USE OF AQUEOUS TWO-PHASE SYSTEM FOR PARTITION OF GALLIC ACID AND 4-HYDROXYBENZOIC ACID FOUND IN THE HERB LEONURUS SIBIRICUS

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ABSTRACT – Leonurus sibiricus is an herb very used as medicinal teas due to its phenolic compounds. An environmentally friendly method for liquid-liquid extraction has been considered due to ecotoxicological effects potentially produced by organic solvents applied in traditional methods. Polyethylene glycol ((PEG 6000) + ammonium sulfate + water) and (Deep eutectic solvent (DES) + dipotassium fosfate + water) ATPS (Aqueous two-phase system) were applied to study partition of gallic and 4-hydroxybenzoic acids. Binodal curves for ATPS composed of PEG were determined by cloud point method at (298.15 and 318.15) K. Liquid-liquid equilibrium data were obtained by applying a gravimetric method. Temperature effect was not relevant on binodal curves, the slopes of tie-line and tie-line length behavior. Partitioning of gallic and 4-hydroxybenzoic acids in ATPS was investigated at different temperatures and tie-lines lengths. It was shown that the partitioning was depended on the temperature and presenting good efficiency being ATPS system promising for these biomolecules partition.

1. INTRODUCTION

One of the most popular drinks in the world used as medicine is tea. It’s been referenced as one of the best sources of antioxidant nutrients such as carotenoids, tocopherols, and flavonoids (Pasha & Reddy, 2005). The Leonurus sibiricus herb known as rubim is found in the entire Brazilian territory and is used as medicinal tea due to its rich composition in phenolic compounds such as gallic, caffeic, 4-hydroxybenzoic and p-coumaric acids (Oliveira et al., 2017). Gallic acid is a phenolic acid used in the pharmaceutical and food industries (Saeki et al., 2000) and 4-hydroxybenzoic acid is also a phenolic acid with antibacterial properties (Vanhoof et al., 1986). One of the ways to purify these compounds from tea solutions is the aqueous two-phase system (ATPS), which are formed when two mutually incompatible systems of polymers/polymers, polymers/salt or salt/salt are dissolved in water above a certain thermodynamic condition (temperature, pressure, and composition). ATPS Polymer/salt combinations have several applications, such as separation, concentration, and fractionation of solutes and biological particles, because they are rich in water and do not degrade biomolecules (De Araujo Sampaio et al., 2016). Thus, the objective of this work was to evaluate the partition coefficient (K) and the separation efficiency of gallic acid and 4-hydroxybenzoic acid in three aqueous two-phase system: DES +K₂PO₄ at 298.15 K; PEG 6000 + (NH₄)₂SO₄ at 298.15 K and 318.15 K.
2. MATERIALS AND METHODS

2.1. Materials

PEG (6000 g.mol\(^{-1}\)) was purchased from Oxiteno. Gallic and 4-hydroxybenzoic acids were purchased from Sigma Aldrich, sucrose and choline chloride (DES) from Anidrol. All of them used as received. \((\text{NH}_4)_2\text{SO}_4\) and \(\text{K}_2\text{PO}_4\) were purchased from Anidrol and dried in an oven at 378.15 K for 2 hours before its use. DES was made according to Abbott et al. (2004) methodology.

2.2. Methods

Binodal curve: Binodal curves were determined for PEG 6000 system at 298.15 and 318.15 K by cloud point method (Kaul, 2000).

Tie-lines (TLs): TLs were determined by a gravimetric method proposed by Merchuck et al., (1998). Three points of overall composition situated in the biphasic region were chosen and prepared in centrifuge tubes (15 mL) as previously described by Sosa et al., 2017. The slope of the tie-line (STL) and tie-line length (TLL) were determined by Equation 1 where \(Y_T\) and \(Y_B\) are DES or PEG mass fraction at the top and bottom phase \(X_T\) and \(X_B\) are salt mass fraction at the top and bottom phase.

\[
STL = \frac{Y_T - Y_B}{X_T - X_B} \quad \text{and} \quad TLL = \left(\frac{(X_T - X_B)^2 + (Y_T - Y_B)^2}{2}\right)^{\frac{1}{2}}
\]  

Partition of gallic and 4-hydroxybenzoic acid: Mixtures were prepared in the biphasic region. The mixtures were mixed by a vortex mixer until the complete dissolution of compounds and maintained in equilibrium for 24 h in a water bath at 298.15 K and 318.15 K, covered by aluminum foil due to photosensitive characteristic of gallic and 4-hydroxybenzoic acids.

Partition coefficients \((K_i)\) and efficiency \((E\%)\) for both acids between the top and bottom phases were calculated at 298.15 K and 318.15 K according to Equation 2, which relates the fraction of the concentration and volume of a biomolecule in the top and bottom phases. For this experiment, water was substituted by aqueous gallic acid/4-hydroxybenzoic solutions with 0.5 g/L and 1 g/L, respectively.

\[
K = \frac{c_T}{c_B} \quad \text{and} \quad E(\%) = \frac{c_T v_T}{c_T v_T + c_B v_B}
\]  

Quantification of gallic and 4-hydroxybenzoic acids content in the top and bottom phases: The quantification was executed in a UV–Vis spectroscopy (Shimadzu-1240), at wavelength 262 nm and 254 nm, respectively.

3. RESULTS AND DISCUSSION

3.1. Liquid-liquid Equilibrium Data

Influence of Temperature: Although an increase in the temperature can promote an increase in the biphasic region since aqueous two-phase formation processes are endothermic, such behavior was not observed in this study as can be seen in Figure 1A. This result is consistent with the previous
report published by De Araujo Sampaio et al. (2016). Figure 1B shows the tie-line experimental data determined at different temperatures and a parallelism observed among them implies that the overall composition of the system has no expressive effect upon the slope of the TLs.

Figure 1- (A) Binodal curves and (B) tie-line of (PEG 6000 + (NH₄)₂SO₄ + water) at 298.15 K and 318.15 K.

Both system phase compositions are shown in Tables 1 and 2. It can be observed that the increase in temperature did not present large effect on STL and TLL values. It is possible that temperature presents some effect on the partitioning of a specific molecule.

Table 1 - Experimental LLE data for the (DES + K₂PO₄ + aqueous Gallic/4-hydroxybenzoic acid solution) system at 298.15 K and p = 91 kPa

| Overall Composition | Top Phase | Bottom Phase |
|---------------------|-----------|--------------|
| TL                  | Salt      | DES          | Solution | Salt | Choline | Solution | Sucrose | Salt | Choline | Solution | Sucrose | TLL | STL |
| 1                   | 28.6      | 28.6         | 42.8      | 14.9  | 33.8    | 44.2      | 7.1      | 35.6  | 10.8    | 42.9     | 10.9    | 0.3  | -1.1 |
| 2                   | 29.9      | 30           | 40.1      | 9.1   | 44.4    | 40.7      | 5.9      | 41.3  | 6.9     | 40       | 12.2    | 0.5  | -1.2 |
| 3                   | 31.8      | 31.7         | 36.5      | 7.4   | 50.6    | 37.2      | 4.8      | 45.1  | 5.5     | 35.7     | 13.6    | 0.6  | -1.2 |

Adapted from Farias et al. (2017)

Table 2 - Experimental LLE data for the (PEG 6000 + (NH₄)₂SO₄ + aqueous Gallic/4-hydroxybenzoic acid solution) two-phase system at T = (298.15 and 318.15) K and pressure p = 91 kPa

| Overall Composition | Top Phase | Bottom Phase |
|---------------------|-----------|--------------|
| TL                  | Salt      | PEG          | Solution | Salt | PEG | Solution | Sucrose | Salt | PEG | Solution | Sucrose | TLL | STL |
| 298.15 K            | 1         | 10.1         | 10.0      | 79.9  | 6.7 | 19.2      | 74.2     | 12.5  | 3.2 | 84.4     | 0.2      | -2.8 |
|                     | 2         | 12.7         | 12.6      | 74.7  | 3.3 | 37.3      | 59.4     | 17.3  | 0.2 | 82.5     | 0.4      | -2.7 |
|                     | 3         | 15.5         | 15.6      | 68.9  | 1.9 | 51.2      | 46.9     | 21.6  | 0.0 | 78.4     | 0.5      | -2.6 |
| 318.15 K            | 1         | 10.1         | 10.1      | 79.8  | 4.4 | 28.0      | 67.6     | 13.2  | 0.4 | 86.4     | 0.3      | -3.1 |
|                     | 2         | 12.6         | 12.6      | 74.7  | 3.1 | 39.9      | 57.0     | 17.1  | 0.0 | 82.9     | 0.4      | -2.9 |
|                     | 3         | 15.6         | 15.5      | 68.9  | 2.1 | 53.4      | 44.5     | 21.2  | 0.0 | 78.8     | 0.6      | -2.8 |
3.2. Partition of Gallic Acid and 4-hydroxybenzoic Acid

The partition coefficient of gallic acid (\(K_{GA}\)) and 4-hydroxybenzoic acid (\(K_{4HA}\)), as well as their extraction efficiencies at gallic acid (\(E_{GA}\) %) and 4-hydroxybenzoic acid separation (\(E_{4HA}\) %), are presented in Figure 2. In all cases, the \(K_{GA}\) and \(K_{4HA}\) values were bigger than 1 which indicates the preference of both acids for the PEG/DES rich phase and higher values of \(K\) results in higher \(E\) % at the top phase.

![Figure 2](image.png)

Figure 2 – (A) Partition coefficients (\(K\)) and (B) extraction efficiencies (\(E\)) of gallic and 4-hydroxybenzoic acid at systems composed by DES and PEG.

Figure 3 showed that PEG 6000 had better results of \(K_{GA}\) and consequently \(E_{GA}\) than the values found for DES systems. The results obtained for 4-hydroxybenzoic acid are very similar for DES and PEG 6000 systems whereas choline chloride’s system presented better results with 4-hydroxybenzoic acid as well as in PEG’s system. The better \(K_{GA}\) and \(E_{GA}\) results of gallic and 4-hydroxybenzoic acids were 40.7 and 95.4%, and 22.2 and 96.5% respectively, both at 298.15 K in a PEG system for the first acid and in choline chloride’s system for the second one.

4. CONCLUSION

This work demonstrates the possibility of using two-phase aqueous systems as a methodology for purification of biomolecules from aqueous solutions of \textit{Leonurus sibiricus}. Several parameters were evaluated to understand the partitioning of phenolic acids in ATPS. The evaluated parameters include the slope of the tie-line, tie-line length, efficiency and partition coefficient. The partition was effective for both biomolecules and the highest efficiency for gallic and 4-hydroxybenzoic acids were 95.4% and 96.5%, respectively. Thus, this system may be promising for purification of such phenolic acids.

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