.1617	0.1816	0.1900	0.2005	0.2250
T/K	329.0	327.6	325.5	324.2	323.2	320.3
(Water + PEG + K_3PO_4) System, w_2/w_1 = 0.0711						
w_3	0.0589	0.0600	0.0639	0.0683	0.0728	0.0800
T/K	324.2	322.8	318.3	312.9	308.2	299.7
(Water + PEG + Na_2HPO_4) System, w_2/w_1 = 0.1114						
w_3	0.0444	0.0459	0.0490	0.0532	0.0562	0.0600
T/K	322.6	320.0	314.7	306.5	301.5	291.2
(Water + PEG + K_2HPO_4) System, w_2/w_1 = 0.1222						
w_3	0.0520	0.0577	0.0638	0.0696	0.0731	0.0780
T/K	322.8	315.2	306.0	298.9	293.7	278.0
(Water + PEG + K_2HPO_4) System, w_2/w_1 = 0.1117						
w_3	0.0551	0.0602	0.0641	0.0665	0.0708	0.0750
T/K	325.7	318.5	313.6	309.7	303.9	298.3
(Water + PEG + Na_2CO_3) System, w_2/w_1 = 0.1000						
w_3	0.0353	0.0385	0.0435	0.0465	0.0490	0.0518
T/K	320.6	313.8	303.3	295.5	289.8	282.9

a sharp decrease in refractive index is observed. After the cloud point, the refractive indexes of the systems again show a slow decreasing trend. The sharp decrease in refractive index at the cloud point can be explained by sudden appearance of particles of the new phases.

The measured cloud points for [water (1) + PEG (2) + salt (3)] ternary mixtures, for salt (3): K_3PO_4, K_2HPO_4, Na_2HPO_4, or Na_2CO_3 are presented in Table 1. The phase separation was also observed by visual inspection of the studied systems. The measured cloud-point temperatures of the [water (1) + PEG (2) + salt (3)] ternary mixtures indicate the existence of a lower critical solution temperature (LCST).

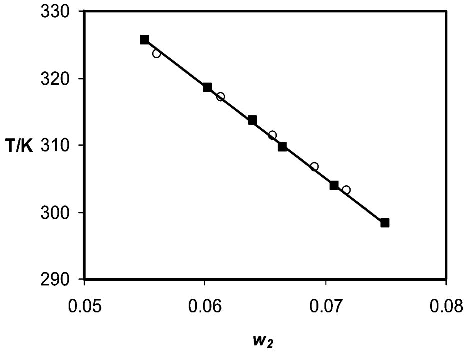

Figure 7. Comparison between measured cloud-point temperature for {water (1) + PEG (2) + K_2HPO_4 (3)} ternary system, (w_2/w_1= 0.1117) obtained by ■ this work and ○ viscosity method.[11] The line indicates the correlated results of this work by eqs 15 to 18.

Table 2. Calculated Parameters for the Studied [Water (1) + PEG (2) + Salt (3)] Ternary Systems

		adjustable parameters				
salt	w_2/w_1	E_0	E_1	F_0	F_1	AAD/K
K_3PO_4	0.0711	−0.03851	1.51044	3.09538	0.04170	0.42
Na_2HPO_4	0.1114	−0.10164	1.35163	−8.71695	0.08881	0.25
K_2HPO_4	0.1222	−0.08557	1.03468	−8.17710	0.10962	0.43
K_2HPO_4	0.1117	−0.09022	1.33379	−0.26912	0.05619	0.10
Na_2CO_3	0.1000	−1.47430	22.2101	1.63185	0.00168	0.34

		adjustable parameters				
salt	w_3/w_1	E_0	E_1	F_0	F_1	AAD/K
K_3PO_4	0.0488	1.97970	2.64860	−1.48467	0.00543	0.55

Table 3. Effect of Sign of the Different Terms and Parameter F_1 of the Interaction Parameter $\chi(T, \phi_2^*)$ on the Type of Phase Diagram

$2E_0 + 3E_1\phi_2^*$	$F_0 + F_1T$	F_1	type of phase diagram
>0	>0	>0	LCST
>0	>0	<0	UCST
<0	<0	>0	UCST
<0	<0	<0	LCST

Cloud-point temperatures measured by refractometry method were in agreement with the visual inspections. The differences between the measured cloud-point temperatures using the refractometry method and visual inspection was up to ± 1 °C. The reproducibility and accuracy of the measured cloud-point temperatures was estimated at ± 0.1 °C.

The linear variations of the obtained cloud-point temperatures versus salt concentration are shown in Figure 5. In Figures 6 and 7, the measured cloud points in this work were compared with those obtained by using the laser beam scattering and viscometry methods.[11] Comparisons indicate that the differences between measured cloud point by different methods are less than ± 1.0 °C.

In Table 2, the coefficients E_0, E_1, F_0, and F_1 of eqs 16 and 17 evaluated from regression method and the AAD are reported. The values of these coefficients are consistent with the phase separation condition expressed by eq 20 that is

$$(2E_0 + 3E_1\phi_2^*)(F_0 + F_1T) > 0 \tag{21}$$

which indicate the existence of LCST for the studied mixtures. Both lower and upper critical solution temperatures (LCST and UCST) may exist in principle. To explain this, we consider the sign of $(2E_0 + 3E_1\phi_2^*)$, $(F_0 + F_1T)$, and slope of $F(T)$, F_1 as presented in Table 3 for two kinds of phase diagrams. However, one may observe only either LCST or UCST behavior in certain experimental temperature ranges.[19]

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