

# Liquid–Liquid Equilibrium of Poly(ethylene glycol) 2000 + Diammonium Hydrogen Citrate + Water System at Different Temperatures

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**ABSTRACT:** Phase diagrams and liquid–liquid equilibrium (LLE) data of the aqueous poly(ethylene glycol) (PEG-2000) + diammonium hydrogen citrate system (aqueous two-phase system) has been determined experimentally at (298.15, 303.15, 308.15, 313.15, and 318.15) K. The effects of temperature on the binodal curve and tie-lines have been studied. The binodal curve was fitted to an empirical equation relating the concentrations of PEG 2000 and diammonium hydrogen citrate, and the coefficients were estimated for the respective temperatures. Tie line compositions were estimated and correlated using Othmer–Tobias and Bancroft equations, and the parameters are reported. The effect of temperature on the phase-forming ability in the investigated system has been studied based on a salting-out coefficient; obtained from fitting the binodal data to a Setschenow-type equation for each temperature. The effective excluded volume values were also calculated from the binodal data.

## INTRODUCTION

In the biotechnological process, downstream processing plays an important role. Conventional techniques for the separation, purification and concentration of biomolecules have some disadvantages such as insufficient selectivity, high energy consumption and possibility of denaturation of biomolecules when organic solvents are used in extraction processes. Most of these difficulties can be overcome by conducting the bio-separations in aqueous two-phase systems. Aqueous two phase systems (ATPS) are formed when two incompatible polymers or one polymer and a salt are mixed together in water at appropriate proportions and at particular temperature, separate into two distinguish phases. ATPS offers a good environment for the separation of biomolecules, due to its high water content in both phases; low interfacial tension and also polymers have a stabilizing influence on the particle structures and the biological activities.<sup>1</sup> ATPS also find wide applications in environmental remediation like removal of color from textile plant wastes, metal ions and volatile organic pollutants from the environment, and aromatics from crude oil. ATPS have also been used for the extraction of metallic ions, extractive crystallization of inorganic salts, recovery of nanoparticles, and extraction of sulfide minerals.<sup>1</sup>

Poly(ethylene glycol) (PEG) is widely used in aqueous two phase separation of biomolecules,<sup>2–7</sup> because it is low cost, nontoxic, and forms an aqueous two phase system with many salts. High molecular weights of the polymer are favorable for aqueous two phase system. However, the use of these high molecular weight polymers leads to high viscosity and density which in turn affects the economics of the separation process. Low molecular weights of polymer would virtually attract all proteins to the PEG phase, which would lead to very poor separation and purification from contaminating proteins.<sup>8</sup> Hence an intermediate polymer is chosen as the phase forming substance in most of the ATPS.

Hofmeister<sup>9</sup> noticed the effect of ions on the partitioning of proteins and reported the Hofmeister series which classifies ions in order of their ability to change water structure. The strength of the cations and anions follows as  $\text{NH}_4^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+}$  and  $\text{PO}_4^{3-} > \text{C}_6\text{H}_5\text{O}_7^{3-} > \text{SO}_4^{2-} > \text{COO}^-$ . The results of Hofmeister<sup>9</sup> clearly suggest that the ammonium and phosphate ion combination may provide more suitable environment for the biomolecule separation. However limited data on binodal and liquid–liquid equilibrium of ammonium salts were available in the literature. Liquid–liquid equilibrium (LLE) data and phase diagrams at different temperatures are essential for the development, optimization, and scale-up of the process. Gao et al.<sup>10</sup> reported the experimental liquid–liquid equilibrium data for systems containing PEG 1000, PEG 1540, PEG 2000, and PEG 4000 with ammonium sulfate. Voros et al.<sup>11</sup> studied the phase behavior of systems composed of PEG 1000 and PEG 2000 with ammonium sulfate and sodium carbonate at (15, 25, 35, and 45) °C. Peng et al.<sup>12</sup> generated and reported the binodal data for the systems composed of PEG 1000, PEG 2000, PEG 4000, and PEG 6000 with a mixture of potassium hydrogen phosphate and potassium dihydrogen phosphate at 25 °C. Berlo et al.<sup>13</sup> suggested volatile salts like ammonium carbamate as a promising alternative, reporting the phase behavior of aqueous PEG2000, PEG 4000, and PEG 10000 with ammonium carbamate at 298.15 K. Graber et al.<sup>14</sup> studied the phase behavior of the ATPS systems with PEG 2000, PEG 6000, and PEG 10 000 using sodium nitrate at 298.15 K. Phosphates and sulfates of sodium, potassium, and ammonium salts are commonly used as inorganic salts to form the ATPS with PEG, but the salts like phosphates and sulfates increase the load on wastewater treatment. Vernau and Kula<sup>15</sup> suggested citrate salts as an alternative since it is biodegradable and can be discharged into wastewater treatment plants. Murugesan et al.<sup>16</sup> studied the effect

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**Table 1. Binodal Data for PEG 2000 + Diammonium Hydrogen Citrate + Water System at Different Temperatures (298.15, 303.15, 308.15, 313.15, and 318.15) K<sup>a</sup>**

T /K = 298.15 K		T/K = 303.15 K		T/K = 308.15 K		T/K = 313.15 K		T/K = 318.15 K	
W <sub>S</sub>	W <sub>P</sub>	W <sub>S</sub>	W <sub>P</sub>	W <sub>S</sub>	W <sub>P</sub>	W <sub>S</sub>	W <sub>P</sub>	W <sub>S</sub>	W <sub>P</sub>
0.3489	0.1460	0.3558	0.1240	0.3345	0.1150	0.3563	0.1020	0.3400	0.0970
0.3037	0.1520	0.2716	0.1461	0.2859	0.1290	0.2734	0.1181	0.3203	0.0940
0.2941	0.1550	0.2656	0.1502	0.2725	0.1300	0.2678	0.1190	0.3000	0.0990
0.2819	0.1550	0.2578	0.1520	0.2500	0.1400	0.2586	0.1240	0.2958	0.0976
0.2705	0.1600	0.2531	0.1570	0.2493	0.1460	0.2437	0.1310	0.2800	0.1000
0.2610	0.1630	0.2412	0.1610	0.2407	0.1500	0.2344	0.1350	0.2725	0.1060
0.2367	0.1760	0.2300	0.1701	0.2287	0.1610	0.2272	0.1390	0.2500	0.1130
0.2308	0.1840	0.2188	0.1770	0.2169	0.1690	0.2109	0.1540	0.2400	0.1170
0.2168	0.1910	0.1985	0.2000	0.2075	0.1790	0.2000	0.1640	0.2136	0.1390
0.2089	0.2010	0.1916	0.2030	0.1875	0.1950	0.1811	0.1830	0.1800	0.1720
0.1979	0.2080	0.1857	0.2100	0.1774	0.2030	0.1661	0.2050	0.1762	0.1760
0.1725	0.2351	0.1804	0.2140	0.1717	0.2120	0.1641	0.2080	0.1633	0.1900
0.1655	0.2460	0.1731	0.2230	0.1692	0.2160	0.1578	0.2160	0.1495	0.2080
0.1585	0.2593	0.1667	0.2340	0.1663	0.2196	0.1449	0.2380	0.1424	0.2141
0.1477	0.2819	0.1520	0.2523	0.1613	0.2257	0.1398	0.2450	0.1360	0.2240
0.1351	0.3034	0.1411	0.2720	0.1548	0.2330	0.1355	0.2540	0.1300	0.2308
0.1275	0.3193	0.1351	0.2821	0.1489	0.2450	0.1332	0.2581	0.1249	0.2420
0.1117	0.3530	0.1275	0.2980	0.1404	0.2620	0.1266	0.2682	0.1192	0.2530
0.0972	0.3770	0.1192	0.3170	0.1330	0.2750	0.1170	0.2889	0.1107	0.2697
0.0825	0.4050	0.1122	0.3310	0.1231	0.2950	0.1100	0.3020	0.1052	0.2810
0.0654	0.4400	0.1037	0.3450	0.1141	0.3100	0.1017	0.3154	0.0963	0.3000
0.0521	0.4707	0.0933	0.3670	0.0989	0.3380	0.0804	0.3574	0.0809	0.3314
0.0392	0.5103	0.0824	0.3870	0.0824	0.3680	0.0675	0.3875	0.0586	0.3812
0.0318	0.5412	0.0506	0.4586	0.0596	0.4210	0.0463	0.4373	0.0500	0.4081
0.0223	0.5746	0.0382	0.4969	0.0462	0.4560	0.0397	0.4560	0.0323	0.4620
0.0179	0.5890	0.0300	0.5351	0.0347	0.4908	0.0328	0.4810	0.0274	0.4864
		0.0233	0.5640	0.0268	0.5200	0.0263	0.5047	0.0184	0.5289
				0.0208	0.5490	0.0194	0.5412	0.0149	0.5519

<sup>a</sup> Standard uncertainties  $u$  are  $u(T) = 0.1$  K and  $u(W) = 0.0001$ .

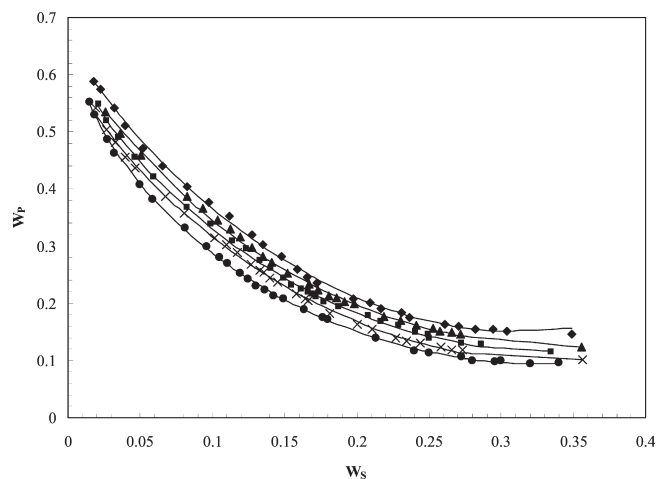
of temperature on the liquid–liquid equilibrium for the system containing PEG 2000 + sodium citrate + water. Malathy et al.<sup>17</sup> reported liquid–liquid equilibrium data for the aqueous PEG 2000–potassium citrate system at (25, 35, and 45) °C. Recently Regupathi et al.<sup>18</sup> reported liquid–liquid equilibrium data for PEG 6000–tri ammonium citrate system at (25, 30, 35, 40, and 45) °C. In this context, the present work intend to study the phase behavior of ATPS of PEG 2000 + diammonium hydrogen citrate at different temperatures (298.15, 303.15, 308.15, 313.15, and 318.15) K. The liquid–liquid equilibrium of the present system at different temperature also studied. The effect of temperature on the phase-forming ability of the PEG 2000 + diammonium hydrogen citrate system also discussed based on the salting-out coefficient and effective excluded volume.

## MATERIALS AND METHODS

**Materials.** Polyethylene glycol [HO-(CH<sub>2</sub>CH<sub>2</sub>O)<sub>*n*</sub>-CH<sub>2</sub>OH], poly(ethane-1,2-diol)-2000 (PEG-2000) (Catalog No. 8.21037-1000) with an average molar mass of 1800 g·mol<sup>-1</sup> purchased from Merck, and diammonium hydrogen citrate [(NH<sub>4</sub>)<sub>2</sub>HC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>] (CAS No. 3012-65-5) with a molar mass of 226.18 g·mol<sup>-1</sup> and minimum purity of 0.99 was purchased from Sigma-Aldrich and

used without further purification. Double distilled water was used for all experiments.

**Apparatus and Procedure.** The cloud point method was used to determine the phase equilibrium concentrations for establishing binodal curves.<sup>19</sup> The experiment was carried out in a jacketed glass vessel. The temperature of the working vessel was maintained by circulating water through an external jacket. The temperature was maintained at 303.15 K with an uncertainty of ± 0.1 K [Refrigerating Bath, JEIO Tech, RW-0525G]. Aqueous stock solution of PEG 2000 of 60 % (w/w) and diammonium hydrogen citrate of 40 % (w/w) were prepared and kept in a constant-temperature bath. To ensure the uniform concentration of the constituents of ATPS in the jacketed vessel, constant stirring was applied by using a magnetic stirrer. A salt solution of known concentration was titrated against the polymer solution or vice versa, until the clear solution turned turbid. A known amount of the salt solution was taken and titrated against PEG. To ensure the end point, PEG was added in drops until the appearance of turbidity, which indicates the two-phase formation. Water was then added until the disappearance of turbidity. The procedure was repeated to get the other binodal points. An analytical balance with a precision of ± 0.1 mg (OHAUS Essae-



**Figure 1.** Effect of temperature on binodal curve for PEG 2000 + diammonium hydrogen citrate + water. Experimental binodal points for  $\blacklozenge$ , 298.15 K;  $\blacktriangle$ , 303.15 K;  $\blacksquare$ , 308.15 K;  $\times$ , 313.15 K;  $\bullet$ , 318.15 K. —, Calculated from eq 1.

Teraoka, model AR2140) was used to determine the composition of the mixture.

For the determination of the tie lines, a total weight of 10 g of samples were prepared by mixing appropriate amounts of PEG2000, diammonium hydrogen citrate, and water in centrifuge tubes. The samples were thoroughly mixed with the help of vortex mixer at lower rpm for 10 min. Then the mixture was allowed to settle for 24 h at constant temperature bath. Top and bottom phases were separated. The concentration of diammonium hydrogen citrate in the phases was determined using conductivity measurements (Bench Conductivity meter, Eutech Instruments, Con510) with an accuracy of  $\pm 0.01$  full scale. The relation between the conductivity and the salt concentrations<sup>18,20</sup> is represented by

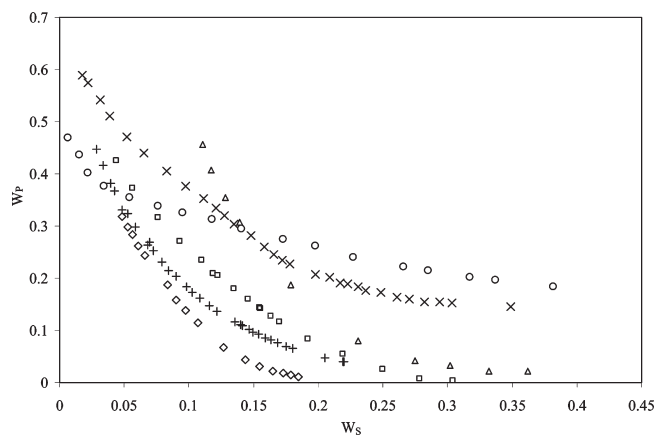
$$\kappa = b_0 + b_1 W_S \quad (1)$$

where  $\kappa$  is the conductivity ( $\mu\text{S} \cdot \text{cm}^{-1}$ ) and the values of  $b_0$  and  $b_1$  for diammonium hydrogen citrate solution are 5.744 and 502.6, respectively. The conductivity predicted by the eq 1 is valid with in the salt weight fraction of 0.15.

The concentration of PEG2000 in both the phases was determined by refractive index measurements (Automatic Digital Refractometer, Atago Co. Ltd., RX-5000 $\alpha$ ) with a precision of  $\pm 0.00004$ . Refractive index of the solution depends on the concentrations of polymer as well as salt. A correlation was developed to measure the refractive index at various compositions of PEG and salt. For dilute aqueous solution containing a polymer and a salt, the relation between the refractive index ( $\eta_D$ ) and the mass fractions of polymer ( $W_P$ ) and salt ( $W_S$ ) is given by

$$\eta_D = a_0 + a_1 W_P + a_2 W_S \quad (2)$$

where  $a_0$ ,  $a_1$ , and  $a_2$  are the fitting parameters. For PEG analysis in the phases, the above form of equation was used successfully in the literature.<sup>15–18,20–22</sup> Based on the known weight percentage of salt and PEG present in the single phase and their refractive index; the correlation coefficients of the above equation were found as  $a_0 = 1.333$ ,  $a_1 = 0.1358$ , and  $a_2 = 0.1625$ . The proposed equation eq 2 is valid only in the range of  $W_P < 0.15$  and  $W_S < 0.10$ .



**Figure 2.** Comparison of various binodal curves of PEG2000 + salt at 298.15 K.  $\diamond$ , Ammonium sulfate;<sup>10</sup>  $\Delta$ , ammonium carbamate;<sup>13</sup>  $\circ$ , sodium nitrate;<sup>15</sup>  $+$ , sodium citrate;<sup>16</sup>  $\square$ , potassium citrate;<sup>17</sup>  $\times$ , diammonium hydrogen citrate.

## RESULTS AND DISCUSSION

Below the critical concentrations of polymer and salt, both the salt and PEG solutions were miscible completely and formed a homogeneous phase. Beyond the critical concentration of salt and PEG, the solution turn to heterogeneous two phase and yield PEG rich upper phase and salt rich lower phase. The binodal curve depicts the boundary between the two immiscible aqueous phase and the single phase regions. Experiments were conducted to obtain the binodal curve at different temperatures (298.15, 303.15, 308.15, 313.15, and 318.15) K for the PEG 2000 + diammonium hydrogen citrate + water based ATPS (Table 1). Further the binodal data at different temperatures for PEG 2000 + diammonium hydrogen citrate + water were plotted as shown in Figure 1 and found that, as reported earlier,<sup>11,16–18,20,21</sup> the biphasic area increases with increase in temperature. The PEG-solvent interaction decreased with an increase in temperature, which then resulted in a decrease in the solubility of PEG in water and an increase in salt solubility. In other words, the critical concentration of a salt required to form an ATPS decreases with increasing the temperature (Figure 1). Further Figure 2 was plotted to compare the present binodal curve with the literature binodal curve of ammonium sulfate,<sup>10</sup> ammonium carbamate,<sup>13</sup> sodium nitrate,<sup>14</sup> sodium citrate,<sup>16</sup> and potassium citrate<sup>17</sup> at the temperature of 298.15 K. Figure 2 reveals that different PEG-salt systems forming different shape of binodal curves, due to the nature of the individual ions present in the salt.

**Correlation of Binodal Data.** The relation between the PEG and salt at binodal curve may be useful to utilize the present system for commercial application. Hence the binodal data at five different temperatures were analyzed and fitted to various form of equations used previously (Table 2). The best fit was obtained with the nonlinear equation.<sup>23</sup>

$$W_P = a + bW_S^{0.5} + cW_S + dW_S^2 \quad (3)$$

The constants of the equation were determined through regression analysis of the binodal data at different temperature and are given in Table 2. The estimated errors are within  $\pm 0.01$ .

From the analysis of the binodal data it was found that the PEG solubility is decreased as the temperature increases; i.e., water becomes a poorer solvent for PEG as the temperature is raised. The increase in temperature enhances PEG and salt concentrations

**Table 2.** Literature Correlations and Their Parameter Values for PEG 2000 + Diammonium Hydrogen Citrate + Water System at Different Temperatures<sup>a</sup>

correlations	T/K	a	b	c	d	AARD/% <sup>*</sup>	R <sup>2</sup>
$1/W_S = a + b(W_P)^{1/2} + cW_P$ (Graber et al. <sup>15</sup> )	298.15	8.8639	-43.7137	76.7320		12.533	0.9253
	303.15	7.5662	-36.1993	68.3311		8.5821	0.9580
	308.15	10.417	-49.4411	85.5127		7.3780	0.9423
	313.15	66.107	-308.103	377.94		23.935	0.9408
	318.15	8.9556	-46.036	90.2336		11.227	0.9321
$W_P = a + bW_S^{0.5} + cW_S$ (Jayapal et al. <sup>17</sup> )	298.15	0.9269	-2.4108	1.8026		3.5036	0.9930
	303.15	0.8778	-2.2122	1.5504		2.5675	0.9950
	308.15	0.8495	-2.1497	1.4835		2.4182	0.9960
	313.15	0.9246	-2.5378	1.8944		3.2097	0.9947
	318.15	0.8345	-2.2924	1.7237		2.7575	0.9977
$W_P = a \exp(bW_S^{0.5} - cW_S^3)$ (Zafarani et al. <sup>23</sup> )	298.15	1.0322	-3.5582	0.2371		4.5759	0.9851
	303.15	1.0301	-3.5953	4.4643		3.5651	0.9871
	308.15	0.9944	-3.6242	7.0523		3.2663	0.9893
	313.15	0.8903	-3.3530	15.2363		4.6184	0.9814
	318.15	0.7948	-2.9863	63.9828		0.6717	0.9890
$W_P = a + bW_S^{0.5} + cW_S + dW_S^2$ (Hu et al. <sup>24</sup> )	298.15	0.6849	-0.4239	-2.2692	4.2140	1.4247	0.9988
	303.15	0.7008	-0.7519	-1.4626	3.1270	1.3061	0.9986
	308.15	0.6509	-0.6569	-1.5920	3.2342	1.2761	0.9991
	313.15	0.6509	-0.6569	-1.5920	3.2342	1.2761	0.9991
	318.15	0.7114	-1.2993	-0.3491	2.2427	1.0436	0.9996
$\ln W_P = a + bW_S^{0.5} + cW_S^3$ (Elisa et al. <sup>25</sup> )	298.15	-0.075	-3.0147	-8.4111		3.9350	0.9851
	303.15	0.0004	-3.5808	-0.8867		3.4067	0.9871
	308.15	-0.0711	-3.4367	-8.8462		2.6405	0.9893
	313.15	-0.1395	-3.2708	-16.206		3.0537	0.9814
	318.15	-0.1054	-3.7187	-7.6791		2.8732	0.9890

<sup>a</sup> Standard uncertainty  $u$  is  $u(T) = 0.1$  K. <sup>\*</sup> Average arithmetic relative deviation (AARD) =  $(\sum |(\text{Exptl} - \text{Cal})| / (\text{Exptl})) / (N) \times 100$ .

in the top and bottom phases, respectively. This is due to the preferential adherence of the water molecules to the polar salt surface instead of the PEG at a higher temperature. Consequently, water molecules available for PEG solvation in the bottom phase, decreased as the temperature increased, thus, reducing the solubility of PEG in the bottom phase. This is known as the “salting-out” effect, which becomes stronger with increasing temperature.<sup>2</sup>

The salting-out strength of the salt could be related to the effective excluded volume (EEV), as proposed by Huddleston et al.<sup>25</sup> The EEV and salting-out phenomena plays a major role in the phase formation of ATPS and application of ATPS for the protein purification. Further these parameters are highly depends on the nature of salt involved in the ATPS. On the basis of statistical geometry methods, the binodal model developed by Guan et al.<sup>26</sup> for aqueous polymer–polymer systems is extended to correlate the experimental binodal data on the PEG 2000 + diammonium hydrogen citrate + water system. This theory is an advance toward the solution of phase separation problems and allows the prediction of the coexistence behavior of two-phase systems using only one parameter, the EEV. The binodal equation for the aqueous polymer-salt systems is defined as

$$\ln \left( V_{123}^* \frac{W_P}{M_P} \right) + V_{123}^* \frac{W_S}{M_S} = 0 \quad (4)$$

where  $V_{123}^*$  is the effective excluded volume (EEV) and  $M_P$  and  $M_S$  are the molar mass of polymer (1800 g·mol<sup>-1</sup>) and salt (226.18 g·mol<sup>-1</sup>), respectively.<sup>20,26,27</sup> The effective excluded

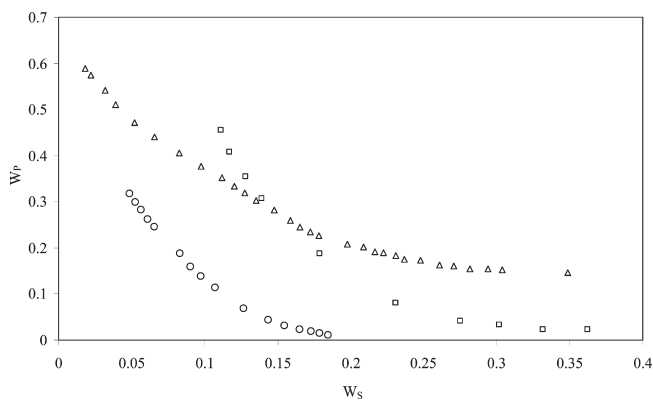
**Table 3.** Effective Excluded Volumes for PEG2000 + Diammonium Hydrogen Citrate + Water System at Different Temperatures (eq 4)<sup>a</sup>

T/K	EEV/g·mol <sup>-1</sup>	AARD/%
298.15	17.96699	2.6084
303.15	18.79349	2.5050
308.15	19.4279	2.2936
313.15	20.3708	2.1069
318.15	21.582	2.0180

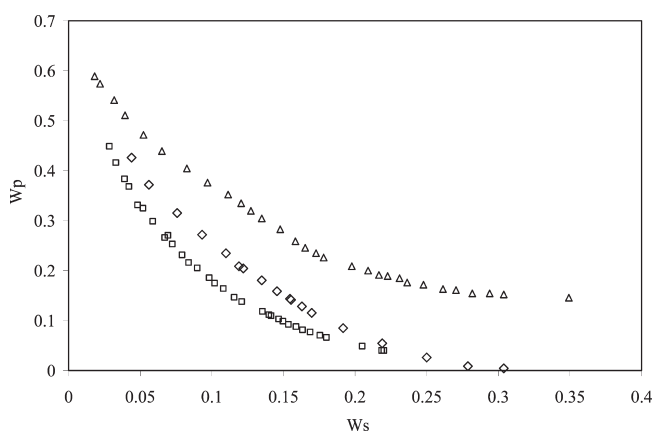
<sup>a</sup> Standard uncertainty  $u$  is  $u(T) = 0.1$  K

volume along with the corresponding AARD were determined (Table 3) for all five temperatures using the experimental binodal data. The EEV represents the acceptability of one component by a network constructed of the other component. Since the molecular packing in a solution is compact, in an ensemble average it is impossible for holes of any significant size to exist, which will admit additional molecules, unless the solution adjusts its structure. For any given system the EEV is determined by the sizes and shapes of the constituent molecules and their molecular interactions.<sup>26</sup> In the present work, the effect of temperature on EEV was analyzed with five different temperatures (Table 3) and it was found that the EEV increases with increasing temperature.<sup>27</sup>

The salting-out strength of the salt increases with temperature<sup>27</sup> and the salts with higher salting-out strength have larger values of EEV at the same temperature.<sup>20</sup> The increase of the EEV value is



**Figure 3.** Effect of anion on the binodal curve for PEG2000 + salt at 298.15 K.  $\circ$ , ammonium sulfate;<sup>10</sup>  $\square$ , ammonium carbamate;<sup>13</sup>  $\Delta$ , diammonium hydrogen citrate.



**Figure 4.** Effect of cation on the binodal curve for PEG2000 + salt at 298.15 K.  $\square$ , sodium citrate;<sup>16</sup>  $\diamond$ , potassium citrate;<sup>17</sup>  $\Delta$ , diammonium hydrogen citrate.

reflected in the phase diagram by a shift in the position of the binodal curve to the left, corresponding to a decrease in the area representing the single-phase mixture; thus, a decrease in the concentration of salt required to form a two-phase system indicates the higher salting-out strength of the salt<sup>28</sup> (Figure 2).

**Effect of Anion and Cation.** Hofmeister<sup>9</sup> noticed the effect of ions on the partitioning of proteins and reported that the Hofmeister series which classifies ions in order of their ability to change water structure and their effect on the stability of the secondary and tertiary structure of proteins. While early members of the series increase solvent surface tension and decrease the solubility of nonpolar molecules (salting-out), later salts in the series act contrariwise by increasing the solubility of nonpolar molecules (salting-in) and increasing the order in water. Due to this effect, they weaken the hydrophobic effect in the system. Anions with higher valence are better salting out agents than anion with lower valence because of their degree of hydration compared to lower valence anion, which decreases the amount of water available to hydrate polymer in case of polymer + salt systems. To understand the effect of anion, Figure 3 was plotted at 298.15 K based on the literature and experimental data by keeping the cation (ammonium) as constant and varying anions of sulfate,<sup>10</sup> carbamate,<sup>13</sup> and citrate. The EEV values of 23.71, 20.11, and 9.96 for ammonium

**Table 4.** Tie Line Data for PEG 2000 + Di-Ammonium Hydrogen Citrate + Water System at (298.15, 303.15, 308.15, 313.15, and 318.15) K<sup>a</sup>

T/K	feed		top		bottom	
	$W_p$	$W_s$	$W_p$	$W_s$	$W_p$	$W_s$
298.15	0.2	0.22	0.333	0.1226	0.174	0.2437
	0.22	0.22	0.363	0.1087	0.156	0.2725
	0.25	0.22	0.435	0.0675	0.156	0.2968
	0.28	0.22	0.487	0.0486	0.156	0.32
303.15	0.2	0.22	0.355	0.0903	0.162	0.2311
	0.22	0.22	0.386	0.0769	0.132	0.2706
	0.25	0.22	0.429	0.0591	0.128	0.2897
308.15	0.18	0.22	0.355	0.0903	0.138	0.2556
	0.2	0.22	0.386	0.0769	0.134	0.2734
	0.22	0.22	0.429	0.0591	0.128	0.2938
313.15	0.16	0.22	0.338	0.0938	0.1412	0.2413
	0.18	0.22	0.394	0.069	0.1402	0.2601
	0.2	0.22	0.4600	0.0422	0.1377	0.2821
	0.22	0.22	0.4860	0.0333	0.1368	0.2909
318.15	0.14	0.22	0.34	0.0809	0.117	0.2387
	0.16	0.22	0.374	0.064	0.115	0.2559
	0.18	0.22	0.413	0.0481	0.099	0.2804
	0.2	0.22	0.451	0.0352	0.103	0.2888
	0.22	0.22	0.487	0.0288	0.1	0.3032

<sup>a</sup> Standard uncertainties  $u$  are  $u(T) = 0.1$  K and  $u(W) = 0.0001$ .

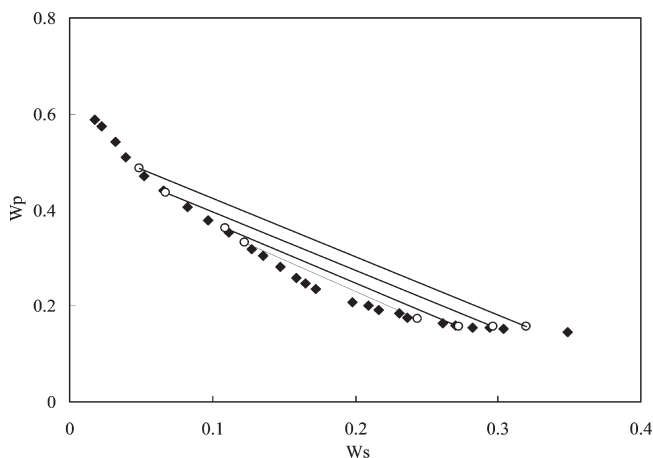
sulfate, di ammonium hydrogen citrate and ammonium carbamate respectively. From the EEV values it was found that the salting out effect of anions follows sulfate > citrate > carbamate, which reveal that the citrate anion also have higher valency and salting out strength when compare to carbamate and lesser salting out strength with comparison of ammonium sulfate.

Similarly to know the effect of cation, the binodal curves of various salts having different cation associated with the common citrate anion (i.e., sodium citrate,<sup>16</sup> potassium citrate,<sup>17</sup> and diammonium hydrogen citrate) was plotted (Figure 4) and the EEV values were obtained through calculation using the literature and experimental data. The EEV values of 30.73, 29.99, and 20.11 for sodium citrate, potassium citrate and diammonium hydrogen citrate respectively at 298.15 K. From Figure 4, it was found that the effect of cation follows as  $\text{Na}^+ > \text{K}^+ > \text{NH}_4^+$ . This effect reveals that lower concentration of Na salt is required to form two phase region, when compared to ammonium salt (Figure 4). Further this effect is similar to Zafarani et al.<sup>29</sup>

**Phase Equilibrium.** The experiments were conducted at different temperature to study equilibrium characteristics of the present ATP system. The initial composition (feed) and the corresponding phase compositions at different temperatures were reported in Table 4.

The Othmer–Tobias and Bancroft equations<sup>30</sup> as given below were earlier successfully applied for tie line compositions of other similar polymer–salt systems; hence, those equations were used to correlate the tie line composition of the PEG 2000 + diammonium hydrogen citrate + water system.

$$\left(\frac{1 - W_p^{\text{top}}}{W_p^{\text{top}}}\right) = K \left(\frac{1 - W_s^{\text{bot}}}{W_s^{\text{bot}}}\right)^n \quad (5)$$



**Figure 5.** Tie-lines for the PEG 2000 + diammonium hydrogen citrate + water system at 298.15 K,  $\blacklozenge$ , Binodal data,  $\circ$  experimental tie line; —, Calculated tie line from eqs 5 and 6.

**Table 5.** Fitting Parameters for the Tie Line Data at Five Different Temperatures<sup>a</sup>

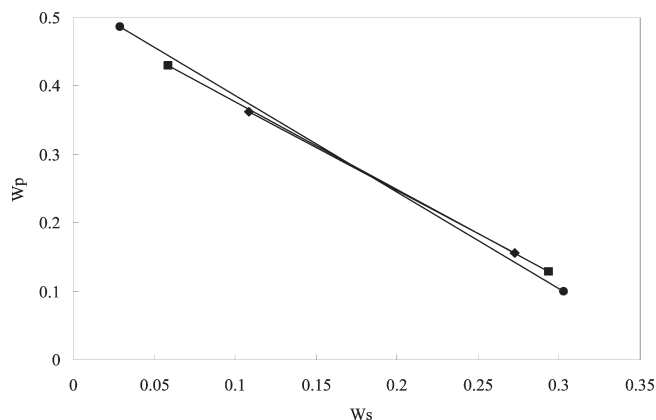
T/ K	Othmer–Tobias equation (eq 5)			Bancroft equation (eq 6)		
	K	N	R <sup>2</sup>	K <sub>1</sub>	r	R <sup>2</sup>
298.15	0.2891	1.7513	0.9602	1.6818	0.6680	0.9818
303.15	0.5858	0.9548	0.9730	1.6649	0.9715	0.9382
308.15	0.3220	1.6236	0.9967	1.7372	0.6897	0.9962
313.15	0.1223	2.4212	0.999	1.9778	0.4957	0.9990
318.15	0.2381	1.8261	0.9750	1.9490	0.5803	0.9807

<sup>a</sup> Standard uncertainty  $u$  is  $u(T) = 0.1$  K.

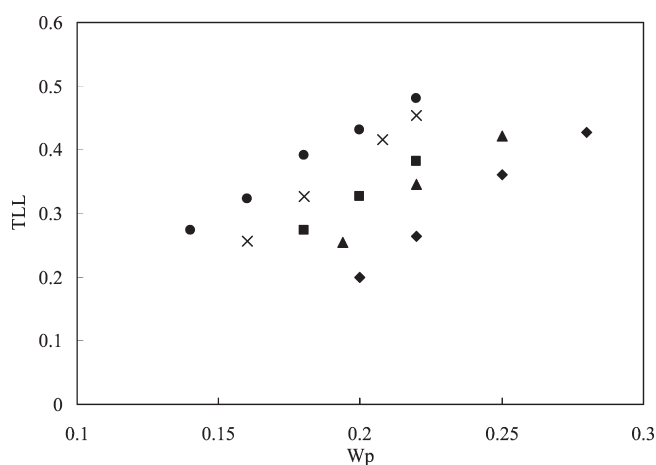
$$\left(\frac{W_W^{\text{bot}}}{W_S^{\text{bot}}}\right) = K_1 \left(\frac{W_W^{\text{top}}}{W_P^{\text{top}}}\right)^r \quad (6)$$

where  $W_P^{\text{top}}$  the weight fraction of PEG 2000 in top phase,  $W_S^{\text{bot}}$  is the weight fraction of salt in bottom phase,  $W_W^{\text{bot}}$  and  $W_W^{\text{top}}$  is weight fraction of water in bottom and top phase respectively.  $K$ ,  $n$ ,  $K_1$ , and  $r$  are the fit parameters. The correlation coefficient and the fitted parameters values are given in Table: 4. The experimental and correlated tie lines (as a coordinate of phase composition) for the temperatures 298.15 K is shown in Figure 5.

**Effect of the Temperature on the Tie Line Length and the Slope of the Tie Line.** The tie-line length (TLL) has the same units as the component concentrations (i.e., % w/w) and is often used to express the effect of system composition on partitioned material. The  $\text{TLL} = (\Delta X^2 + \Delta Y^2)^{1/2}$ ,  $X$  denotes the concentration of diammonium hydrogen citrate and  $Y$ , the concentration of poly ethylene glycol. However, if the total composition and weight ratio are known, then analysis of one phase is sufficient by using,  $V_t \rho_t / V_b \rho_b = (X_b - X_0) / (X_0 - X_t)$  where  $X$  denotes the concentration of diammonium hydrogen citrate in the top phase (t), bottom phase (b), and the total system (0) (the same holds true for poly ethylene glycol). Tie-lines are commonly parallel for most of the organic solvent system but considerable deviation occurs for the ATPS at different temperature (Figure 6). Hence the slope of the tie-line (STL) can also be calculated,  $\text{STL} = \Delta Y / \Delta X$ , where  $X$



**Figure 6.** Effect of temperature on TLL and STL for feed composition of  $W_P = 0.22$  and  $W_S = 0.22$ .  $\blacklozenge$ , 298.15 K;  $\blacksquare$ , 308.15 K;  $\bullet$ , 318.15 K.

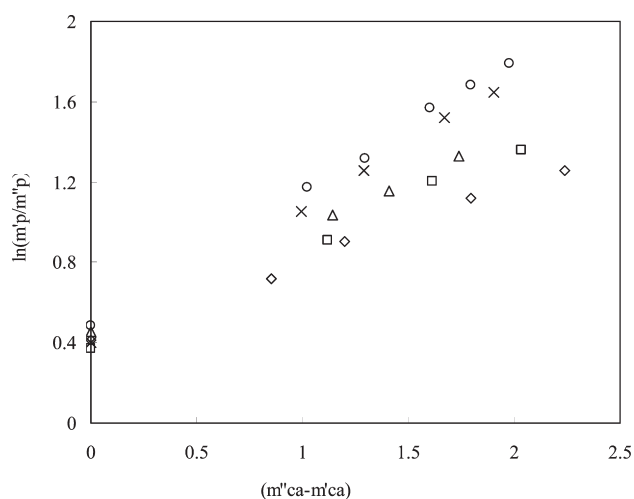


**Figure 7.** Effect of temperature on the tie-line length (TLL),  $\blacklozenge$ , 298.15 K;  $\blacktriangle$ , 303.15 K;  $\blacksquare$ , 308.15 K;  $\times$ , 313.15 K;  $\bullet$ , 318.15 K.

denotes the concentration of diammonium hydrogen citrate and  $Y$ , the concentration of poly ethylene glycol. STL and TLL increases with increasing temperature and polymer concentration (Figure 6 and 7). The STL change was due to the transfer of water from top phase to bottom phase. This is because PEG becomes more hydrophobic with an increase in temperature; that is, water is driven out from the PEG rich phase to the salt phase. As a result, the PEG concentration at the top phase increases, while the salt concentration at the bottom phase decreases and the volume of the salt-rich phase increases at the expense of the PEG-rich phase.<sup>31</sup>

Depression of the hydrophilic nature of PEG with increasing temperature may be related to the salting-out phenomenon. The critical concentration of a salt required to form a two-phase system which in turn indicates the effectiveness of the salt in inducing the formation of two phases (i.e., the salting-out strength of the salt) which is increased at increase in temperature.<sup>31</sup> Salting-out effects are usually quantified to the empirical equation of modified Setschenow equation. The equation used has the following form.

$$\ln \left( \frac{m_p'}{m_p''} \right) = k_p (m_p'' - m_p') + k_{ca} (m_{ca}'' - m_{ca}') \quad (7)$$



**Figure 8.** Setschenow-type plots for the tie-line data of the PEG 2000 + ammonium citrate dibasic + H<sub>2</sub>O system. Upper and lower phase concentrations are denoted by single and double primes, respectively:  $\diamond$ , 298.15 K;  $\square$ , 303.15 K;  $\Delta$ , 308.15 K;  $\times$ , 313.15 K;  $\circ$ , 318.15 K.

**Table 6.** Values of Parameters to the Setschenow Type eq 7 for PEG2000 + Ammonium Citrate Dibasic + Water at Different Temperatures<sup>a</sup>

T/K	$k_{ca}/\text{kg}\cdot\text{mol}^{-1}$	intercept	$R^2$
298.15	0.3861	0.4121	0.9887
303.15	0.4952	0.3695	0.9841
308.15	0.5029	0.455	0.9986
313.15	0.6621	0.3996	0.998
318.15	0.6646	0.4848	0.9956

<sup>a</sup> Standard uncertainty  $u$  is  $u(T) = 0.1$  K.

where  $m_p$ ,  $m_{ca}$ ,  $k_p$ , and  $k_{ca}$  represent the molality of PEG, the molality of the salt, a parameter relating the activity coefficient of PEG to its concentration, and the salting-out coefficient, respectively. Single primes denote the upper phase and double prime for the lower phase. If the first term on the right-hand side of this equation is small compared to the second term, a Setschenow-type equation is obtained. This would imply that  $k_p \ll K_{ca}$  since the absolute values of  $(m_p'' - m_p')$  exceed the  $(m_{ca}' - m_{ca}'')$  values. This equation is previously applied to PEG- Sodium tungstate system by Sadeghi et al.<sup>31</sup> Setschenow-type plots for the tie-line data of the PEG 2000 + diammonium hydrogen citrate + H<sub>2</sub>O system are shown in Figure 8. Values of parameters to the Setschenow type equation (eq 7) for PEG 2000 + diammonium hydrogen citrate + H<sub>2</sub>O system are shown in Table 6. It is found that the values of salting-out coefficients increase by increasing temperature. This trend is in agreement with our experimental observations in which, at higher temperatures, aqueous two-phase formation occurs at lower concentrations of the polymer and salt. Also this results confirmed the salting-out phenomena, which was initially explained with EEV.

## CONCLUSION

The experimental binodal data were satisfactorily correlated with empirical equation relating the concentration of PEG and diammonium hydrogen citrate at different temperatures. It was found that an increase in temperature caused the expansion of the

two-phase region. It was also found that the concentration of salt which is in equilibrium with a certain concentration of PEG decreases by increasing temperature. The tie lines were satisfactorily correlated with the Bancroft and Othmer-Tobias equations. An increase in temperature promotes an increase in the slope of the tie-line (STL). It is also concluded that the STL change was due to the transfer of water from the top to the bottom phase.

Therefore, the polymer concentration increases in the upper phase, and the salt content decreases in the lower phase, which is in agreement with the previous results. The salting out coefficient values increases with increasing temperature due to that the aqueous two-phase formation occurs at lower concentrations of the polymer and salt, which is in agreement with experimental observations. The effect of temperature on EEV was analyzed with five different temperatures, and it was found that the EEV increases with increasing temperature. The EEV results also confirm the salting-out phenomena of the present system.

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