

AQUEOUS TWO PHASE (POLY ETHYLENE GLYCOL + SODIUM CITRATE + WATER) SYSTEM: INFLUENCE OF pH, MOLECULAR WEIGHT OF PEG ON BINODAL CURVE AT 30°C

Ramya Devi D., Settu Saravanan

Department of Chemical Engineering, National Institute of Technology, Trichy 620 015

Email: ramyachem@rediffmail.com

ABSTRACT

Aqueous two-phase systems of Poly (ethylene glycol) and a salt are considered as extraction media for large scale downstream processing of industrially important biomolecules. The present paper deals with such kind of systems with different molecular weights of Poly (ethylene glycol) and sodium citrate salt. The binodal curves corresponding to systems of sodium citrate and Poly (ethylene glycol) of different molecular weights (2000, 3000, 4000, 6000, 10000) $\text{g}\cdot\text{mol}^{-1}$, were determined at pH (5, 7, 9). Moreover, the effect of pH and different molecular weights of Poly (ethylene glycol) on binodal data is observed. An increase in pH caused the expansion of the two-phase area. The binodal curves became more asymmetric and close to the origin with an increase in Poly (ethylene glycol) molecular weight.

Key words: ATPS, Poly ethylene glycol, Sodium citrate, Binodal curve.

I. INTRODUCTION

Aqueous two phase systems (ATPSs) are formed when two mutually incompatible water- soluble polymers (e.g., Dextran and Poly ethylene glycol) or one polymer and one inorganic salt (e.g., Polyethylene glycol, PEG and Potassium phosphate) are dissolved in water above a critical concentration. ATPSs have advantages over conventional extraction using organic solvents and those merits include low interfacial tension, non-toxicity nature, biocompatible environment, high water content, and their ease of scale up of the process and availability of commercial separators which allow a faster and continuous protein separation. Among ATPSs suitable for bio separation, those formed by polymer and salt are the most widely used for large scale extraction due to their low cost, low viscosity and rapid disengagement. Several studies [1-7] have combined Polyethylene glycol with different types of salts, the majority with sulfate or phosphate salts, but to a lesser extent, citrate salt has been used as an alternative to substitute phosphate or sulfate in the ATPS formation. Citrate is biodegradable and non-toxic and can be discharged in to biological waste water treatment plants. Nevertheless, for Poly (ethylene glycol) + Citrate salts + Water systems, a limited number of experimental data are reported in the literature[8-14]. Vernau and Kula[8] have measured equilibrium data for aqueous PEG 1550 + Sodium citrate system at room temperature. Zafarani- Moattar

et al [9] determined the phase equilibrium compositions for the PEG 6000 + Sodium citrate aqueous system at different temperatures. Jayapal et al[14] reported liquid-liquid equilibrium data for PEG 6000 + Potassium citrate. In the present work, we report the binodal data for PEG (2000, 3000, 4000, 6000 & 10000) + Sodium citrate + Water system at 30°C. Moreover, the influence of pH (5, 7, and 9) on binodal curve was also studied.

II. EXPERIMENTAL SECTION

A. Materials

Poly (ethylene glycol) of molecular weights of 2000, 3000, 4000, 6000 and 10 000 was obtained from Merck-Schuchardt (Munich, Germany). Sodium citrate and other chemicals were of analytical grade. The polymers used for the present work were without any further purification. Milli-Q water was used throughout the experiments.

B. Experimental Procedure

The determination of binodal curve was carried out by Cloud point method[1]. Stock aqueous solutions of PEG of different molecular weight with mass fraction of 40% and mass fraction of sodium citrate of 30% were prepared by weighing known quantities of the polymer and citrate salt, respectively. The pH of the sodium citrate stock solutions was adjusted to 5, 7 and 9, respectively, by the addition of appropriate quantities

of sodium hydroxide. Small aliquots, approximately (0.01 to 0.05) g, of the polymer stock solution were added to 1 g of the sodium citrate stock solution and placed in a glass tube. After each aliquot addition, the system was thoroughly mixed. The first appearance of turbidity (the cloud point) indicated that the system was about to enter to the two- phase area. With knowledge of the composition of the starting polymer and salt solutions and of the added amounts (grams), the total system composition, just prior to the two-phase formation, was calculated and provided a point on the binodal curve. The starting and added solution masses were measured on an analytical balance with a precision of 1.10^{-4} g. Additional binodal points were obtained by adding a small amount of water to clear the system and then enough drops of the PEG stock solution to produce turbidity again. To obtain the binodal points corresponding to higher polymer concentration, the- mentioned procedure was inverted, thus titrating the stock polymer solution with the stock salt solution. The system temperature was maintained constant and controlled to within $\pm 0.05^{\circ}\text{C}$ by immersing the glass tube and stock solutions in a thermostatic bath.

III. RESULTS AND DISCUSSION

A. Effect of pH on Binodal curve

The effect of pH on the phase forming ability for the systems with different molecular weights of PEG is illustrated in Figure 1-5. The locus for the experimental binodals shown in Figure 1-5 indicates that the two-phase area is expanded with an increase pH which is attributed to the decrease in phase- forming ability in the studied systems. This is because the PEG becomes more hydrophobic with an increase in pH. Thus, by increasing the pH, water is driven from the PEG-rich phase to the salt- rich phase. So, the PEG concentration at the PEG-rich phase increases, while the salt-rich phase will be somewhat more diluted (i.e., the salt concentration will be decreased). In other words water becomes a poorer solvent for PEG as the pH is raised. Depression of the hydrophilicity of PEG with increasing pH may be related to the salting-out phenomenon because a decrease in the solubility of PEG in water with an increase in pH is reflected by a decrease in the critical concentration of a salt required to form a two-phase 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). The binodal curves showed similar shape for the several pH values and tend to superimpose at high PEG or Citrate concentrations, thus indicating that either the exclusion or the salting-out effect respectively prevail in phase separation. When PEG and Citrate concentrations adopt intermediate values, a smaller concentration of citrate is needed for two-phase formation at basic pH values with higher ratios between trivalent and divalent citrate ions. This is in agreement with the well- known fact that trivalent ions are more efficient than divalent ions in promoting the phase separation[15].

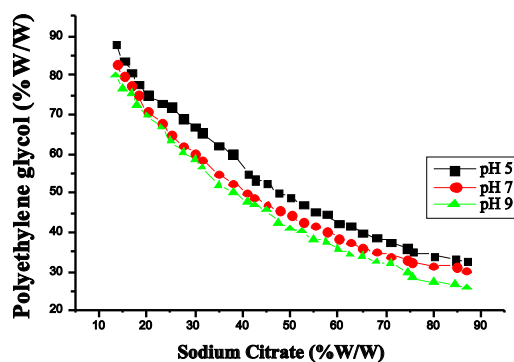


Fig. 1 Effect of pH on Binodal curve for PEG 2000 + Sodium citrate + water system at 30°C

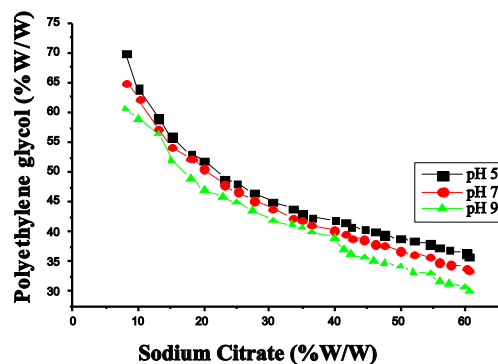


Fig. 2 Effect of pH on Binodal curve for PEG 3000 + sodium citrate + water system at 30°C

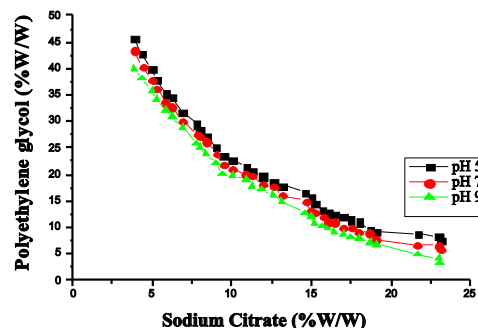


Fig. 3 Effect of pH on Binodal curve for PEG 4000 + sodium citrate + water system at 30°C

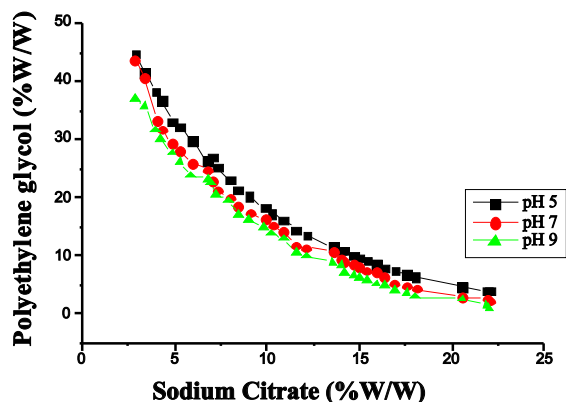


Fig. 4 Effect of pH on Binodal curve for PEG 6000 + sodium citrate + water system at 30°C

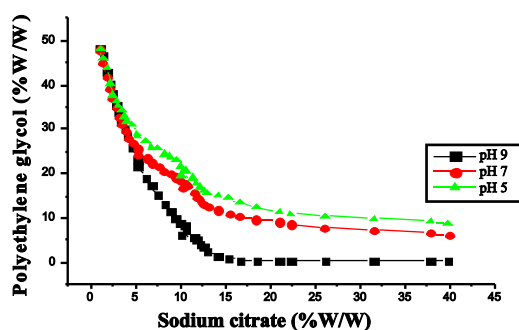


Fig. 5 Effect of pH on Binodal curve for PEG 10000 + sodium citrate + water system at 30°C

B. Effect of PEG Molecular weight on Binodal curve

The Phase compositions for the systems with PEG at three different pH values at 30°C are shown in table 1. For most systems, the PEG concentration in the bottom phase is very small, and in some cases, PEG is almost excluded from this phase while the opposite behavior is observed in the top phase. A similar behavior was observed for the traditional PEG

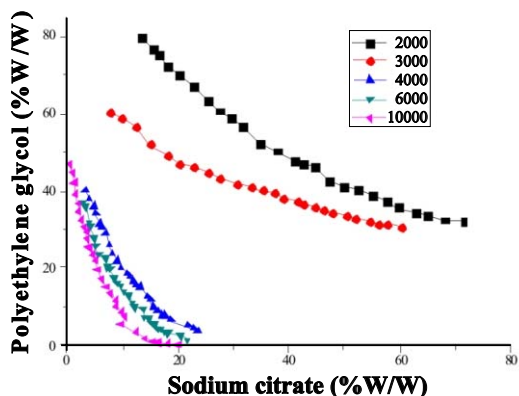


Fig. 6 Effect of Molecular weight on Binodal curve for PEG of varying molecular weight + sodium citrate + water system at 30°C and pH 9.

+ phosphate ATPSs [16]. The binodal curves for ATPSs of sodium citrate and PEGs of different molecular weight at 30°C and at pH 9 are shown in Figure 6.

Table 1. Binodal data for PEG 4000 + sodium citrate + water system at different pH (5,7,9)

pH 5		pH 7		pH 9	
100W _s	100W _p	100W _s	100W _p	100W _s	100W _p
3.72	45.73	3.59	43.27	3.31	39.92
4.35	42.15	4.17	40.82	4.04	37.90
4.90	39.92	4.99	38.13	4.90	35.89
5.16	37.90	5.16	36.34	5.16	34.10
5.89	35.22	5.89	33.87	5.89	31.64
6.34	34.32	6.25	32.76	6.25	30.74
6.79	31.41	6.79	30.07	6.88	28.95
7.70	29.62	7.88	27.60	7.70	25.81
7.79	28.28	8.14	26.48	8.24	23.79
8.87	24.69	8.87	23.58	9.14	22.00
8.96	25.14	9.50	21.97	9.68	20.21
9.41	23.58	10.13	21.70	10.95	18.65
10.04	22.90	10.86	20.05	11.31	17.70
10.77	21.11	11.40	19.77	11.94	16.63
11.31	20.67	11.94	18.65	12.48	15.51
11.76	19.55	12.48	17.30	13.11	15.30
12.48	18.42	13.11	16.19	14.65	12.83
13.30	18.20	14.56	15.07	15.01	11.72
14.74	16.63	15.19	13.28	15.65	10.14
15.46	13.28	15.74	12.38	16.37	9.03
16.37	12.16	16.01	11.04	17.09	7.91
16.91	12.00	16.55	10.37	17.72	7.89
17.45	11.26	17.18	10.14	18.79	6.56
19.26	8.80	18.00	9.03	21.79	5.00
21.76	8.58	19.08	7.91	22.87	4.33
22.97	7.68	21.61	6.50	23.55	3.51

It can be seen from the figure 6 that binodal curves became more asymmetric and close to the origin with increase in PEG molecular weight, thus requiring lower concentrations for phase separation. This may be caused by the increase in the incompatibility between the system components due to the more hydrophobic character of PEGs of higher molecular weight [17].

IV. CONCLUSION

The aqueous two-phase partitioning method of liquid-liquid extraction is useful for separating material of biological origin. Poly (ethylene glycol) + Sodium citrate + Water systems are attractive for protein purification because of low cost, rapid phase separation, and the biodegradability of citrate. However, reliable and complete data on the composition and properties of these systems were not available at

present. Since such information is necessary for the design of an extraction process, we analyzed the effect of different medium conditions such as pH, varying molecular weight of PEG binodal curve of these PEG+ sodium citrate salt systems. An increase in pH and PEG molecular weight caused the expansion of the two-phase area. The trivalent citrate showed to be more effective in phase separation since, at higher pH values, smaller concentrations of citrate were needed for two phase formation.

REFERENCES

- [1] Albertsson, P. A. 1987 Partition of cell particles and macromolecules, 3rd ed.; John Wiley & Sons: New York.
- [2] Hey, M. J.; Jackson, D. P.; Yan, H. 2005 The salting out effect and phase separation in aqueous solutions of electrolytes and poly (ethylene glycol). *Polymer*, 46pp 2567–2572.
- [3] Carvalho, C. P.; Coimbra, J. S. R.; Costa, I. A. F.; Minim, L. A.; Silva, L. H. M.; Maffia, M. C. 2007 Equilibrium Data for PEG4000 + Salt + Water Systems from (278.15 to 318.15) K. *J. Chem. Eng. Data*, 52 pp 351–356 .
- [4] Costa, M. J. L.; Cunha, M. T.; Cabral, J. M. S.; Aires-Barros, M.R. 2000 Scale-up of recombinant cutinase recovery by whole broth extraction with PEG-phosphate aqueous two-phase. *Bioseparation*, 9 pp 231–238.
- [5] Pico, G.; Romanini, D.; Nerli, B.; Farruggia, B. 2006 Polyethyleneglycol molecular mass and polydispersivity effect on protein partitioning in aqueous two- phase systems. *J. Chromatogr. B*, 2 pp286–292.
- [6] Ho-Gutierrez, I. V.; Cheluget, E. L.; Vera, J. H.; Weber, M. E.;1994Liquid-liquid equilibrium of aqueous mixtures of poly(ethylene glycol) with Na₂SO₄ or NaCl. *J. Chem. Eng. Data*, 39pp 245–248.
- [7] Gonzales-Tello, P.; Camacho, F.; Blazquez, G.; Alarcon, F. J.1996 Liquid-liquid equilibrium in the system poly (ethylene glycol) + MgSO₄ + H₂O at 298 K. *J. Chem. Eng. Data*, 41pp 1333–1336.
- [8] Vernau, J.; Kula, M. R. 1990 Extraction of proteins from biological raw material using aqueous poly (ethylene glycol) - citrate phase systems. *Biotechnol. Appl. Biochem*, 12 pp 397–404.
- [9] Zafarani-Moattar, M. T.; Sadeghi, R. Hamidi, A. A. 2004 Liquid-liquid equilibria of an aqueous two-phase system containing polyethylene glycol and sodium citrate: experiment and correlation. *Fluid Phase Equilib.*, 219pp 149–155.
- [10] Perumalsamy, M.; Bathmalakshmi, A.; Murugesan, T. 2007 Experiment and correlation of liquid-liquid equilibria of an aqueous salt polymer system containing PEG6000 + sodium citrate. *J. Chem. Eng. Data*, 52pp 1186–1188.
- [11] Murugesan, T.; Perumalsamy, 2005 M. Liquid-liquid equilibria of poly (ethylene glycol) 2000 + sodium citrate + water at (25, 30, 35, 40, and 45) °C. *J. Chem. Eng. Data*, 50 pp1392–1395.
- [12] Tubío, G.; Pellegrini, L.; Nerli, B. B.; Pico, G. A. 2006 Liquid-liquid equilibria of aqueous two-phase systems containing poly (ethylene glycols) of different molecular weight and sodium citrate. *J. Chem. Eng. Data*, 51, pp 209–212.
- [13] Zafarani-Moattar, M. T.; Hamidi, A. A. 2003 Liquid-liquid equilibria of aqueous two-phase poly (ethylene glycol)-potassium citrate system. *J. Chem. Eng. Data*, 48pp 262–265.
- [14] Jayapal, M.; Regupathi, I.; Murugesan, T. 2007 Liquid-liquid equilibrium of poly (ethylene glycol) 2000 + potassium citrate + water at (25, 35, and 45) °C. *J. Chem. Eng. Data*, 52 pp 56–59.
- [15] Mishima, K.; Nakatani, K.; Nomiya, T.; Matsuyama, K.; Nagatani, M.; Nishikawa, H.1996 liquid-liquid equilibria of aqueous two-phase systems. *J.Chromatogr. B*, 680, pp 3-30.
- [16] Lei, X.; Diamond, A.D.; Hsu, J.T.1990 Equilibrium phase behavior of the poly (ethylene glycol)/potassium phosphate/ water two-phase system at 4°C. *J.Chem.Eng.Data*, 35, pp 420-423.
- [17] Harris, M., 1992 Ed. *Poly (ethylene glycol) chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York.