

## PHASE COMPOSITIONS OF AQUEOUS TWO-PHASE SYSTEMS FORMED BY POLY (ETHYLENE GLYCOL) AND MALTODEXTRIN AT DIFFERENT TEMPERATURES

D. Ramyadevi, A. Subathira and S. Saravanan\*

Department of Chemical Engineering, National Institute of Technology,  
Tiruchirappalli - 620 015, India.

\*Corresponding author: Tel: +919894140206, Fax- 0431-2500133 E-mail: saravanans@nitt.edu

[Received-01/06/2012, Accepted-19/06/2012]

### ABSTRACT:

The phase compositions in liquid-liquid equilibria of aqueous two-phase systems (ATPS) composed of the poly (ethylene glycol) - Maltodextrine at several temperatures (20°C, 30°C and 40°C) are presented. The influence of temperature in the binodal position was pronounced for almost all of the ATPS's, with the phase-separation process being endothermic. An increase in temperature and 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:** liquid-liquid equilibrium, Poly (ethylene glycol), Maltodextrine, Binodal curve,

### INTRODUCTION

Extraction by aqueous two-phase systems (ATPS) has become an important emerging technique for separation, concentration and purification of proteins, biomolecules and pharmaceutical products. Aqueous two-phase system was first applied to recover Bio molecules by Albertson, which later applied to recover the metallic ions, and recently in the extractive crystallization of inorganic salts and in the extraction process of sulfide minerals [1]. The basis of separation using an ATPS is the selective distribution of substances between the two phases which may be affected by different factors like the nature and size of the Bio compound, molecular structure and chain size of the polymer, type of salt, pH, system initial composition, and temperature.

When the biomolecules such as proteins, enzymes, and nucleic acids are purified by the conventional methods, it is usually expensive, difficult to scale up and the product may degrade. To overcome it the bio molecules are separated by ATPSs which provides bio compatibility. Aqueous two-phase systems arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt. When two certain polymers, e.g., Dextran and polyethylene glycol, are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. There is a clear interfacial boundary separating two distinct aqueous-based phases, each preferentially rich with one of the polymers. The aqueous solvent in both phases was demonstrated to provide a media suitable for

biological products [2-4]. For the large-scale isolation of enzymes, the inexpensive polyethylene glycol (PEG)/salt systems are being used, but they are not very selective and can damage fragile proteins. In some cases, when high salt concentration is used, they become a waste disposal problem [5]. The most common polymer-polymer system is composed by dextran and PEG, but this system is very expensive (due to the high cost of dextran) for scaling up. This problem may be solved by the use of alternative economical polymers that substitute for dextran with equivalent partition properties. Maltodextrin (Mdx) can provide a low cost alternative to the fractionated dextran. Maltodextrin (Mdx) is a polysaccharide obtained by the hydrolysis of starch, it is water-soluble and commercially available with polydispersity near that of dextran [6]. The current work is aimed to work on phase diagrams of aqueous two-phase systems formed by water, Maltodextrin, and poly ethylene glycol of different molecular weights determined and the temperature on the binodal curve was also analyzed.

## MATERIALS AND METHODS

### 2.1. Materials

Polyethylene glycol with molecular weights of 4000, 6000, and 10000 (Da) was obtained from Merck-Schuchardt (Munich, Germany). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). Maltodextrine was obtained from Hi Media, India. For the present work the polymers were used without further purification. Milli-Q water was used throughout the experiments.

### 2.2. Apparatus and Procedure

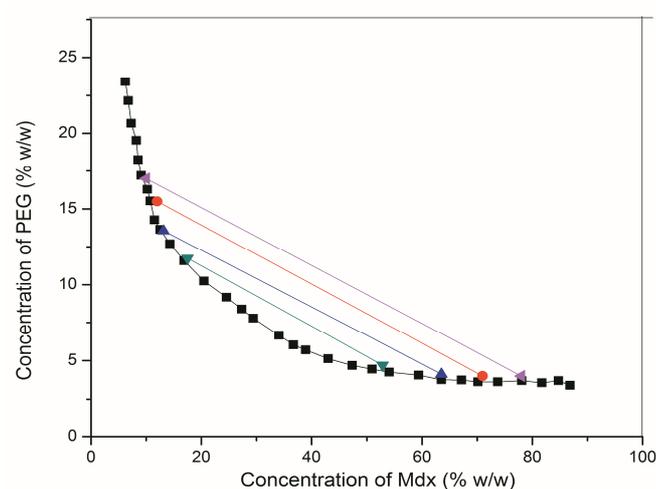
The binodal curves were determined by the cloud point method [7]. The experimental apparatus employed is well known and essentially similar to the one used previously. Initially the stock solution of PEG and Maltodextrine of 40% and 35% respective concentration by weight prepared. A known amount of the salt solution was taken and titrated against PEG. To ensure the end point, PEG was added in drops till the appearance of turbidity. The first appearance of turbidity indicates that the

system is about to enter the two-phase area. Water was then added until the disappearance of turbidity. The procedure was repeated to get the other binodal points.

## 3. RESULTS AND DISCUSSIONS

### 3.1. Binodal Curve:

Binodal curve is a border line between the homogeneous phase and Heterogeneous two phase of ternary mixture. The two phase region formation is due to the incompatibility of system components. Liquid - Liquid Equilibrium gives a brief idea of phase forming composition which is very essential and plays vital role in the development and scale-up of the extraction process using ATPS.

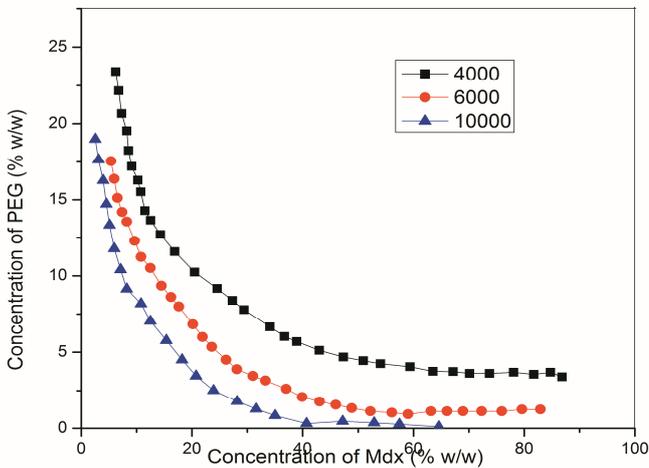


**Fig.1.** Binodal curve and tie lines for polyethylene glycol 4000 (1) + Maltodextrine (2) + water (3) systems at 30°C. The Binodal curve was determined using the cloud-point method. Known masses of polymer and Maltodextrine stock solutions were added to an assay tube to obtain a heterogeneous system. The phase region may vary with the variation of molecular weight of PEG and Temperature there by influences the separation efficiency of the process. Phase diagrams comprise a binodal curve and different parallel tie-lines. While the binodal represents the borderline between the one-phase and the two-phase regions, the tie-line describes the compositions of the two phases in equilibrium. Fig. 1 shows the phase diagrams determined for PEG 4000/ Maltodextrine ATPS, at pH 7. It can be noticed that the tie-lines are practically parallel, with

minor deviations due to experimental errors. In addition, the composition of phases, determined by refractometry and polarimetry, correlates well with the binodal points, obtained by the cloud point method.

**3.2. Effect of Molecular Weight**

The data for Binodal curve represented in table 1 and in Fig.2 The figure 2 which plotted concentration of PEG as ordinate and that of Maltodextrine as abscissa for the molecular weights of PEG of 4000, 6000 and 10000 shows, as the molecular weight of PEG increases the Binodal curve moves closer to the origin stating that lower concentrations are adequate for the two phase formation there by resulted in the expanded area of two phase region. This variation can be explained in terms of excluded volume effect in which the increase in molecular weight of PEG reduces the volume available to bind up the water molecules there by increases the incompatibility between the system components. Luiza et al [8] reported the similar results for different PEG + Maltodextrine system.

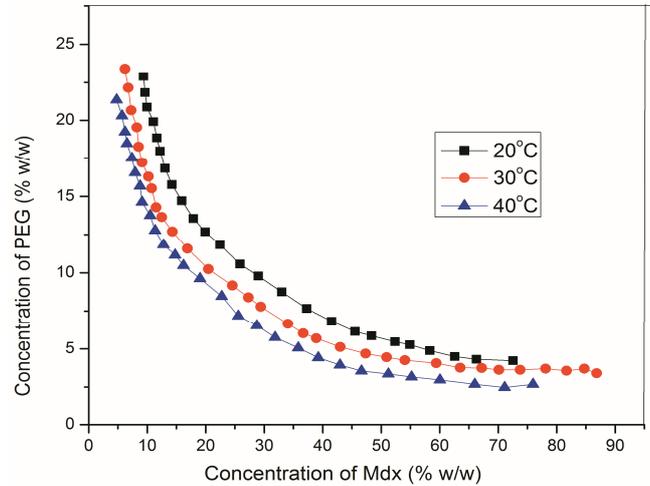


**Fig.2.** Effect of Molecular weight on the binodal curves for polyethylene glycol (1) + Maltodextrine (2) + water (3) systems.

**3.3. Effect of Temperature**

The effect of Temperature on the Biphasic behavior observed through the series of experiments conducting on the system of PEG 4000 + Maltodextrine + Water. The experiments were

conducted at the temperatures of 20°C, 30°C and 40°C.



**Fig.3.** Effect of temperature on the binodal curves for polyethylene glycol (1) + Maltodextrine (2) + water (3) systems.

The Fig.3. shows that minimum concentration required for higher temperature with expanded Biphasic region. With an increase in temperature PEG becomes more hydrophobic. Water becomes a poorer solvent for PEG as the system temperature is raised.

**Table: 1.** Binodal Data for the Polyethylene glycol 4000 (1) + Maltodextrine (2) + Water (3) system at Temperature (20°C, 30°C and 40°C).

T = 20°C		T = 30°C		T = 40°C	
100W <sub>1</sub>	100W <sub>2</sub>	100W <sub>1</sub>	100W <sub>2</sub>	100W <sub>1</sub>	100W <sub>2</sub>
9.37	22.9	6.21	23.41	4.79	21.35
9.64	21.83	6.76	22.15	5.68	20.3
9.95	20.87	7.27	20.67	6.22	19.23
11.06	19.91	8.21	19.54	6.53	18.47
11.63	18.85	8.53	18.26	7.37	17.51
12.21	17.98	9.11	17.21	7.95	16.54
13.06	16.83	10.23	16.29	8.79	15.67
14.21	15.77	10.75	15.53	9.1	14.62
15.9	14.71	11.51	14.27	10.52	13.75
17.9	13.55	12.51	13.64	11.37	12.78
19.9	12.69	14.31	12.7	12.79	11.82
22.47	11.82	16.87	11.57	14.79	11.15
25.89	10.57	20.47	10.24	16.21	10.47
29	9.79	24.58	9.17	19.05	9.61
33	8.74	27.32	8.39	22.74	8.46

37.27	7.67	29.42	7.78	25.58	7.19
41.53	6.82	34.06	6.65	28.74	6.53
45.53	6.14	36.69	6.02	31.85	5.75
48.37	5.86	38.9	5.7	35.85	5.07
52.37	5.47	43.02	5.13	39.27	4.42
54.9	5.27	47.37	4.69	42.95	3.94
58.32	4.89	50.96	4.45	46.64	3.54
62.59	4.5	54.05	4.26	51.22	3.35
66.32	4.31	59.41	4.06	55.21	3.15
72.54	4.22	63.53	3.75	60.06	2.96
		67.17	3.74	66.01	2.67
		70.16	3.62	71.12	2.47
		73.76	3.62	75.96	2.67
		78.12	3.69		
		81.72	3.56		
		84.76	3.69		
		86.84	3.4		

This decrease in solubility of PEG in water decreases the critical concentration of a Maltodextrine required to form a two-phase system, which in turn indicates the effectiveness of the Maltodextrine in inducing the formation of two phases. According to the model proposed by Kjellander and Florin, [9] the entropically unfavorable structuring of water produced by PEG at low temperatures is overcome due to the large decrease in enthalpy (due to the energetically favorable and highly directional interactions, such as hydrogen bonding, between unlike molecules). Even at higher temperatures, the effect of temperature does not break down the structure of water in the PEG hydration shell rapidly, and the unfavorable entropy contribution makes the system phase to separate itself. This trend also agrees with experimental results from the literature for other ATPS [10-11].

#### 4. CONCLUSION

From the experimental studies it has observed that the Biphasic behavior is sensitive in nature and the variation is very significant even with the minute changes in system parameters such as pH,

Temperature and the system composition. The variation of curve with molecular weight had explained in terms of excluded volume effect, and Temperature in terms of hydrophobic nature of PEG. This all represents that higher molecular weight and Temperature are feasible for the formation of two phase system.

#### REFERENCES

- [1] Osseo-Azare K, Zeng X, [2000] Partition of pyrite in aqueous biphasic systems. *Int. J. Miner. Process.* 58: 319-330.
- [2] Albertsson PA, [1986] *Partition of Cell Particles and Macromolecules*, third. ed., Wiley, New York.
- [3] Zaslavsky BY, [1994] *Aqueous Two-Phase Partitioning: Physical Chemistry and Bioanalytical Applications*, Marcel Dekker, New York, 1994.
- [4] Walter H, Brooks DE, Fisher D, [1985] *Partitioning in Aqueous Two-Phase Systems: Theory, Methods, Use, and Applications to Biotechnology*, Academic Press, Orlando.
- [5] Vernau J, Kula MR, [1990] Extraction of Proteins from Biological Raw Material Using Aqueous PEG/citrate Phase Systems, *Biotechnol. Appl. Biochem.* 12: 397-404.
- [6] Luiza HM, da Silva, Antonio JA, Meirelles, [2000] Bovine serum albumin,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin partitioning in polyethylene glycol/maltodextrin aqueous-two-phase systems, *Carbohydr. Polym.* 42:279-282.
- [7] Hatti- Kaul R, [2000] *Aqueous Two-Phase Systems: Methods and Protocols; Methods in biotechnology*; Humana Press: Totowa, NJ,
- [8] Luiza HM, da Silva, Antonio JA, Meirelles, [2000] Phase equilibrium in polyethylene glycol/maltodextrin aqueous two-phase systems *Carbohydr. Polym.* 42: 273-278.
- [9] Kjellander R, Florin E, [1981] Water structure and change in thermal stability of the system poly(ethyleneoxide)-water. *J. chem. Soc. Faraday. Trans. 77*: 2053-2077.
- [10] Se AG, Aznar M, [2002] Liquid-Liquid Equilibrium of Aqueous Two-Phase System water + PEG 4000+ Potassium phosphate at four Temperatures: Experimental determination and Thermodynamic Modeling. *J. Chem. Eng. Data* 47: 1401-1405.
- [11] Ramya devi D, Subathira A, Saravanan S, [2012] Equilibrium phase Behavior of the Poly (ethylene glycol) PEG 4000 and Biodegradable salts at Temperatures of (20°C, 30°C and 40°C). *J. Chem. Eng. Data* 57: 1112-1117.