Phase Equilibrium in Aqueous Two-phase Systems Containing Poly (propylene glycol) and Sodium Citrate at Different pH

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Abstract—The phase diagrams and compositions of coexisting phases have been determined for aqueous two-phase systems containing poly(propylene glycol) with average molecular weight of 425 and sodium citrate at various pH of 3.93, 4.44, 4.6, 4.97, 5.1, 8.22. The effect of pH on the salting-out effect of poly (propylene glycol) by sodium citrate has been studied. It was found that, an increasing in pH caused the expansion of two-phase region. Increasing pH also increases the concentration of PPG in the PPG-rich phase, while the salt-rich phase will be somewhat mole diluted.

Keywords—Aqueous two-phase system, Phase equilibrium, Biomolecules purification

I. INTRODUCTION

AN aqueous two-phase system (ATPS) is produced when appropriate small amounts of two chemically different water soluble polymers, or a water soluble polymer and inorganic salts, are added to water, causing the system to separate into two immiscible water-rich phases [1]. The high water content in aqueous two-phase systems, typically greater than 80 wt.%, coupled with low interfacial tension provide a benign environment for biomolecules not attained in solvent extraction. The system offers a technically simple, energy efficient, easily scalable and mild separation technique for product recovery in biotechnology [2].

Its major use has been in the concentration and purification of proteins and in the extractive bioconversion of enzymes. The separation technique is also becoming important in non-biotechnology areas such as industrial waste remediation. Notable examples include utilization of aqueous phase systems in removal of color from textile plant wastes [3], metal ions [4] and organic pollutants from the environment [5], and aromatics from crude oil [4]. Liquid–liquid equilibrium (LLE) data for some aqueous PPG + inorganic salt two-phase systems have been reported in the literature [6-7]. In these studies, salting out of PPG has been accomplished by the use of either carbonate or sulfates.

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This work was supported in part by the research vice departments of Razi University.

These salts, however, lead to high phosphate or sulfate concentration in the effluent streams and therefore, to environmental concern. One way to reduce the salt discharged in the wastewater is to recycle the chemicals. In this regard, Hustedt [8] and Greve and Kula [9] have published useful papers. Recently, as an alternative approach, Vernau and Kula [10] and Rahimpour et al. [11] have investigated citrates as a substitute for inorganic salts and found that sodium and potassium citrates form aqueous two-phase systems with PEG which is suitable for biomolecules extraction. Citrate is biodegradable and nontoxic and could be discharged into biological wastewater treatment plants and therefore the citrates can be considered as a substitute for inorganic salts, because the citrate forms aqueous two-phase system with PPG which are suitable for biomolecules extraction.

As far as we know, there is no report on the pH effect on phase diagram of the PPG425 + sodium citrate + water system in the literature. This work is devoted to obtaining phase equilibrium data for the PPG425 + sodium citrate + water system at pH of 3.93, 4.44, 4.6, 4.97, 5.1, and 8.22. These results can be used to develop thermodynamic models of aqueous two-phase systems and also to obtaining experimental data for choosing suitable systems for purification of biomolecules.

II. EXPERIMENTS

A. Materials

PPG of molecular weight 425, tri-sodium citrate, and citric acid were obtained from Merck. The polymer and salt were used without further purification, and double distilled, deionized water was used. All other materials used were of analytical grade.

B. Preparation of the Aqueous Two-Phase Systems

Aqueous two-phase systems (ATPS) were prepared by a mixture of PPG425 and sodium citrate salt solution at required pH. The pH of the salt solution was adjusted by mixing appropriate ratio of sodium citrate and citric acid.

All components were added into a graduated 15 ml test tube as stock solution at constant pH, resulting in a 10 g system. All experiments were carried out at 25 °C. The pH values of the solutions were measured precisely with a pH meter of JENWAY 3345 model. In order to speed up phase separation the resulted solution was mixed by rigorous vortexing the test tube for 2 min. The tubes were placed in a room temperature for 4 h and then were centrifuges at 1500 rpm for 10 min; the

solution reach to equilibrium and the samples of the top and bottom phase were carefully withdrawn, with care being taken to leave a layer of solution at least 0.2 cm thick above the interface.

C. Measurement of Salt and PPG Concentration

The analysis methods for salt concentrations were determined by using atomic absorption spectroscopy (AAS), shimatsu AA-6300 model.

The concentration of PPG was determined by refractive index measurements at 298.15K using an ATAGO-DTM1model. Since the refractive index of phase samples depends on PEG, and salt concentration, calibration plots of refractive index versus polymer concentration were prepared for deferent concentrations of salt.

A. Procedure

The binodal curves were determined by a titration method. A salt solution of known concentration was titrated with the polymer solution or vice versa, until the solution turned turbid. The composition of the mixture was determined by mass using an analytical balance with a precision of $\pm 1 \times 10^{-7}$ kg. To determine the compositions of coexisting phases, feed samples (about 10 gr) were prepared by mixing appropriate amounts of polymer, salt and water in the tube. After separation of the two transparent phases, the concentrations of the salts and PPG in the top and bottom phases were determined.

III. RESULTS AND DISCUSSION

Experimental results

For the aqueous PPG - sodium citrate two-phase system the binodal data obtained from turbidimetric titrations and the tie line data for the compositions of conjugate solutions at pH of 3.93, 4.44, 4.6, 4.97, 5.1, and 8.22 are shown in Tables 1 and 2, respectively. The complete phase diagrams of all studied systems are shown in Figs. 1.

It can be seen that the binodal curve shifts downward as the pH of the medium becomes lower, indicating that smaller concentration of the phase polymers is required to form aqueous two-phase system. This behavior is probably due to decrease in hydrodynamic volume of the polymer as the pH of the solution decreases. It was shown that decreasing the pH leads to reduction in intrinsic viscosity of the polymer solutions [12]. Since it is well known that hydrodynamic volume of polymers in solution is directly proportional to their intrinsic viscosity, it can be deduced that decreasing the pH causes the polymer chains to assume a more compact structure.

The formation of aqueous two-phase systems clearly indicates the mutual exclusion of the ions and the polymer and their high affinity for the solvent. It is possible that even in homogeneous systems (below the phase boundary) the ions are excluded from the near surface region of the polymer in solution. With increase in the concentration of the polymer or the salt, the extent of exclusion will increase. Ultimately, the system could reach a state where, for entropic reasons, phase formation would become favorable. In fact, salting-out effects are related to a reduction in entropy resulting from the exclusion of ions from volume elements immediately adjacent

to the solute molecules. Exclusion of ions from the polymer molecule–water interface itself can occur for a number of reasons. Both the polymer and the ion are strongly hydrated in solution. Because of the hydration sheath, the near-surface region of the polymer may not be accessible to structure-making ions. The pH could affect the binodal location, either by changing the charge of the solute or by altering the ratio of the charged species present. Fig. 1 shows that the two-phase area is expanded with increasing pH. In other words, if we take a sample with a known composition on the binodal curve, this mixture becomes a two-phase system at increasing pH as has been observed experimentally. It was found that, at higher pH, hydrogen bond interactions of PPG are weakened [7]. Depression of the cloud point by increasing pH is may be related to the salting-out phenomenon resulting from the weakening of the PPG–solvent interaction.

The tie-line length- TLL, calculated by the following equation:

$$ TLL = \sqrt{\left(w_s^{top} - w_s^{bot}\right)^2 + \left(w_p^{top} - w_p^{bot}\right)^2} \qquad (1) $$

The tie lines are determined by connecting each corresponding set of total, bottom-, and top-phase points. As an example, the tie lines for the systems with pH of 3.93 and 5.1 are shown in Fig. 2 and 3. As shown in these figures, the slope and the length of the equilibrium tie-lines increased with increasing pH. Also, by increasing pH the volume of salt-rich phase increases at the expense of the PPG-rich phase, as we observed experimentally. This is because the compositions of the phases in equilibrium change with varying pH. Increasing the pH of the aqueous PPG-salt two-phase system will cause an increase of the concentration in the PPG-rich phase and a decrease of the concentration in the salt-rich phase.

TABLE I
TIE LINE DATA FOR SYSTEMS OF
PPG425- CITRATE- WATER AT DIFFERENT pH

| pH	Total compositions		Top phase		Bottom phase	
	Salt [w/w%]	PPG [w/w%]	Salt [w/w%]	PPG [w/w%]	Salt [w/w%]	PPG [[w/w%]
3.93	8.02	15.98	2.08	36.20	9.20	12.80
	9.85	13.75	1.95	39.34	10.96	11.12
	11.42	12.04	1.65	42.14	12.60	9.02
	13.25	11.02	1.50	45.05	13.84	9.91
4.44	8.41	14.02	2.00	37.21	9.86	10.40
	10.00	12.00	1.40	40.57	11.20	9.60
	12.50	10.00	1.24	43.03	13.10	8.25
	13.50	9.80	1.20	46.87	14.20	8.40
4.60	8.41	14.02	1.60	38.74	10.6	7.35
	10.57	12.36	1.04	42.81	12.00	7.50
	12.5	10.00	1.20	45.21	14.40	5.18
	14.91	8.72	0.85	51.18	16.50	6.40
4.97	10.32	12.22	0.847	40.85	11.20	7.08
	11.47	11.16	0.60	45.62	12.60	7.10
	13.35	10.14	0.40	52.05	14.20	6.10
	15.51	8.91	0.30	55.23	17.80	4.90
5.10	10.12	10.54	0.80	42.61	11.60	5.01
	11.77	9.26	0.51	48.77	12.86	5.70
	13.45	8.26	0.504	53.41	15.32	5.04
	16.04	8.03	0.488	56.25	18.40	4.07
8.22	14.13	9.02	0.24	47.31	16.40	3.05
	15.32	8.26	0.35	51.71	17.50	3.35
	17.50	7.14	0.60	56.2	19.50	2.45
	18.35	6.51	0.26	61.4	20.20	2.34
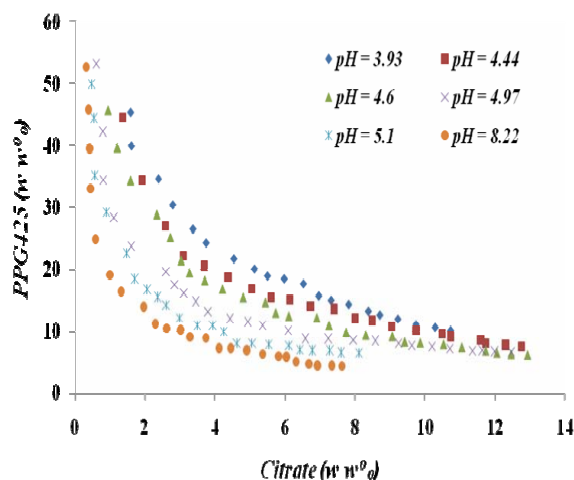
Fig. 1 Biondals of aqueous two-phase systems of
PPG425- citrate- water at different pH.

TABLE II
EQUILIBRIUM DATA FOR SYSTEMS OF
PPG425- CITRATE- WATER AT DIFFERENT pH

pH=3.93		pH=4.44		pH=4.6	
$100\omega_1$	$100\omega_2$	$100\omega_1$	$100\omega_2$	$100\omega_1$	$100\omega_2$
1.589	45.415	1.351	44.528	0.940	45.761
1.612	39.927	1.898	34.426	1.202	39.657
2.386	34.603	2.571	26.992	1.582	34.366
2.793	30.447	3.084	22.128	2.332	28.895
3.368	26.528	3.679	20.548	2.718	25.194
3.742	24.276	4.357	18.750	3.026	21.443
4.533	21.708	5.033	16.884	3.267	19.433
5.122	20.089	5.589	15.484	3.703	18.213
5.492	18.954	6.127	15.064	4.207	16.813
5.969	18.496	6.713	14.043	4.799	15.473
6.518	17.712	7.386	13.654	5.433	14.721
6.956	15.756	7.990	12.089	5.727	13.054
7.307	15.066	8.475	11.771	6.101	12.524
7.809	14.409	9.031	10.782	6.901	12.235
8.368	13.342	9.740	10.124	7.250	10.994
8.698	12.725	10.489	9.658	7.738	9.864
9.211	12.071	10.723	9.154	8.302	9.412
9.728	11.145	11.581	8.527	9.057	9.197
10.274	10.677	11.728	8.0522	9.409	8.311
10.715	10.202	12.297	7.815	9.855	8.177
		12.746	7.563	10.522	7.904
				11.044	7.446
				11.726	6.876
				12.045	6.522
				12.452	6.312
				12.922	6.215

| pH=4.97		pH=5.1		pH=8.22	
$100\omega_1$	$100\omega_2$	$100\omega_1$	$100\omega_2$	$100\omega_1$	$100\omega_2$
0.615	0.615	0.471	49.942	0.326	52.855
0.799	0.799	0.531	44.526	0.3958	45.895
0.806	0.806	0.555	35.243	0.421	39.557
1.108	1.108	0.896	29.341	0.442	33.025
1.616	1.616	1.467	22.687	0.585	24.876
2.594	2.594	1.697	18.525	0.998	19.142
2.837	2.837	2.051	16.796	1.325	16.418
3.104	3.104	2.356	15.607	1.955	14.025
3.448	3.448	2.588	14.314	2.298	11.261
3.792	3.792	2.980	12.276	2.617	10.521
4.422	4.422	3.487	11.028	3.014	10.221
4.938	4.938	3.922	11.000	3.282	9.066
5.352	5.352	4.241	9.988	3.731	8.951
6.092	6.092	4.609	8.111	4.111	7.307
6.580	6.580	5.048	8.100	4.440	7.291
7.234	7.234	5.525	7.998	4.896	6.955
7.938	7.938	6.094	7.839	5.348	6.396
8.569	8.569	6.416	7.143	5.800	6.053
9.244	9.244	6.773	7.008	6.021	5.952
9.610	9.610	7.267	6.999	6.299	5.140
10.193	10.193	7.595	6.653	6.667	4.842
10.707	10.707	8.098	6.605	6.910	4.574
11.348	11.348			7.326	4.562
11.619	11.619			7.604	4.497
11.977	11.977				
12.449	12.449				
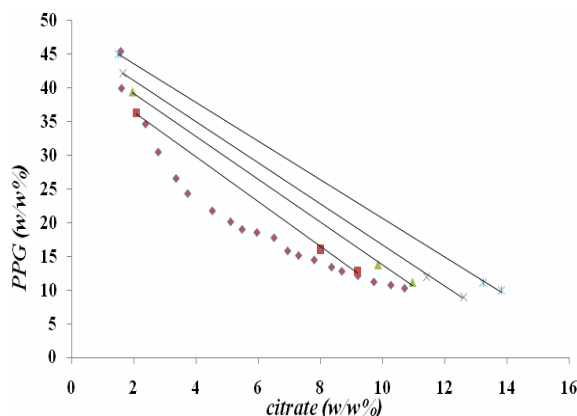
Fig. 2 Tie lines of aqueous two-phase systems of
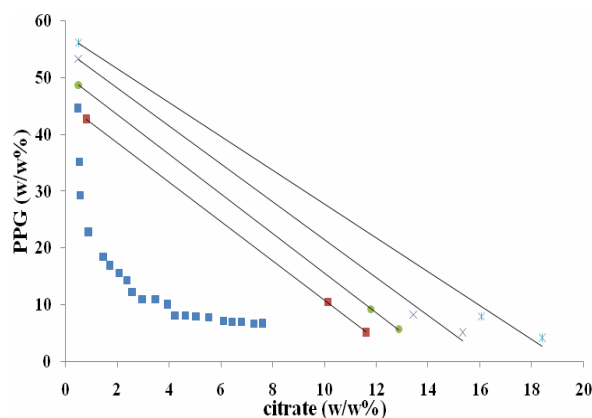PPG425- citrate- water at pH of 3.93.

Fig. 3 Tie lines of aqueous two-phase systems of
PPG425- citrate- water at pH of 5.10.

As mentioned above, the attraction between PPG and water will decrease with an increase in pH and therefore by increasing pH of the aqueous PPG-salt two-phase system water is driven from the PPG-rich phase to the salt-rich phase, so the PPG concentration of the PPG-rich phase increases, while the salt-rich phase will be somewhat more diluted (i.e. the salt concentration will be decreased), and the volume of the salt-rich phase increases at expense of the PPG-rich phase. Close examination of the tie-line data given in Table 1 indicate that, the PPG has been observed to enrich the upper phase, while the salt has been observed to enrich the lower

phase for the aqueous PPG-salt systems at pH 3.93, 4.44, 4.6, 4.97, 5.1, and 8.22.

IV. CONCLUSION

LLE data for the system poly(propylene glycol) + sodium citrate + water, at different pH have been determined experimentally. It was found that the two phase area is expanded with increasing pH. It was also observed that the slope and length of all equilibrium tie-lines increased with increasing pH for the investigated system.

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