

Influence Double Protic Ionic Liquid on the Formation Aqueous Two-Phase Systems

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In this work, we propose the use ATPS based on double protic ionic liquid (2-hydroxyethylammonium acetate (2HEAA), Bis 2-hydroxyethylammonium acetate (BHEAA), 2-hydroxyethyl ammonium butanoate (2HEAB), Bis 2-hydroxyethylammonium butanoate, (BHEAB)). The system initially aims to evaluate the influence of the mixture of two protic ionic liquids in the formation of ATPS. The ternary phase diagrams were determined at 298 (± 1) K and at atmospheric pressure, by the cloud point titration method. Posteriorly, were selected 2HEAA + BHEAB and 2HEAA + BHEAA, where formed another system pure at 2.5 M and these IL were mixed (each at 1.25 M) in different proportions by weight (100% and 0%,) > (75% and 25%) > (50% and 50%) > (25% and 75%) > (0% and 100%). For all the phase diagrams the biphasic region is localized above the binodal curve while the monophasic region is presented below. In general, the larger the biphasic region the higher is the capacity of the PIL induce the liquid-liquid demixing due to different molecular weights of PIL and ACN. The TL data are reliable because the correlation coefficients are bigger than 0.935, showing a good degree of consistency in the experimental data.

1. Introduction

The application, of aqueous biphasic systems (ATPS) for liquid-liquid extraction processes was originally proposed by Albertsson in 1958 (Albertsson, 1958). ATPS are composed of two immiscible aqueous liquid phases macroscopic in concentrations higher than the critical concentration of each component, when they are dissolved in water. The scientific literature describes studies on the application of ATPS for the extraction and purification of biomolecules such as antibiotics (Mokhtarani *et al.*, 2008), enzymes (Souza *et al.*, 2010) and partition of herbicide (Sousa *et al.*, 2017). In the past decade, ionic liquid-(IL)-based ATPS appeared as a novel alternative to polymer-rich systems because of their enhanced performance in extraction and purification approaches (Freire *et al.*, 2012) In fact, the use of ILs as phase-forming components of ATPS permits the tuning of the polarities and affinities of the coexisting phases and improved extractions and selectivity can be foreseen. (Quental *et al.*, 2015).

Ionic liquids (ILs) are molten salts with a melting point below 100 °C, which contain at least one organic cation and organic or inorganic anion (Losetty *et al.*, 2017). A large array of structures owing to the combination of cations (cationic family and alkyl chain) and anions allows the synthesis of over one million different ionic liquids, conferring to this class of solvents the denomination of "design solvents" (Baker *et al.*, 2005). When compared with organic solvent, ILs possess fascinating properties such as non-flammability, high solvation capacity, low vapor pressure, large electrochemical window, excellent chemical stability, and high thermal and electrical conductivity (Chhotaray *et al.*, 2014; Flieger *et al.*, 2014).

IL can be classified into two main categories known as aprotic ionic liquid (AIL) and protic ionic liquid (PIL). The subset PILs are easily synthesized by the equimolar combination of a Brønsted acid and a Brønsted base through the transfer of proton, leading to recognition of a proton-donor and -acceptor site, which generates a hydrogen-bonded network (Álvarez *et al.*, 2010). Other systems have been studied for the formation of ATPS, mainly those formed by organic solvents + salts (Tan *et al.*, 2013) or organic solvents + carbohydrates (Sousa

et al., 2017). However, depending on the type of organic solvents, the second constituent and the mixing point chosen, the condition for having an ATPS (high water content in both phases) may be breaks and the systems become only biphasic systems.

In this work, we propose the use ATPS based on double protic ionic liquid (2-hydroxyethylammonium acetate (2HEAA), Bis 2-hydroxyethylammonium acetate (BHEAA), 2-hydroxyethyl ammonium butanoate (2HEAB), Bis 2-hydroxyethylammonium butanoate, (BHEAB)) and acetonitrile. The system initially aims to evaluate the influence of the mixture of two protic ionic liquids in the formation of ATPS.

2. Material and Methods

2.1 Material

Protic ionic liquids (PIL) (2-hydroxyethylammonium acetate - 2HEAA, bis(2-hydroxyethyl) ammonium acetate - 2BHEAA, bis(2-hydroxyethyl) ammonium butyrate - 2BHEAB) were kindly provided by the Federal University of Bahia. They were synthesized for this work following the methodology of Alvarez and co-workers (Alvarez *et al.*, 2010). ATPS were prepared using the protic ionic liquid and acetonitrile (HPLC grade, 99.9 wt%), which was purchased from Sigma-Aldrich®. In all experiments ultrapure water (type 1 ultrapure water - Direct-Q 3UV) was used.

2.2 Phase Diagrams

Binodal curves data were determined gravimetrically at 298.15 ± 1.00 K and 0.10 ± 0.01 MPa within an uncertainty of $\pm 10^{-5}$ g, using the cloud point method, which was already established in our previous works (Dinis *et al.*, 2015; Santos *et al.*, 2015). Aqueous solutions of acetonitrile - ACN (≈ 80 wt%) and different protic ionic liquids - PIL (≈ 60 – 80 wt%) were prepared. Drop-wise addition of the PIL was carried out to each solution of ACN until the visual detection of a cloud point (biphasic region) was obtained. Subsequently, drop-wise addition of water was carried out until the solution became clear (monophasic region). This methodology was performed under constant stirring and was repeated several times in order to obtain sufficient data for the construction of a binodal curve. The experimental binodal curves were fitted according to Eq. 1: (Merchuck *et al.*, 1998).

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

where [ACN] and [PIL] are, respectively, the acetonitrile and protic ionic liquid mass fraction percentages, and A, B and C are the adjustable parameters.

In order to build the tie-lines (TLs), points in biphasic region of the ternary system was chosen. The constituents of biphasic systems were vigorously stirred, and centrifuged at 400 rpm for 10 min. The tubes were brought to equilibrium in a thermostatic bath at 298.15 K for 4 h at least. The tubes were closed during this period to avoid vaporization of the ACN. After that, the top and bottom phases were carefully separated and weighed and their volume was determined. Individually, each TL was determined by mass balance using a relation to describe the weight of the top phase and the total weight of the system. The TLs determination was then accomplished by solving the following system of four Equations (2)–(5).

$$[\text{ACN}]_T = A \times \exp(B[\text{PIL}]_T^{0.5} - C[\text{PIL}]_T^3) \quad (2)$$

$$[\text{ACN}]_B = A \times \exp(B[\text{PIL}]_B^{0.5} - C[\text{PIL}]_B^3) \quad (3)$$

$$[\text{PIL}]_T = \left(\frac{[\text{PIL}]_M}{\alpha} \right) - \left(\frac{1-\alpha}{\alpha} \right) [\text{PIL}]_B \quad (4)$$

$$[\text{ACN}]_T = \left(\frac{[\text{ACN}]_M}{\alpha} \right) - \left(\frac{1-\alpha}{\alpha} \right) [\text{ACN}]_B \quad (5)$$

where the subscripts M, T, and B indicate the initial mixture and the top and bottom phases, respectively. The value of α is the ratio of the mass of the top phase to the total mass of mixture.

The tie line length (TLL) was determined by applying Equation (6):

$$\text{TLL} = \sqrt{([\text{ACN}]_T - [\text{ACN}]_B)^2 + ([\text{PIL}]_T - [\text{PIL}]_B)^2} \quad (6)$$

The critical point of the ternary phase system was determined by extrapolating the TLs of individual systems by applying Equation (7).

$$[\text{ACN}] = f + g[\text{PIL}] \quad (7)$$

where f and g are fitting parameter.

The measured TLs consistencies of the pure and commercial fructose were determined using the Othmer–Tobias Equation (8) and the Bancroft Equation (9):

$$\left(\frac{100 - [\text{ACN}]_{\text{T}}}{[\text{ACN}]_{\text{T}}} \right) = k_1 \left(\frac{100 - [\text{PIL}]_{\text{B}}}{[\text{PIL}]_{\text{B}}} \right)^{\eta} \quad (8)$$

$$\left(\frac{100 - [\text{ACN}]_{\text{T}} - [\text{PIL}]_{\text{T}}}{[\text{ACN}]_{\text{T}}} \right) = k_2 \left(\frac{100 - [\text{PIL}]_{\text{B}} - [\text{PIL}]_{\text{B}}}{[\text{PIL}]_{\text{B}}} \right)^r \quad (9)$$

where k_1 , η , k_2 , and r are the fitting parameters. Linear dependence indicates the consistency of the results.

3. Results and Discussions

3.1 Phase Diagrams

The binodal curves for the system composed by ACN + PIL were measured at 298.15 K and atmospheric pressure (0.10 MPa). O ATPS were constituted of ACN and different PIL, therefore the easy phase formation was attributed only to PIL. This study presents a great evidence that PILs based on ethanolamine, diethanolamine and methyl-monoethanolamine have the ability to induce liquid-liquid separation in systems formed of PIL and acetonitrile.

The protonic IL anion chain demonstrated a higher ability to form ATPS by acetate, followed by propanoate and butanoate, respectively. Posteriorly, the extremes curves were selected 2HEAA + BHEAB and formed another system pure at 2.5 M and these IL were mixed (each at 1.25 M) in different proportions by weight (100% and 0%,) > (75% and 25%) > (50% and 50%) > (25% and 75%) > (0% and 100%) (Figure 1), these curves binodal followed the order of the higher concentration of 2HEAA is greater region biphasic of system. The study of the mixing of two ILs of the same anion chain was evaluated, which (2HEAA + BHEAA) were selected and formed new systems pure at 2.5 M and posteriorly, systems were formed with ILs mixed (each at 1.25 M) in different proportions by weight, (100% and 0%,) > (75% and 25%) > (50% and 50%) > (25% and 75%) > (0% and 100%), as show in Figure 1 The curves followed the standard order, the higher the concentration of 2HEAA is greater the biphasic region of the system.

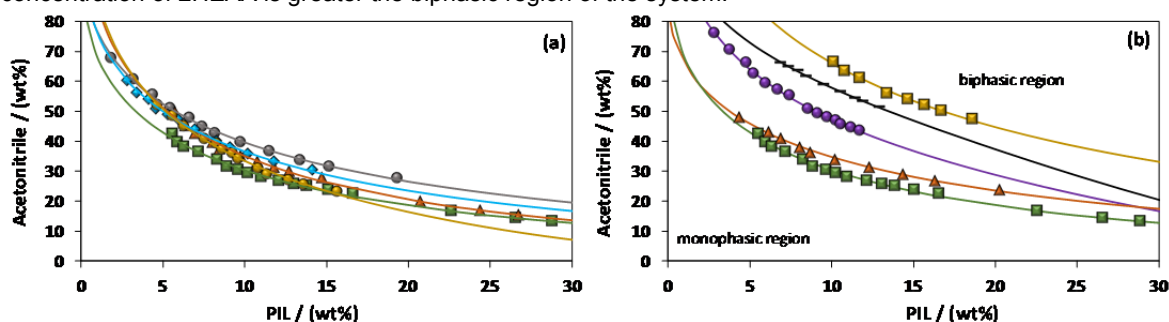


Figure 1: Phase diagram composed of acetonitrile + protic ionic liquids + water: (a) BHEAA + 2HEAA (100% + 0% (■), 75%+ 25% (●), 50%+50% (▲), 25%+75% (◆), 0% + 100% (●)); (b) 2HEAA + BHEAB (100% + 0% (■), 75%+ 25% (▲), 50%+50% (●), 25%+75% (◆), 0% + 100% (■)).

The nanoscale aggregates present in protic ionic liquids were stable in size on dilution to high concentrations of water, indicating that the water is localized in the ionic region and has little effect on the nonpolar domains. The water solution of precursors of PIL (Brønsted acid and Brønsted base amines containing multiple alkyl chains or hydroxyl groups) did not display nanostructure at any water concentration. Primary amine Brønsted bases formed aggregates in water, which generally displayed characteristics of poorly structured microemulsions or a form of bicontinuous phase (Tokuda *et al.*, 2004). As the nanostructure of the protic ionic liquid is little changed, the influence of the partial hydrolysis on the phase separation is probably small.

Table 1: Correlation parameters of equation 1 used to describe the experimental binodal data for system based on acetonitrile and double protic ionic liquids at 298.15 K and 0.1 MPa, standard deviation (σ) and correlation coefficient (R^2)

| Ionic liquid | | Regression Parameters | | | R^2 |
|---------------|-------------|-----------------------|-------------------|--|--------|
| | | A $\pm \sigma$ | B $\pm \sigma$ | C $\pm \sigma$ | |
| 2HEAA+ BHEAA | 0% and 100% | 103.59 \pm 1.55 | -0.31 \pm 0.007 | $1.84 \times 10^{-14} \pm 3.97 \times 10^{-6}$ | 0.9988 |
| | 25% and 75% | 105.65 \pm 2.42 | -0.34 \pm 0.01 | $6.5 \times 10^{-14} \pm 1.01 \times 10^{-5}$ | 0.9980 |
| | 50% and 50% | 127.39 \pm 8.69 | -0.41 \pm 0.02 | $2.3 \times 10^{-14} \pm 4.69 \times 10^{-6}$ | 0.9934 |
| | 75% and 25% | 134.07 \pm 8.23 | -0.43 \pm 0.02 | $2.09 \times 10^{-5} \pm 1.53 \times 10^{-5}$ | 0.9951 |
| | 100% and 0% | 99.02 \pm 0.46 | -0.37 \pm 0.003 | $5.2 \times 10^{-15} \pm 1.49 \times 10^{-6}$ | 0.9994 |
| 2HEAA + BHEAB | 75% and 25% | 89.05 \pm 3.17 | -0.29 \pm 0.01 | $7.29 \times 10^{-16} \pm 4.89 \times 10^{-6}$ | 0.9969 |
| | 50% and 50% | 129.76 \pm 2.80 | -0.31 \pm 0.01 | $1.2 \times 10^{-5} \pm 1.24 \times 10^{-5}$ | 0.9983 |
| | 25% and 75% | 122.21 \pm 4.57 | -0.23 \pm 0.01 | $1.94 \times 10^{-5} \pm 8.09 \times 10^{-6}$ | 0.9993 |
| | 0% and 100% | 169.1 \pm 25.47 | -0.29 \pm 0.04 | $1.77 \times 10^{-15} \pm 1.11 \times 10^{-5}$ | 0.9941 |

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 acetonitrile ([ACN]) and mixture protic ionic liquid ([PIL]) at 298.15 K and 0.1 MPa.

| Ionic liquid | | Weight fraction composition / wt% | | | | | | |
|--------------|-------------|-----------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|
| | | [ACN] _M | [PIL] _M | [ACN] _T | [PIL] _T | [ACN] _B | [PIL] _B | TLL |
| 2HEAA+ BHEAA | 0% and 100% | 44.56 | 10.39 | 65.46 | 2.32 | 31.75 | 15.43 | 34.32 |
| | | 50.13 | 9.91 | 71.74 | 1.45 | 26.86 | 19.21 | 47.11 |
| | | 54.32 | 10.50 | 77.84 | 0.88 | 24.21 | 22.81 | 57.94 |
| | | 55.01 | 12.78 | 84.04 | 0.52 | 20.65 | 27.33 | 67.04 |
| | 25% and 75% | 44.56 | 10.39 | 69.30 | 1.57 | 25.92 | 17.29 | 41.80 |
| | | 49.87 | 10.37 | 75.92 | 0.97 | 23.78 | 19.79 | 55.43 |
| | | 54.23 | 10.72 | 84.01 | 0.47 | 22.11 | 22.07 | 65.46 |
| | | 54.69 | 12.66 | 89.27 | 0.31 | 19.59 | 24.91 | 72.05 |
| | 50% and 50% | 44.92 | 9.95 | 69.03 | 2.26 | 23.76 | 17.02 | 43.94 |
| | | 50.10 | 10.15 | 76.43 | 1.57 | 20.87 | 19.58 | 58.43 |
| | | 54.93 | 10.11 | 82.48 | 1.02 | 19.29 | 21.56 | 67.79 |
| | | 54.93 | 12.45 | 90.90 | 0.63 | 15.97 | 25.24 | 76.48 |
| | 75% and 25% | 44.76 | 10.30 | 70.49 | 2.16 | 18.40 | 18.95 | 55.54 |
| | | 49.89 | 10.09 | 77.14 | 1.54 | 16.13 | 20.93 | 64.95 |
| | | 54.65 | 10.40 | 84.58 | 1.09 | 13.60 | 23.08 | 77.16 |
| | | 55.35 | 11.95 | 91.02 | 0.90 | 10.37 | 25.94 | 83.83 |
| 100% and 0% | 44.81 | 9.95 | 73.89 | 0.68 | 19.95 | 18.28 | 51.39 | |
| | 50.09 | 9.88 | 79.84 | 0.37 | 18.37 | 20.27 | 63.69 | |
| | 54.85 | 10.08 | 85.16 | 0.15 | 17.04 | 22.22 | 72.18 | |
| | 54.88 | 12.44 | 92.01 | 0.04 | 15.10 | 25.06 | 81.13 | |
| 2HEAA+ BHEAB | 75% and 25% | 59.43 | 10.28 | 70.72 | 0.65 | 28.35 | 14.58 | 34.66 |
| | | 64.76 | 10.45 | 75.70 | 0.31 | 26.27 | 16.42 | 44.77 |
| | | 69.47 | 10.40 | 81.02 | 0.07 | 25.78 | 18.29 | 55.43 |
| | | 69.59 | 12.71 | 87.78 | 0.01 | 22.25 | 21.86 | 66.08 |
| | 50% and 50% | 59.27 | 10.37 | 77.46 | 2.66 | 20.01 | 27.23 | 60.51 |
| | | 64.17 | 10.64 | 84.96 | 1.65 | 15.84 | 31.55 | 73.46 |
| | | 69.63 | 10.15 | 89.42 | 1.24 | 11.14 | 36.32 | 82.96 |
| | | 70.06 | 12.61 | 93.55 | 1.22 | 7.16 | 41.71 | 96.16 |
| | 25% and 75% | 40.17 | 10.49 | 68.06 | 6.31 | 32.5 | 22.62 | 39.09 |
| | | 44.50 | 10.44 | 80.70 | 3.21 | 17.25 | 32.15 | 60.12 |
| | | 49.87 | 10.37 | 87.02 | 2.22 | 11.21 | 36.95 | 76.16 |
| | | 49.38 | 12.93 | 95.29 | 1.16 | 7.04 | 41.51 | 95.99 |
| | 0% and 100% | 70.12 | 9.95 | 78.75 | 6.59 | 45.20 | 19.65 | 35.99 |
| | | 70.01 | 12.51 | 91.43 | 4.23 | 37.17 | 26.06 | 61.23 |
| | | 69.74 | 14.92 | 96.07 | 3.69 | 33.16 | 29.97 | 69.87 |

The nanoscale aggregates present in protic ionic liquids were stable in size on dilution to high concentrations of water, indicating that the water is localized in the ionic region and has little effect on the nonpolar domains. The water solution of precursors of PIL (Brønsted acid and Brønsted base amines containing multiple alkyl chains or hydroxyl groups) did not display nanostructure at any water concentration. Primary amine Brønsted bases formed aggregates in water, which generally displayed characteristics of poorly structured microemulsions or a form of bicontinuous phase (Tokuda *et al.*, 2004). As the nanostructure of the protic ionic liquid is little changed, the influence of the partial hydrolysis on the phase separation is probably small. The precision of the TLs for the system was verified by applying the Othmer–Tobias and Bancroft equations (7) and (8), and the respective fitting parameters and correlation coefficients (R^2) are shown in Table 3. However, the TL data are reliable because the correlation coefficients are bigger than 0.935, showing a good degree of consistency in the experimental data.

Table 3. Values of the parameters of Othmer-Tobias and Bancroft equations for aqueous two-phase system based on acetonitrile + protic ionic liquid + water and their correlation coefficients (R^2) at 298.15 K and 0.1 MPa.

| Ionic liquid | | Othmer-Tobias | | | Bancroft | | |
|--------------|-------------|---------------|--------|-------|----------|--------|-------|
| | | k_1 | n | R^2 | k_2 | R | R^2 |
| 2HEAA+ BHEAA | 0% and 100% | 1.813 | -3.599 | 0.950 | 2.116 | -3.206 | 0.960 |
| | 25% and 75% | 3.115 | -5.600 | 0.992 | 3.578 | -5.032 | 0.993 |
| | 50% and 50% | 3.029 | -5.528 | 0.985 | 3.734 | -5.461 | 0.983 |
| | 75% and 25% | 3.613 | -6.075 | 0.994 | 5.003 | -6.886 | 0.989 |
| | 100% and 0% | -8.055 | 10.95 | 0.984 | -30.57 | 35.945 | 0.972 |
| 2HEAA+ BHEAB | 75% and 25% | 2.624 | -5.435 | 0.997 | 3.005 | -4.916 | 0.995 |
| | 50% and 50% | 3.232 | -5.187 | 0.989 | 4.006 | -5.765 | 0.988 |
| | 25% and 75% | 1.145 | 2.641 | 0.994 | -0.923 | -1.047 | 0.993 |
| | 0% and 100% | 4.224 | -7.133 | 0.935 | 4.632 | -4.446 | 0.963 |

4. Conclusion

This work has studied the ability of protic double ionic liquids based on amines and organic acids to form biphasic systems with acetonitrile. The phase diagrams were determined at 298.15 K and atmospheric pressure. The curves followed the standard order of hydrophilicity, the higher the concentration of 2HEAA, greater the biphasic region of the system. Therefore, the 2HEAA (largest biphasic area) acts as phase-forming and the other PILs (BHEAA and BHEAB) as adjuvant in the biphasic system. For all the phase diagrams the biphasic region is localized above the binodal curve while the monophasic region is presented below. In general, the larger the biphasic region the higher is the capacity of the PIL induce the liquid-liquid demixing due to different molecular weights of PIL and ACN. The TL data are reliable because the correlation coefficients are bigger than 0.935, showing a good degree of consistency in the experimental data.

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