

NOVEL PHASE DIAGRAMS FOR WATER-FREE TWO-PHASE SYSTEMS BASED ON POLYMERS + IONIC LIQUID + ACETONITRILE

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ABSTRACT

The phase diagram is an essential tool for characterizing a biphasic system applied in separating, concentrating, or purifying biomolecules from biomass. Hydrophobic biomolecules are not applied in aqueous two-phase systems because they precipitate at the interface. In this sense, this work presents novel water-free hydrophobic systems using acetonitrile, ionic liquids (IL), and polymers such as polyethylene glycol (PEG) and polypropylene glycol (PPG) at 298.15 ± 1.00 K and 0.10 ± 0.01 MPA. The hydrophilic characteristic of acetate of 1-ethyl-3-methyl-imidazolium ([C₂mim][OAc]) allows its use as a salting-out agent. Polymers can form a biphasic system, and those with a longer alkyl chain (highest molecular weight) provide larger biphasic areas. Comparing systems formed with different polymers, PPG, being more hydrophobic than PEG, more easily separates the phases of the system.

Keywords: biphasic system, phase diagrams, separation

1 INTRODUCTION

Aqueous two-phase systems (ATPS) are already widely studied in biomolecule separation processes because they have advantages such as biocompatibility, low cost, and easy scale-up¹. However, the separation of hydrophobic molecules in ATPS is questionable since they precipitate at the interface². To overcome this disadvantage, water-free two-phase systems (WFTPS) have recently been introduced, with the complete replacement of water by ethanol (Ethanolic Two-Phase Systems – ETPS)³ and expanding this replacement with other alcohols⁴.

WFTPS have been applied to separating vitamins⁵ and bixin², presenting the same advantages as an ATPS, such as biocompatibility, ease of operation, and scale-up. However, the main disadvantage of the system is the limitation on the constituents that can be used. Therefore, polymers and ionic liquids have been used to construct phase diagrams, which are fundamental in choosing the system for application in extraction and purification processes⁶.

Thus, this work addresses the construction of WFTPS not yet reported in the literature, using acetonitrile as a solvent for diluting the other constituents such as polymers (PEG and PPG) and ionic liquid as 1-ethyl-3-methyl-imidazolium acetate ([C₂mim][OAc]) at 298.15 ± 1.00 K and 0.10 ± 0.01 MPA

2 MATERIAL & METHODS

The binodal curves for water-free two-phase systems based on polymers (PPG and PEG) with different molecular weight + [C₂mim][OAc] + ACN were determined by the cloud point method at 298.15 ± 1.00 K and 0.10 ± 0.01 MPA. Aqueous solution of polymer (60-80 wt%) and IL (80 wt%) were prepared. Repetitive drop-wise addition of the IL to each polymer solution in acetonitrile was carried out until the detection of a cloudy mixture under vigorous magnetic stirring. Then, repetitive drop-wise of acetonitrile was added until the detection of a clear and limpid mixture. The process was performed until a sufficient set of data was obtained to form the binodal curves. The experimental data for each alcohol were adjusted to the empirical equation proposed by (Equation 1)⁷.

$$[\text{Polymer}] = A \times \exp [B \times [\text{IL}]^{0.5} - C \times [\text{IL}]^3] \quad (1)$$

Where [Polymer] and [IL] correspond to mass fraction percentages of polymers (PEG and PPG) and ionic liquid ([C₂mim][AOc]), respectively. A, B and C are constants parameters obtained by non-linear regression.

Points in the biphasic region were used to determine the tie line (TL). Appropriate amounts of the constituents were weighed, dissolved in ACN and added to 3 mL vials. After stirring, centrifugation 2000 rpm for 10 min and thermodynamic equilibrium (4h), the top and bottom phases are separated and the weight and volume of each phase are measured. (TL) was determined individually by solving a system of equations applying the lever arm rule, which describes the relationship between the weight of the upper stage and the total weight and composition of the system. To complete the graph, the length of each tie line (TLL) was calculated, representing the distance between the compositional differences between the polymer-rich and IL-rich phases.

3 RESULTS & DISCUSSION

The determination of phase diagrams constitutes the initial step in the characterization of two-phase systems, and it provides data on the concentrations of polymers (PPG and PEG) and IL necessary for forming distinct phases. ACN is the solvent capable of solubilizing the constituents (polymers and ionic liquid), and for this reason removed from the graphical representation to present the binodal curve (Figure 1 and 2). The Figures were expressed in molality units to avoid ambiguities in the evaluation due to disparities in molecular weights.

The proximity of the binodal curve to the origin of the Cartesian axis indicates the ease of phase separation and consequently the larger area above the binodal curve, which represent the biphasic area⁸. Therefore, the decreasing order of biphasic area size is PPG 4000 > PPG 2000 > PPG 1000 > PPG 725 and PEG 20000 > PEG 8000 > PEG 6000 > PEG 4000⁵. The increase in molecular mass increases the hydrophobicity of the polymer and consequently the incompatibility between the constituents of the system. Therefore, a smaller amount of salting-out agent (ionic liquid) is required to separate the phases.

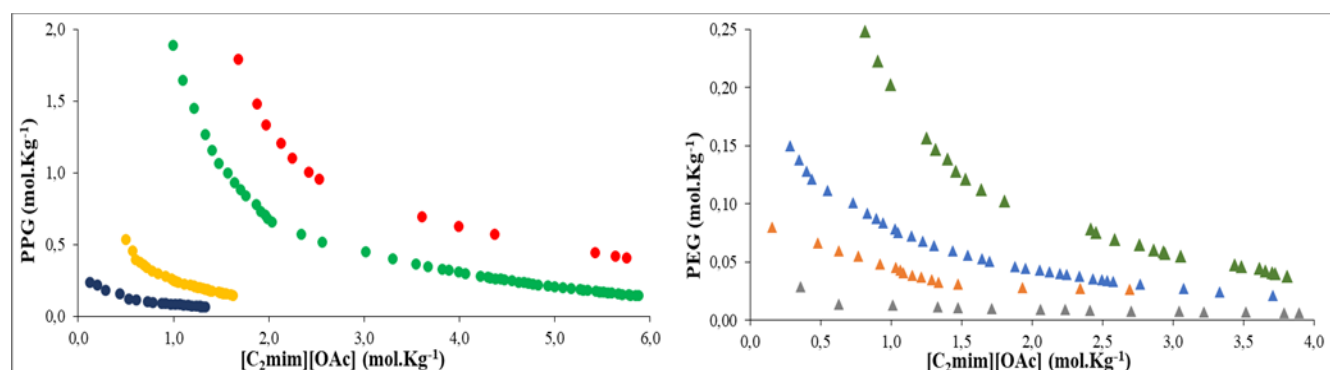


Figure 1: Binodal curves of biphasic systems formed polymers + $[C_2mim][OAc]$ + ACN. ● PPG ($g.gmol^{-1}$): PPG 725; ● PPG 1000; ● PPG 2000 and ● PPG 4000; or PEG ($g.gmol^{-1}$): ▲ PEG 4000; ▲ PEG 6000; ▲ PEG 8000 and ▲ PEG 20000.

Figure 2 depicts a comparison between the binodal curves for the system formed by PEG and PPG with the same molecular mass. It is observed that the additional presence of the methyl group per PPG monomer increased the hydrophobicity of PPG, and repulsion between the molecules, favoring phase separation in relation to PEG¹⁰.

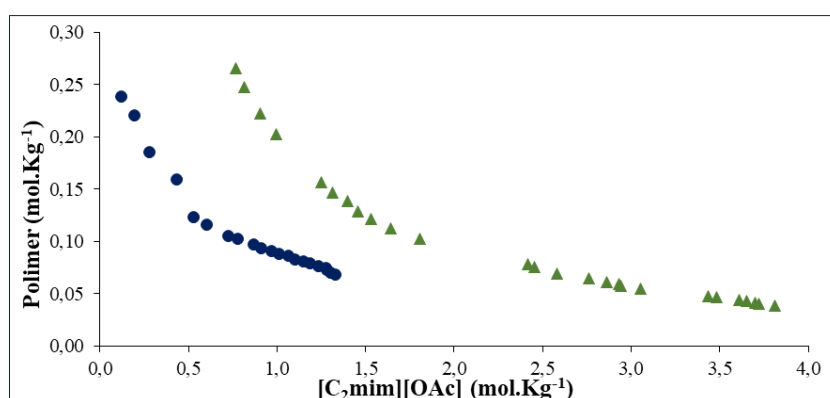


Figure 2: Comparison of binodal curves using different polymers. ● PPG 4000 $g.gmol^{-1}$ and ▲ PEG 4000 $g.gmol^{-1}$

The binodal curves were fit using the mathematical adjustment originally described by Merchuck et al.⁷, through the application of Equation (1). Table 1 presents the regression parameters A, B and C, the respective standard deviations (σ) and the correlation coefficients (R^2), in which it is possible to verify that the adjustment of the equation to the experimental data is satisfactory, with a coefficient of correlation between 0.991 and 0.999. The composition of the mixing point, the top and bottom phases, in addition to the TLL are presented in Table 2.

4 CONCLUSION

For the systems presented (Polymer + $[C_2mim][OAc]$ + ACN at 298.15 ± 1.00 K and 0.10 ± 0.01 MPa, the hydrophilic-hydrophobic balance of the polymer is the driving force for the phase separation of the systems. For this reason, the ease of separation follows the increase in the molecular mass of the polymer. PPG are more hydrophobic than PEG, therefore, they have the largest biphasic area.

Table 1: Regression parameters (A, B and C) and related standard deviations (σ), obtained by applying the Merchuck equation and coefficient of determination (R^2) for ternary systems, at 298.15 ± 1.00 K and 0.10 ± 0.01 MPa.

Systems	(A \pm σ)	(B \pm σ)	$10^{-6}(C \pm \sigma)$	R^2
PPG 750	99 ± 1	$-0,12 \pm 0,01$	$5,18 \pm 0,45$	0,996
PPG 1000	107 ± 2	$-0,16 \pm 0,01$	$7,55 \pm 0,45$	0,999
PPG 2000	118 ± 4	$-0,03 \pm 0,01$	$0,57 \pm 1,82$	0,995
PPG 4000	72 ± 1	$-0,02 \pm 0,04$	$0,11 \pm 0,07$	0,994
PEG 4000	107 ± 6	$-0,23 \pm 0,09$	$9,85 \pm 0,55$	0,998
PEG 6000	78 ± 1	$-0,22 \pm 0,03$	$0,10 \pm 0,05$	0,996
PEG 8000	74 ± 7	$-0,25 \pm 0,02$	$0,17 \pm 2,16$	0,985
PEG 20000	46 ± 1	$-0,15 \pm 0,04$	$0,77 \pm 0,45$	0,999

Table 2: Mass fraction composition for the TLs and respective TLLs, at the top (T) and bottom (B) phase, and initial biphasic composition of the mixture (M), composed of Polymer (PPG or PEG) + IL ([C₂mim][OAc]) + Acetonitrile (ACN) at 298.15 K and 0.1 MPa.

System	Mass Fraction wt%									TLL
	[Polymer] _M	[IL] _M	[ACN] _M	[Polymer] _T	[IL] _T	[ACN] _T	[Polymer] _B	[IL] _B	[ACN] _B	
PPG 725	40.11	39.99	19.90	6.41	69.18	24.41	84.03	1.95	14.02	102.70
	39.89	45.09	15.02	3.57	75.75	20.68	92.89	0.34	6.77	116.89
	39.86	50.23	9.91	1.71	82.80	15.49	96.69	0.01	3.30	127.51
PPG 1000	40.10	39.99	16.61	1.30	73.48	25.22	83.97	2.16	13.87	109.18
	39.94	44.94	15.12	0.30	83.96	15.74	84.58	2.15	13.27	114.84
	39.49	50.25	10.26	0.05	92.87	7.08	86.00	2.15	11.85	123.60
PPG 2000	40.00	40.03	19.97	0.06	78.15	21.79	82.28	1.04	16.68	117.68
	39.91	44.68	15.41	0.72	89.97	9.31	79.73	0.00	20.27	119.73
	39.66	49.55	10.79	0.00	88.46	11.54	91.50	0.86	7.64	123.04
PPG 4000	39.81	39.90	20.29	0.40	85.46	14.14	74.11	0.00	25.89	91.83
	40.08	44.78	15.14	0.04	95.37	4.59	76.18	0.00	23.82	107.51
	39.49	50.52	9.99	0.10	99.71	0.19	79.71	0.01	20.28	117.55
PEG 4000	35.33	29.91	34.76	9.96	43.63	46.41	89.62	0.55	9.83	90.56
	35.20	34.38	30.42	3.71	53.95	42.34	90.64	0.78	8.58	97.05
	34.99	40.15	24.86	0.42	63.98	35.60	93.05	0.44	6.51	103.21
PEG 6000	34.28	34.81	30.91	0.86	65.29	33.85	72.09	0.05	27.86	97.21
	30.17	35.01	34.82	1.33	61.35	37.32	68.28	0.24	31.48	90.58
	40.16	34.67	25.17	0.40	71.34	28.26	78.47	0.24	21.29	104.04
PPG 8000	34.71	29.73	35.56	12.92	46.49	40.59	74.87	0.00	25.13	80.70
	34.82	35.32	29.86	10.51	54.30	35.19	79.64	0.06	20.30	102.79
	34.75	25.34	39.91	14.17	41.41	44.42	66.98	0.17	32.85	77.00
PPG 20000	29.71	30.13	40.16	0.01	47.72	52.27	60.55	11.74	27.71	70.44
	35.02	29.99	34.99	0.02	48.90	51.08	90.26	5.73	4.01	90.85
	39.77	29.73	30.50	5.49	94.37	0.14	55.54	0.02	44.44	103.17

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