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PII: S1383-5866(17)33966-7
DOI: <https://doi.org/10.1016/j.seppur.2018.02.029>
Reference: SEPPUR 14393

To appear in: *Separation and Purification Technology*

Received Date: 3 December 2017
Revised Date: 9 February 2018
Accepted Date: 13 February 2018

Please cite this article as: F. Oliveira Farias, H. Passos, M. Gomes Sanglard, L. Igarashi-Mafra, J.A.P. Coutinho, M.R. Mafra, Designer solvent ability of alcohols in aqueous biphasic systems composed of deep eutectic solvents and potassium phosphate, *Separation and Purification Technology* (2018), doi: <https://doi.org/10.1016/j.seppur.2018.02.029>

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Designer solvent ability of alcohols in aqueous biphasic systems composed of deep eutectic solvents and potassium phosphate

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Abstract

Deep eutectic solvents (DES) have been proposed as phase forming components of aqueous biphasic systems (ABS). However, the DES hydrogen bonding complexes are not stable in the high concentrations of water present in this type of systems. Therefore, as previously shown, DES-based ABS should be treated as quaternary systems. This confers DES-based ABS with an extra degree of freedom for the design of separation processes since while one of the DES components acts as a phase forming component, the other could induce the modification of the ABS phase properties and, consequently, the control of the partition of various biomolecules. In this context, the designer solvent effect of the hydrogen bond donor (HBD), using four different alcohols – ethanol, *n*-propanol 1,2-propanediol and ethylene glycol – mixed at three different molar fractions (2:1; 1:1 and 1:2) with cholinium chloride (the hydrogen bond acceptor, HBA) in quaternary systems composed of K₂HPO₄ and water, was evaluated in this work. The results show that the presence of the HBD has an impact upon the liquid-liquid equilibrium, and these changes are dependent on the alcohol nature. The NRTL model was correlated to the tie-line experimental data with a low mean deviation. Moreover, several biomolecules (phenolic compounds, alkaloids, and amino acids) were used as molecular probes to evaluate the ability of alcohols to tune the partition in the studied systems. The alcohol presence changes the properties of the ABS's phases and it is here shown that the HBD of the DES can indeed be used to modulate the partition behavior of target molecules.

Keywords: aqueous biphasic system, quaternary systems, deep eutectic solvents, designer solvents, partition, biomolecules.

1. INTRODUCTION

Traditional aqueous biphasic systems (ABS) are constituted by immiscible aqueous solutions of two polymers, two salts or a polymer and a salt, and have been intensively explored and used in the separation and purification of several biomolecules.[1–5] Due to their renewable character, high biodegradability, and easy synthesis, deep eutectic solvents (DES) were also proposed as phase former components of ABS.[6–13] DES are solvents that result from the formation of strong hydrogen bonding complexes between a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD).[14–16]

DES-based ABS were successfully applied in the extraction of a large range of compounds, such as proteins,[6–10] textile dyes,[11] phenolic compounds,[12,13] amino acids and alkaloids.[13] However, Coutinho and co-workers[11,12] showed that DES' complexes are disrupted in both DES/polymer [11] and DES/salt-based ABS, [12] due to a nonstoichiometric partition of the HBA and the HBD between the phases in equilibrium, in accordance with their affinities for each phase. More recently, the same authors [13] reported that in some ABS, depending on their phase forming components, it is possible to preserve the stoichiometry of the DES starting materials. Although this type of systems can be considered a pseudo ternary system, there is no evidence that the DES integrity is maintained.[13] Furthermore, independently of the HBA:HBD stoichiometry be maintained or not, in all the cases studied it is the HBA that works as a phase forming component of the ABS. The HBD may, or may not, participate in the ABS formation, presenting always an influence in the liquid-liquid equilibrium and in the solutes partition on these systems.[11–13]

The choice of ABS composition depends on the ability to manipulate the properties of the phases to obtain the appropriate partition coefficient of a target biomolecule. There are several ways to adjust the properties of the phases in equilibrium to obtain the desired partition, such as the use of different phase former compounds, the manipulation of their concentrations, the addition of a fourth component, or even the change of the pH or temperature of the system.[17–19] Thus, and considering the HBA and HBD ability to modulate the phases' properties in a DES-based ABS,[11–13] the goal of this work is to evaluate the designer solvent ability of the DES as ABS phase formers, by studying DES of four different alcohols (acting as HBD) – ethanol, *n*-propanol, 1,2-propanediol and ethylene glycol – mixed at different molar fractions (2:1, 1:1 and 1:2) with cholinium chloride (the HBA), in ABS composed of K_2HPO_4 and water. In order to evaluate the alcohol ability to tune the extraction in the studied systems, the partition

coefficients and extraction efficiencies of several biomolecules (amino acids, alkaloids, and phenolic compounds) were measured.

2. MATERIAL AND METHODS

2.1 Materials

The liquid-liquid phase diagrams were prepared using aqueous solutions of K_2HPO_4 (98 wt % pure from Alphatec), cholinium chloride, $[N_{111}(2OH)]Cl$ (98 wt % pure from Acros Organics), and the following alcohols: ethanol (99.8 wt % pure from Fisher Scientific), *n*-propanol (99.5 wt % pure from Lab-Scan) 1,2-propanediol (99.5 wt % pure from Panreac) and ethylene glycol (99.5 wt % pure from Sigma Aldrich). All chemicals were used without further purification. The chemical structure, molecular weight (M_w) and logarithm of octanol-water partition coefficient ($\log(K_{OW})$) of each alcohol considered in this work are presented in Figure 1. $[N_{111}(2OH)]Cl$ and alcohols 1H and ^{13}C NMR spectra are provided in the Supplementary material.

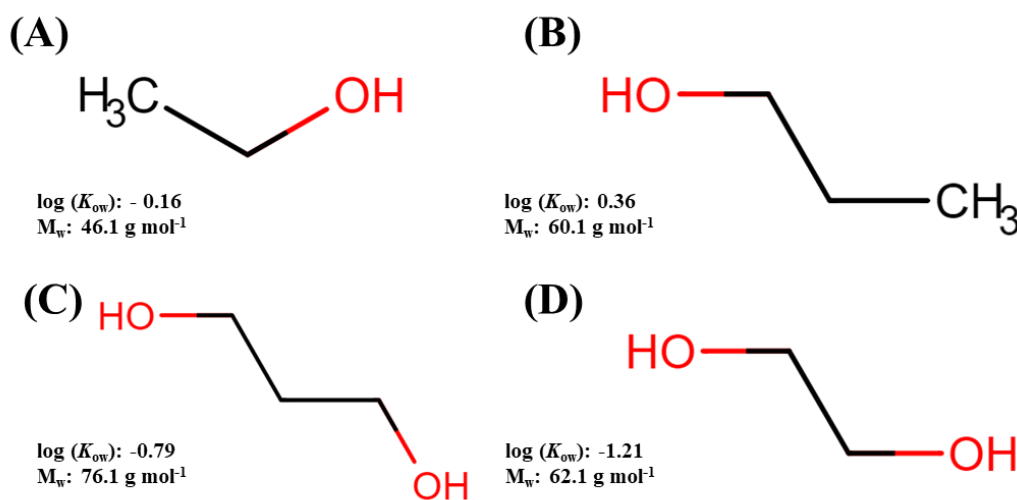
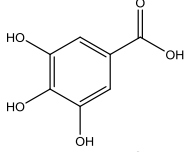
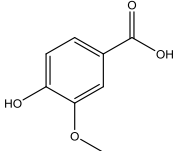
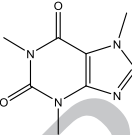
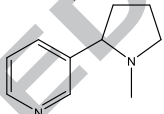
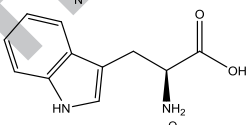
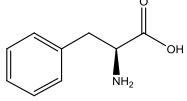
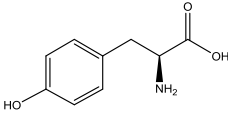
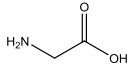
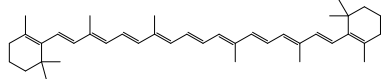


Figure 1. Chemical structure, molecular weight (M_w) and logarithm of octanol-water partition coefficient ($\log(K_{OW})$)[20] of the alcohols (A) ethanol, (B) *n*-propanol, (C) 1,2-propanediol, and (D) ethylene glycol.

The biomolecules studied were the phenolic compounds vanillic acid (97 wt % pure) from Sigma-Aldrich and the gallic acid (99.5 wt % pure) from Merck; the alkaloids were nicotine (99 wt % pure) and caffeine (99 wt % pure) from Fluka; the amino acids were L-tryptophan (99 wt % pure) and L-phenylalanine (99 wt % pure) from Sigma-Aldrich, L-tyrosine (99 wt % pure) from Fluka and glycine (99 wt % pure)

from Acros Organic; and the carotenoid was β -carotene (97 wt % pure) from Fluka. The chemical structure of the biomolecules investigated and their characteristics, such as molecular weight (M_w), octanol-water partition coefficient ($\log(K_{OW})$), and acid dissociation constants (pK_a), are presented in Table 1.

Table 1. Biomolecules chemical structure, molecular weight (M_w), logarithm of octanol-water partition coefficient ($\log(K_{OW})$), and acid dissociation constants

Name	Chemical structure	M_w (g mol ⁻¹)	$\log(K_{OW})$	pK_{a1}/pK_{a2}
Gallic Acid		170.1	0.72	3.9/9.0
Vanillic Acid		168.1	1.17	4.2/10.1
Caffeine		194.2	-0.55	--- ^a
Nicotine		162.2	1.16	2.7/8.6
L-Tryptophan		204.2	-1.09	2.5/9.4
L-Phenylalanine		165.2	-1.18	2.5/9.5
L-Tyrosine		181.2	-1.49	2.0/9.2
Glycine		75.1	-3.41	2.3/9.2
β -Carotene		536.9	11.12	--- ^a

(pK_a).[20]

^a the biomolecule does not suffer speciation.

2.2. Methods

2.2.1. Determination of phase diagrams and tie-lines

The phase diagrams were obtained by the cloud point titration method [11,12] at (298 ± 1) K and atmospheric pressure. It was previously shown [12,13] that DES-based

ABS final equilibrium is not dependent on the path used to attain it, and ABS can be prepared by the direct dissolution of the HBA and HBD in the salt aqueous solution, without previous preparation of DES. Thus, aqueous solutions at 75 wt % of the mixtures of $[N_{111(2OH)}]Cl$ and alcohols at three different molar fractions (2:1, 1:1 and 1:2) and aqueous solutions at 60 wt % of K_2HPO_4 were prepared with an uncertainty of $\pm 10^{-4}$ g. This method consists of a repetitive dropwise addition of the K_2HPO_4 aqueous solution to the $[N_{111(2OH)}]Cl$ and alcohols solution until the detection of a cloudy point (biphasic region), followed by the dropwise addition of water until the detection of a clear and limpid solution (monophasic region), under constant stirring. The system composition was determined by weight quantification of all pure components added with an uncertainty of $\pm 10^{-4}$ g. The water content of the ABS constituents was measured using a Metrohm 831 Karl Fischer coulometer and taken into consideration during the preparation of each solution. The phase diagrams of ternary systems composed of $[N_{111(2OH)}]Cl + K_2HPO_4 + H_2O$ and alcohol + $K_2HPO_4 + H_2O$ were also determined.

One tie-line was determined for each ABS composed of $[N_{111(2OH)}]Cl$, alcohol (at different molar fractions), K_2HPO_4 and water. For each tie-line, a quaternary or a ternary mixture at the biphasic region was gravimetrically prepared within $\pm 10^{-4}$ g, vigorously stirred and centrifuged at 3500 rpm for 30 min at (298 ± 1) K. After reaching the equilibrium, both phases were carefully separated and individually weighted. The coexisting phases' composition was analytically determined. The systems composed of ethanol and *n*-propanol were analyzed by 1H NMR and Karl Fischer. The 1H NMR analysis was carried out to quantify the amount of $[N_{111(2OH)}]Cl$ and mono-alcohol present in the ABS, using a Bruker Avance 300 at 300.13 MHz, with deuterated dimethylsulfoxide as solvent and tetramethylsilane (TMS) as the internal reference. The Karl Fischer titration (Metrohm 831 Karl-Fischer coulometer) to water quantification was made using the Hydranal[®] - Coulomat AG reagent from Riedel-de Haën as the analyte. The systems composed of 1,2-propanediol and ethylene glycol were analyzed by a thermogravimetric analysis (TGA), using a Perkin Elmer TGA 400 and following the methodology previously proposed by Farias et al.[12]. In the end, a mass balance was performed between the initial mass of each component and the amounts present in the top and bottom phases to confirm the accuracy of the obtained results.

The tie-line length (TLL) of each tie-line was calculated according to the Equation 1. Three components were taken into account to the TLL calculation, considering that in quaternary systems the tie-lines are in the space.[13]

$$TLL = \sqrt{([X]_{top} - [X]_{bot})^2 + ([Y]_{top} - [Y]_{bot})^2 + ([Z]_{top} - [Z]_{bot})^2} \quad (1)$$

where $[X]$, $[Y]$ and $[Z]$ are K_2HPO_4 , $[N_{111(2OH)}]Cl$, and alcohol weight fraction percentages, respectively. The subscripts *top* and *bot* designate the top and bottom phases, respectively.

The parameter α was calculated as the mass ratio between the top and the bottom phases.

2.2.2. Biomolecules' partition

Aqueous solutions of each biomolecule were prepared at the following concentrations and used as part of the water composition in each tie-line: 2.94×10^{-3} mol L⁻¹ for gallic acid, 2.97×10^{-3} mol L⁻¹ for vanillic acid, 5.15×10^{-3} mol L⁻¹ for caffeine, 6.16×10^{-3} mol L⁻¹ for nicotine, 4.90×10^{-3} mol L⁻¹ for L-tryptophan, 1.82×10^{-2} mol L⁻¹ for L-phenylalanine, 5.52×10^{-4} mol L⁻¹ for L-tyrosine, 9.99×10^{-3} mol L⁻¹ for glycine, and 3.10×10^{-5} mol L⁻¹ for β -carotene. All phase-forming components were weighted within $\pm 10^{-4}$ g and vigorously stirred for 5 min. After the total dissolution, it was centrifuged at 3500 rpm for 30 min at (298 ± 1) K to achieve the complete partitioning of each biomolecule between the two phases. The biomolecules concentration in each phase was measured by UV-visible spectroscopy (BioTeck Synergy HT microplate reader), at the wavelength of 262 nm for gallic acid, 259 nm for vanillic acid, 273 nm for caffeine, 260 nm for nicotine, 279 nm for L-tryptophan, 258 nm for L-phenylalanine, 275 nm for L-tyrosine, and 512 nm for β -carotene, using calibration curves previously established. Possible interferences of the phase forming components with the analytical method were considered, and control samples were prepared at the same weight fraction composition, using pure water instead of biomolecule aqueous solutions.

To the quantification of glycine it was necessary to use the ninhydrin reagent. For this, 75 μ L of ninhydrin reagent was added to 100 μ L of an adequately diluted sample of each ABS phase containing glycine. The samples were incubated at 80°C for 30 min, after which 100 μ L of ethanol at 50 % (v/v) was added to stop the reaction. The glycine

concentration was also quantified by UV-visible spectroscopy at the wavelength of 570 nm.[21,22] At least two individual experiments were carried out for each ABS, allowing the determination of the average of the partition coefficient (K) and the extraction efficiency percentages (EE %), as well as, and the respective standard deviations (σ).

The biomolecules partition coefficients were determined as the ratio between the concentration of each biomolecule in the top phase and that in the bottom phase, according to the equation (2):

$$K = \frac{[Biom]_{top}}{[Biom]_{bot}} \quad (2)$$

where $[Biom]_{top}$ and $[Biom]_{bot}$ are the concentration of the extracted biomolecule in the top and in the bottom phase respectively. The extraction efficiencies percentage are defined as the percentage ratio between the amount of each biomolecule in the phase to which it preferentially partitioned (top or bottom phase) and that in the total mixture, according to equation (3):

$$EE \% = \frac{w_{Biom.}^{top\ or\ bot}}{w_{Biom.}^{top} + w_{Biom.}^{bot}} \times 100 \quad (3)$$

where w are the weights of the extracted biomolecules in a specific phase: top or bottom (bot) phase.

The selectivity (S) of each system was defined as the ratio between the partition coefficient of two biomolecules ($K_{Biom.}$):

$$S_{1/2} = \frac{K_{Biom.1}}{K_{Biom.2}} \quad (4)$$

3. RESULTS AND DISCUSSION

3.1. Phase diagrams

The binodal curves of the ternary and quaternary systems composed of $[N_{111}(2OH)]Cl$, alcohols (OH), K_2HPO_4 and water are represented in Figure 2. These ABS are quaternary systems and should be represented in a three-dimensional phase diagram. However, their representation as pseudo ternary systems, as depicted in Figure 2,

remains the best option to an easier reading and interpretation of the obtained data.[13] The detailed experimental weight fraction data and three-dimensional representation are reported in the Supplementary material.

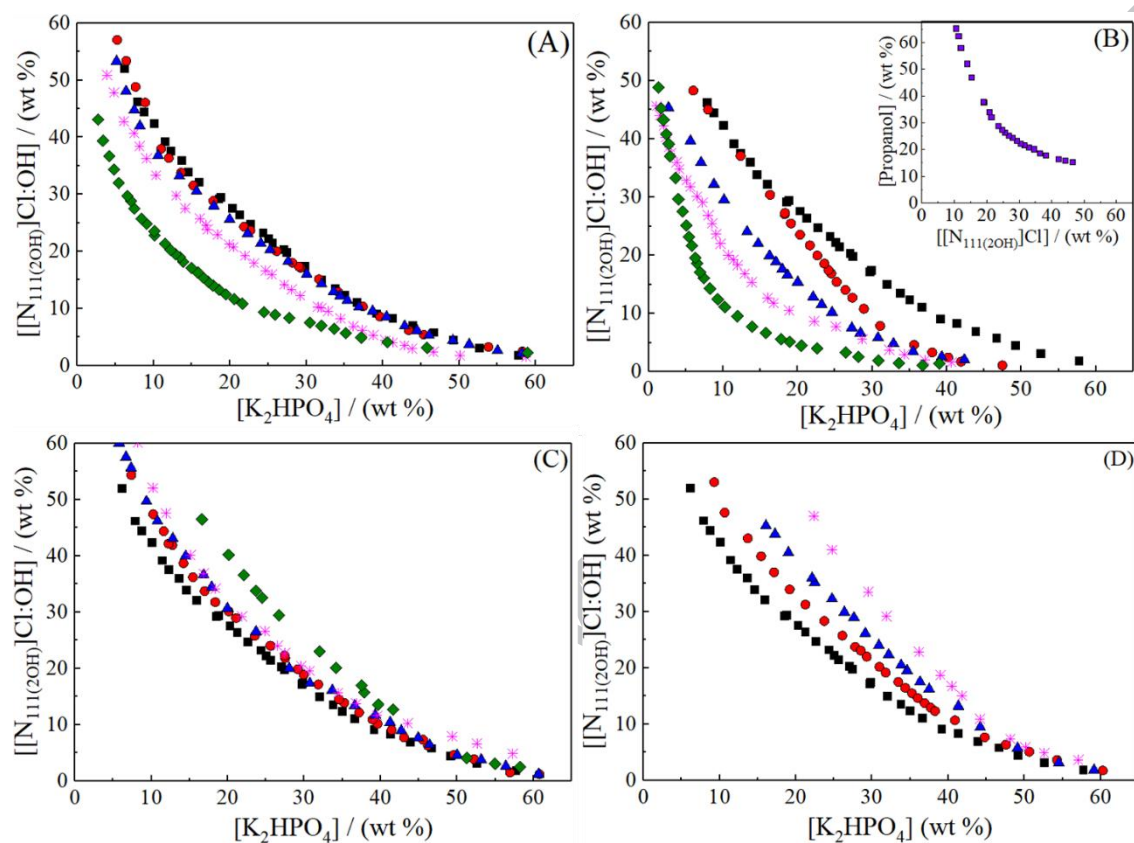


Figure 2. Phase diagrams at 298 K and atmospheric pressure of ABS composed of K_2HPO_4 , water and mixtures of $[N_{111(2OH)}]Cl$ and different alcohols (OH): (A) ethanol; (B) *n*-propanol, (C) 1,2-propanediol, and (D) ethylene glycol at different molar ratios – 2:1 (●), 1:1 (▲), 1:2 (*); $[N_{111(2OH)}]Cl + K_2HPO_4 + H_2O$ (■) [12], alcohol + $K_2HPO_4 + H_2O$ (◆), and *n*-propanol + $[N_{111(2OH)}]Cl + H_2O$ (■).

Figure 2 depicts the alcohol effect on the biphasic region of the ternary system composed of $[N_{111(2OH)}]Cl$, K_2HPO_4 and water. It is possible to see that both, the concentration and the nature of the alcohol present a significant effect in the binodal curves. In what concerns the alcohol nature, if the same alcohol molar fraction (for example 1:1) is considered, the ability of $[N_{111(2OH)}]Cl:OH$ mixtures to form an ABS with the K_2HPO_4 presents the following trend: $[N_{111(2OH)}]:propanol > [N_{111(2OH)}]:ethanol > [N_{111(2OH)}]$ (without alcohol) $> [N_{111(2OH)}]:1,2-propanediol > [N_{111(2OH)}]:ethylene\ glycol$. It is clear that the presence of the mono-alcohols (ethanol and *n*-propanol)

induces an increase in the biphasic region of $[N_{111(2OH)}]Cl + K_2HPO_4 + H_2O$ ternary system (Figure 2 A and B), while the presence of the di-alcohols (1,2-propanediol and ethylene glycol) results in a decrease (Figure 2 C and D). Furthermore, these effects are enhanced by the alcohol concentration.

The demixing of two aqueous phases, and consequent formation of an ABS, occurs due to the competition of different species (the solutes) for the formation of hydration complexes. Thus, the ability to induce the demixing of the phases is directly related with the chemical nature of the ABS components.[23] In a ternary system constituted by two salts, such as $[N_{111(2OH)}]Cl$ and K_2HPO_4 , the competition for the hydration is dominated by the higher charge density inorganic ions of K_2HPO_4 –capable of stronger interactions with water – resulting in the salting-out of $[N_{111(2OH)}]Cl$ to a new aqueous phase. The addition of a fourth component to the system will change the strength of this effect.[17,24–26] In fact, a good relationship between the influence of the alcohol nature on the binodal curve, and the alcohol hydrophilicity/hydrophobicity (measured through the octanol-water partition coefficient, $\log(K_{OW})$ – cf. Figure 1) can be observed in the systems under study. It is clear that the addition of hydrophilic alcohols ($\log(K_{OW}) < 0$), such as the di-alcohols 1,2-propanediol and the ethylene glycol, difficulties the formation of ABS, due to their stronger interactions with water. On the other hand, the hydrophobic character of the mono-alcohols *n*-propanol and ethanol ($\log(K_{OW}) \geq 0$) facilitates the ABS formation. This trend is also in good agreement with the ability of each alcohol to induce the formation of an ABS when mixed only with the K_2HPO_4 (without $[N_{111(2OH)}]Cl$). While *n*-propanol (the most hydrophobic alcohol under study) presents the larger biphasic region, the most hydrophilic alcohol – the ethylene glycol – is not able to form an ABS with K_2HPO_4 – cf. Figure 2D. These results are also in good agreement with data previously reported in the literature. [27,28]

Remarkably it was observed the formation of three phases in equilibrium in specific regions of the quaternary system composed of $[N_{111(2OH)}]Cl + n$ -propanol + $K_2HPO_4 + H_2O$. It was recently proposed by Passos et al.[29] that a triple salting-out effect is required to the formation of an aqueous multiphase systems (MuPS) – a system with more than two aqueous phases in equilibrium. In fact, among the studied alcohols, *n*-propanol is the only one able to induce the formation of two phases when mixed with an aqueous solution of $[N_{111(2OH)}]Cl$, in absence of the inorganic salt – cf. Figure 2B. Despite its low salting-out character, $[N_{111(2OH)}]Cl$ is still able to induce the exclusion of an alcohol with a high hydrophobic character, such as the *n*-propanol. Thus, in the

quaternary system constituted by $[N_{111(2OH)}]Cl$, *n*-propanol, K_2HPO_4 and water three different salting-out effects can occur simultaneously – the salting-out effect of the $[N_{111(2OH)}]Cl$ over the *n*-propanol and the salting-out effect of the K_2HPO_4 over the $[N_{111(2OH)}]Cl$ and the *n*-propanol – allowing the formation of a MuPS.

The results obtained for the experimental tie-lines of each ternary and quaternary system studied, as well as their respective TLL and α parameter, are presented in Table 2. The binodal curves and respective tie-lines representation is given in the Supplementary material. For all the two-phase systems studied, the bottom phases are richer in K_2HPO_4 while the top phases are in general mainly composed of $[N_{111(2OH)}]Cl$. However, $[N_{111(2OH)}]Cl$:propanol-based ABS present a very distinct behavior, since the top phases are mainly composed of *n*-propanol, and $[N_{111(2OH)}]Cl$ remains in the bottom phase with the inorganic salt. This is a good indication of the strong ability of K_2HPO_4 to salt-out *n*-propanol, compared with its ability to exclude the $[N_{111(2OH)}]Cl$ to the other phase – *cf.* Figure 2B. However, due to this type of behavior, the tie-lines of *n*-propanol-based ABS only make sense when presented in a three-dimensional representation – *cf.* Supplementary material.

Table 2. Liquid-liquid equilibrium experimental data of K_2HPO_4 (1) + $[N_{111(2OH)}]Cl$ (2) + alcohol (3) + H_2O (4) systems at 298.15 K and atmospheric pressure.

Alcohol molar fraction	Overall composition / wt %		Top phase composition / wt %			Bottom phase composition / wt %			TLL	α
	K_2HPO_4	$[N_{111(2OH)}]Cl:OH$	w1	w2	w3	w1	w2	w3		
Without alcohol ^a										
---	25	30	6.7	50.4	-	50.1	3.9	-	73.4	0.92
---	40	20	3.1	57.6	-	55.0	4.4	-	75.6	0.50
Ethanol										
0.33	25	30	6.5	42.3	6.1	51.4	2.0	0.1	70.8	0.88
0.50			7.7	37.6	10.7	46.3	2.3	0.3	65.4	0.85
0.67			1.6	34.1	21.4	51.4	2.6	0.0	65.3	0.80
1.00 ^b			1.6	-	65.2	46.1	-	1.1	79.9	0.54
<i>n</i> -Propanol										
0.50	25	20	2.6	4.9	79.1	27.1	14.7	1.7	83.4	0.04
0.67			1.0	4.6	80.1	25.8	14.2	2.4	82.8	0.10
1.00 ^b			1.0	-	77.9	33.4	-	1.0	84.2	0.35
1,2-Propanediol										
0.33	40	20	4.4	50.6	12.8	54.9	3.1	1.6	76.8	0.49
0.50			6.6	35.6	22.9	54.8	2.4	1.8	72.9	0.47
0.67			6.9	26.8	29.1	53.8	2.8	0.8	71.2	0.49
1.00 ^b			14.4	-	53.8	52.3	-	3.0	84.2	0.53
Ethylene glycol										
0.33	40	20	6.7	48.9	8.7	51.9	3.1	3.1	74.6	0.47
0.50			10.0	48.5	7.9	51.2	1.2	5.3	77.4	0.42
0.67			21.0	21.4	28.8	45.0	7.8	4.2	71.7	0.20

^a ternary system composed of K_2HPO_4 + $[N_{111(2OH)}]Cl$ + H_2O ;

^b ternary system composed of $K_2HPO_4 + \text{alcohol (OH)} + H_2O$.

To infer on the final stoichiometry of the HBA and HBD in the systems studied, the $[N_{111(2OH)}]Cl:OH$ molar ratio was calculated for both phases of all the tie-lines studied. The obtained results are presented in Figure 3. Remarkably, the stoichiometry of the HBA ($[N_{111(2OH)}]Cl$) and the HBD (alcohol) of the initial mixture is maintained in the top phase of ethanol- and 1,2-propanediol-based ABS. Similarly to the observed in previous works,[13] these two alcohols are mainly partitioned to the top phase, which is richer in $[N_{111(2OH)}]Cl$. Thus, the initial molar ratio is maintained in this phase, while on the bottom phase, due to the very low concentration of both HBA and HBD is not possible to observe the same behavior. On the other hand, since the ethylene glycol presents a high hydrophilic character, it presents a higher partition to the bottom phase, and consequently the HBA:HBD molar ratio is not maintained in any phase for $[N_{111(2OH)}]:\text{alcohol}$ 1:1 and 1:2 mixtures. However, at a molar ratio of 1:2 the stoichiometry was maintained in both phases. In what concerns the *n*-propanol-based systems, the stoichiometry in both phases is completely different of the initial mixture. In fact, since in these systems the top phase is richer in *n*-propanol, while the $[N_{111(2OH)}]Cl$ remains in the bottom phase with the inorganic salt (K_2HPO_4), it is not possible to keep the HBA:HBD molar ratio.

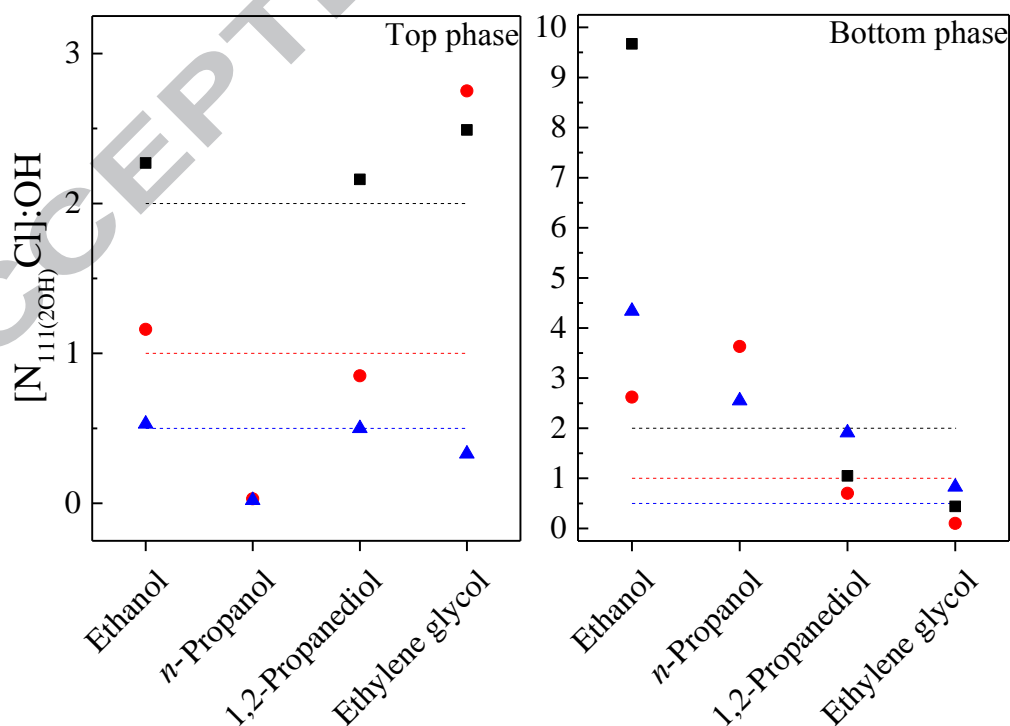


Figure 3. Molar ratio between the HBA ($[N_{111(2OH)}]Cl$) and the HBD

(alcohols) in the initial mixture composition (dashed lines) and the coexisting phases of ABS composed of $[N_{111(2OH)}]Cl:OH + K_2HPO_4 + H_2O$: 2:1 (■), 1:1 (●), and 1:2 (▲).

A mixture point in the triphasic region of the quaternary system composed of 20 wt % of $[N_{111(2OH)}]Cl$:propanol (at 1:1 molar ratio) + 30 wt % of K_2HPO_4 + 50 wt % of H_2O , was also characterized. The obtained results showed that the top phase of this system is mainly composed of *n*-propanol (84.0 wt % of *n*-propanol, 6.1 wt % of $[N_{111(2OH)}]Cl$, 0.5 wt % of K_2HPO_4 , and 9.4 wt % of H_2O), the middle phase is richer in $[N_{111(2OH)}]Cl$ (8.3 wt % of *n*-propanol, 41.2 wt % of $[N_{111(2OH)}]Cl$, 2.2 wt % of K_2HPO_4 and 48.3 wt % of H_2O), and the bottom phase is rich in K_2HPO_4 (0.1 wt % of *n*-propanol, 3.8 wt % of $[N_{111(2OH)}]Cl$, 45.6 wt % of K_2HPO_4 and 50.5 wt % of H_2O), which is in good agreement with the data previously reported in the literature.[29] The respective “tie surface” is presented in the Supplementary material.

3.2. Thermodynamics modelling

The Non-Random Two-Liquid (NRTL) model was used to describe the liquid-liquid equilibrium phase diagrams measured. The NRTL model was proposed by Renon and Prausnitz [30] and is based on the local composition concept. This model applies to partially miscible, as well as totally miscible systems, allowing a good representation of the experimental data.

The equations for calculating the NRTL activity coefficients for ATPS were proposed by Sé and Aznar.[31] The parameters for this model were estimated using TML-LLE 2.0 software developed by Stragevitch and D'Ávila [32] which estimate the parameters by the minimization of the objective function described in equation (5), using the modified Simplex numeric method. [33]

$$OF = \sum_k^D \sum_j^M \sum_i^{N-1} \left(w_{ijk}^{I.exp} - w_{ijk}^{I.calc} \right)^2 + \left(w_{ijk}^{II.exp} - w_{ijk}^{II.calc} \right)^2 \quad (5)$$

where M and N are the TLs and the number of components, respectively. The superscript, I and II refer to the two liquid phases in equilibrium, w_i represents the experimental and NRTL calculate mass fractions of component i in the phases.

The Table 3 presents the binary interaction parameters for the NRTL model for the quaternary systems composed of $[N_{111(2OH)}]Cl$, alcohol, K_2HPO_4 and water at 298.15 K.

Table 3. NRTL binary interaction parameters for the quaternary systems K_2HPO_4 (1) + $[\text{N}_{111}(\text{2OH})]\text{Cl}$ (2) + alcohol (3) + H_2O (4) at 298.15 K and atmospheric pressure.

Parameters (i/j)	$A_{0,ij}/\text{K}$	$A_{0,ji}/\text{K}$	α_{ii}	RMSD ^a (%)
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ + ethanol + K_2HPO_4 + H_2O				
ethanol / K_2HPO_4	1205.4	1884.4	0.20000	0.87
ethanol / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	-75.224	-15.987	0.30081	
ethanol / H_2O	-113.84	1206.3	0.29941	
K_2HPO_4 / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	487.94	854.83	0.20843	
K_2HPO_4 / H_2O	-28.688	1113.3	0.45402	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ / H_2O	1956.5	3350.7	0.36196	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ + <i>n</i> -propanol + K_2HPO_4 + H_2O				
<i>n</i> -propanol / K_2HPO_4	1115.5	344.76	0.46422	0.63
<i>n</i> -propanol / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	1337.7	-11.701	0.20000	
<i>n</i> -propanol / H_2O	950.79	1571.0	0.36984	
K_2HPO_4 / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	2959.9	325.90	0.20000	
K_2HPO_4 / H_2O	133.72	2954.7	0.41414	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ / H_2O	920.97	1155.7	0.44224	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ + 1,2-propanediol + K_2HPO_4 + H_2O				
1,2-propanediol / K_2HPO_4	698.04	614.60	0.21102	1.13
1,2-propanediol / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	3247.7	-110.18	0.20000	
1,2-propanediol / H_2O	8974.6	2119.9	0.40378	
K_2HPO_4 / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	1807.1	2132.8	0.20381	
K_2HPO_4 / H_2O	-193.93	631.22	0.47000	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ / H_2O	-412.47	1284.7	0.44715	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ + ethylene glycol + K_2HPO_4 + H_2O				
ethylene glycol / K_2HPO_4	422.18	6387.7	0.45279	1.10
ethylene glycol / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	2364.2	408.25	0.31057	
ethylene glycol / H_2O	1182.7	1228.2	0.43317	
K_2HPO_4 / $[\text{N}_{111}(\text{2OH})]\text{Cl}$	483.26	541.88	0.39818	
K_2HPO_4 / H_2O	1463.0	4552.9	0.28290	
$[\text{N}_{111}(\text{2OH})]\text{Cl}$ / H_2O	-178.09	1863.1	0.42718	

$$^a \text{Root mean square deviation } \delta_w = 100 \cdot \sqrt{\frac{\sum_i^M \sum_j^N (w_{ij}^{I.exp} - w_{ij}^{I.calc})^2 + (w_{ij}^{II.exp} - w_{ij}^{II.calc})^2}{2MN}}$$

The NRTL model provides a very good description of the experimental data for all the studied systems, with a mean deviation below 1.13 %.

3.3. Biomolecules' partition on DES-based ABS

In order to evaluate the designer solvent ability of different alcohols on the properties of the phases in equilibrium, the partition of eight biomolecules – two phenolic acids (gallic acid and vanillic acid), two alkaloids (nicotine and caffeine), and four amino acids (L-tyrosine, L-tryptophan, L-phenylalanine and glycine) – was determined in the mixture points reported in Table 2. The biomolecules partition coefficients (K) in function of the nature and concentration of each alcohol are presented in Figure 4. Due to the very distinct equilibrium behavior observed in the quaternary mixtures composed of $[N_{111}(2OH)]Cl$, *n*-propanol, K_2HPO_4 , and water, *n*-propanol-based systems were not considered in this first analysis, and will be discussed separately. The partition coefficients and extraction efficiency percentages values are detailed in the Supplementary material.

Through the results presented in Figure 4 it is possible to see that all the biomolecules under study, with the exception of glycine, are preferentially partitioned ($K > 1$) to the top phase, which is mainly composed of $[N_{111}(2OH)]Cl$ and alcohol (*cf.* Table 2). Furthermore, both the nature and the concentration of the alcohol added could affect significantly the biomolecules partition, depending on their physicochemical properties.

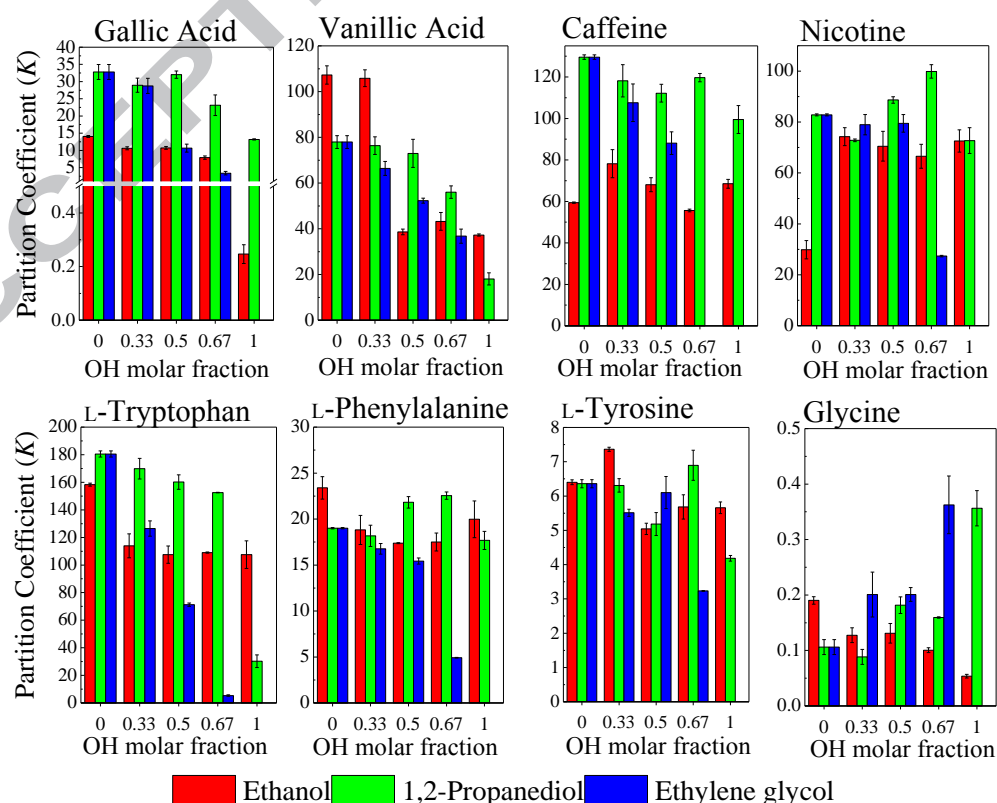


Figure 4. Biomolecules partition coefficients (K) to the top phase of ABS composed of $[N_{111(2OH)}]Cl + \text{alcohol (OH)} + K_2HPO_4 + H_2O$ at different $[N_{111(2OH)}]Cl:OH$ molar fraction.

In general, the addition of ethanol, 1,2-propanediol or ethylene glycol to the $[N_{111(2OH)}]Cl + K_2HPO_4 + H_2O$ system results in a decrease of the partition of the phenolic compounds gallic and vanillic acid. At the systems pH – which ranges between 9.0 and 10.6 – gallic and vanillic acid are negatively charged, conferring them a significant hydrophilic character. Thus, the decrease of the top phase hydrophilic character, by increasing the alcohol concentration (*cf.* Table 2), is not favorable to the partition of phenolic compounds at this pH. Furthermore, in what concerns the alcohol nature, it seems that the application of a mono-alcohol vs a di-alcohol presents a significant effect on the partition of the biomolecules. However, it is important to take into account that the extractions in ethanol-based systems were carried out at a different mixture point, which could result in different partition coefficients, not due to the alcohol nature but due to the distinct phases' composition. In fact, it is possible to see in Figure 4 that, for almost all the biomolecules under study, when the molar fraction of alcohol is equal to 0, *i.e.*, the partition is carried out in the ternary system composed of $[N_{111(2OH)}]Cl + K_2HPO_4 + H_2O$, there is already significant differences in the partition coefficient values derived only of different starting mixture points – *cf.* Table 2. Nevertheless, considering the phenolic compounds partition in di-alcohol-based systems, the application of 1,2-propanediol is the most favorable.

The alkaloids partition is not as much affected by the alcohols presence and concentration as phenolic compounds – *cf.* Figure 4. In general, the caffeine partition coefficient is slightly improved by the addition of the ethanol, while the presence of di-alcohols reduces its extraction to the top phase. On the other hand, the addition of ethanol to the extraction mixture results in a significant increase of nicotine partition (which is independent of the alcohol concentration), while the addition of di-alcohols only induces significant changes in the biomolecule partition at a molar fraction of 0.67. These results indicate that the presence of ethanol – the alcohol with the higher hydrophobic character – is more favorable to the alkaloids partition when compared with 1,2-propanediol and ethylene glycol. Furthermore, despite its hydrophobic character, nicotine is positively charged at the systems pH. Thus, its partition coefficient values are lower than those observed for caffeine, which does not suffer speciation and

is always in its neutral form (*cf.* Table 1). In the quaternary system composed of ethylene glycol at 0.67 molar fraction, there are no results for the caffeine partition due to the caffeine precipitation. This suggests a means to recover caffeine from the extraction phase.

In what concerns the amino acids partition, it is possible to observe a direct relation between the obtained results – $K(\text{L-tryptophan}) > K(\text{L-phenylalanine}) > K(\text{L-tyrosine}) > K(\text{glycine})$ – and the octanol-water partition coefficients of these biomolecules, which is presented in Figure 5. In fact, the partition coefficients increase with the hydrophobic character of each biomolecule (higher values of $\log(K_{OW})$), with L-tryptophan presenting the largest partition coefficient values among all the biomolecules studied. A similar behavior for L-tryptophan partition was previously described by Farias et al.[13] in DES-based ABS composed of $[\text{N}_{111}(2\text{OH})]\text{Cl}$, glucose, polypropylene glycol and water. The authors[13] proposed that specific interactions between this amino acid and the $[\text{N}_{111}(2\text{OH})]\text{Cl}$ are the main responsible for the high partition of L-tryptophan to the $[\text{N}_{111}(2\text{OH})]\text{Cl}$ -rich phase. On the other hand, glycine is the only aliphatic amino acid studied here, and all the partition coefficients were $\ll 1$. Due to its high hydrophilic character ($\log(K_{OW}) = -3.41$), glycine is partitioned to the salt-rich phase, with extraction efficiencies ranging between 75.0 and 98.9 % – *cf.* the Supplementary material.

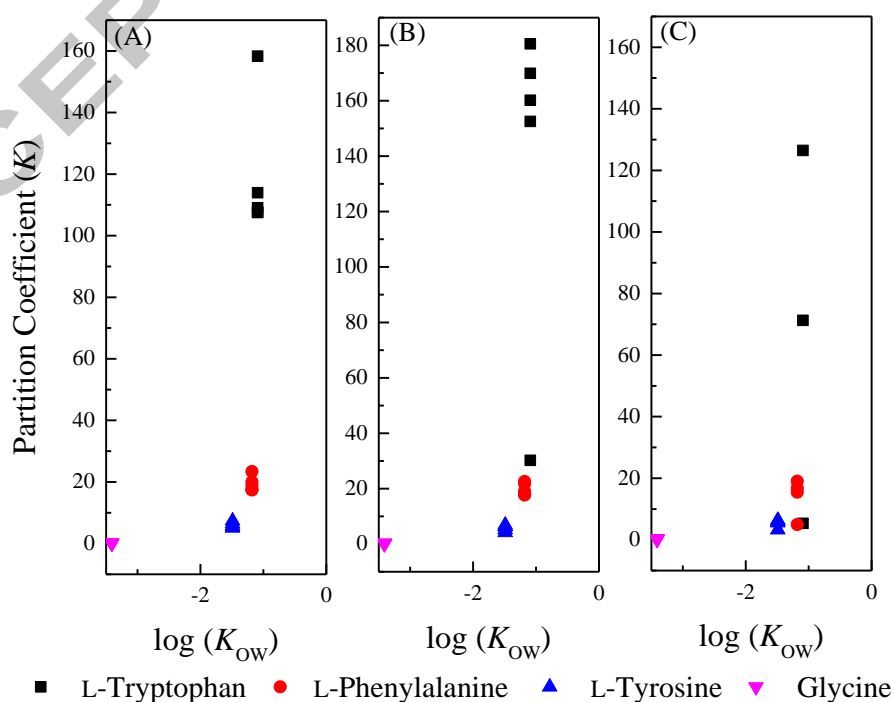


Figure 5. Partition coefficient (K) in function of $\log(K_{OW})$ for each amino acid studied in ABS composed of $[N_{111(2OH)}]Cl$, K_2HPO_4 , water and different alcohols: (A) ethanol, (B) 1,2-propanediol, and (C) ethylene glycol.

The alcohol nature and concentration effect over the amino acids partition is highly dependent on the biomolecule hydrophilic/hydrophobic character. Considering L-tryptophan, L-phenylalanine, and L-tyrosine, which are mainly partitioned to the top phase, the addition of ethanol induces only slight changes on the partition coefficients. However, when di-alcohols are added to the mixture, specially the ethylene glycol, there is a significant reduction of amino acids partition. Nevertheless, due to its hydrophilic character, the addition of di-alcohols is favorable to the partition of glycine to the top phase – *cf.* Figure 4.

The systems selectivities (S) to separate molecules of the same family (phenolic compounds, alkaloids and amino acids), was also evaluated. The obtained results are presented in Figure 6 and reported in the Supplementary material. As expected, very distinct selectivities were obtained depending on the biomolecules family considered. For example, the $S_{Caf/Nic}$ values are always near to 1 (*cf.* Supporting Information), which indicates a low ability of these systems to selectively separate the alkaloids under study. On the other hand, the selectivity parameter of the phenolic compounds ($S_{Van/Gal}$), is always higher than 2 and the best result was obtained in the ternary system composed of ethanol + K_2HPO_4 + H_2O , in which $S_{Van/Gal} = 151.1$. Considering the selectivity of the studied systems to the separation of the aromatic amino acids, the $S_{Tryp/Tyr}$ presents the best results, since the L-tryptophan ($\log(K_{OW}) = -1.06$) and L-tyrosine ($\log(K_{OW}) = -2.26$) are the most and the less hydrophobic aromatic amino acids, respectively. Furthermore, the higher value ($S_{Tryp/Tyr} = 30.9$) was obtained when a mixture of $[N_{111(2OH)}]Cl$ + 1,2-propanediol is used, showing the alcohol potential as a designer solvent to attain a better separation. As expected, the best results of selectivity were obtained in the separation of aromatic (L-tryptophan, L-phenylalanine, and L-tyrosine) and aliphatic amino acids (glycine), ranging between 8.9 and 2006. Due to the glycine high hydrophilic character and preferential partition to the salt-rich phase, a high selectivity is obtained in its separation from the other amino acids here studied – *cf.* the Supplementary material. Furthermore, independently of the biomolecules pairs considered, the selectivity is always highly dependent of the alcohols nature and concentration. For example, through the data presented in Figure 6, it is possible to

observe that while the presence of ethanol induces an increase on the selectivity, the addition of di-alcohols to the ternary system composed of $[N_{111(2OH)}]Cl$, K_2HPO_4 and water results in a decrease of these values. In summary, these results shows the versatility of the studied systems, once by the change of the nature and concentration of the alcohol in the quaternary mixtures is possible to tune the separation of very distinct (such as glycine and the remain amino acids) or similar biomolecules (for example, the vanillic and gallic acid).

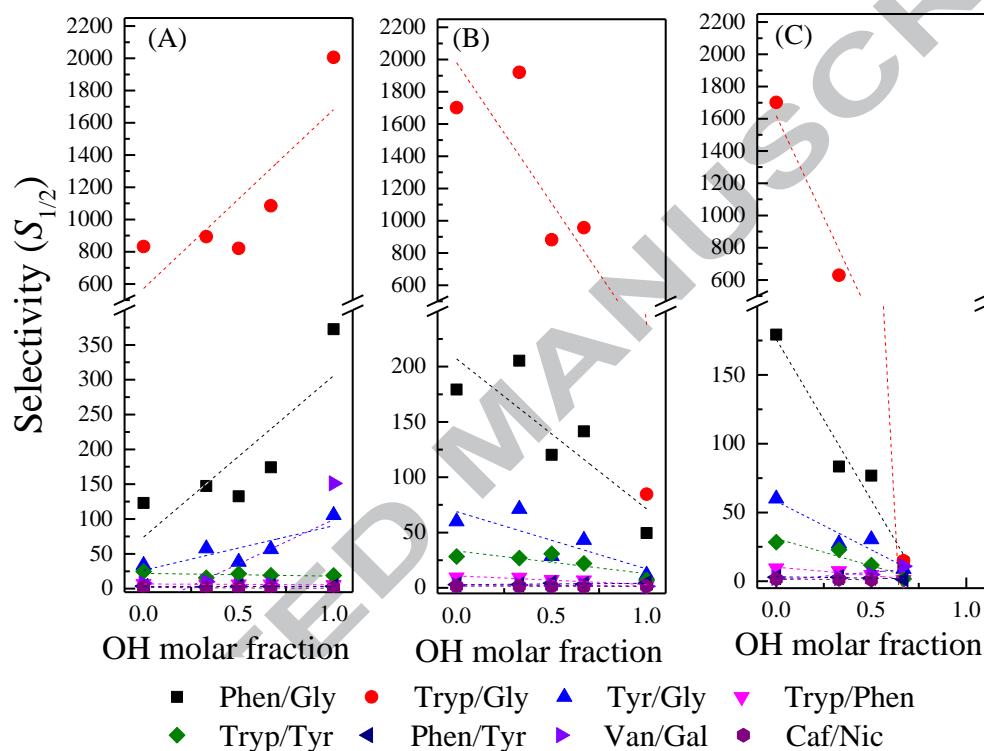


Figure 6. Selectivity (S) of ABS composed of $[N_{111(2OH)}]Cl$, K_2HPO_4 , water and alcohols – (A) ethanol, (B) 1,2-propanediol and (C) ethylene glycol – to the extraction of different biomolecules: vanillic acid (Van), gallic acid (Gal), nicotine (Nic), caffeine (Caf), L-tryptophan (Tryp), L-phenylalanine (Phen), L-tyrosine (Tyr), and glycine (Gly).

As mentioned previously, the *n*-propanol-based quaternary systems present a very distinct phase equilibrium behavior when compared with the remaining alcohols, since the top phase is highly concentrated in *n*-propanol and most of $[N_{111(2OH)}]Cl$ remains in the bottom phase together with the inorganic salt – *cf.* Table 2. This behavior has a high impact on the partition of the biomolecules under study, as it is possible to see through the results presented in Figure 7.

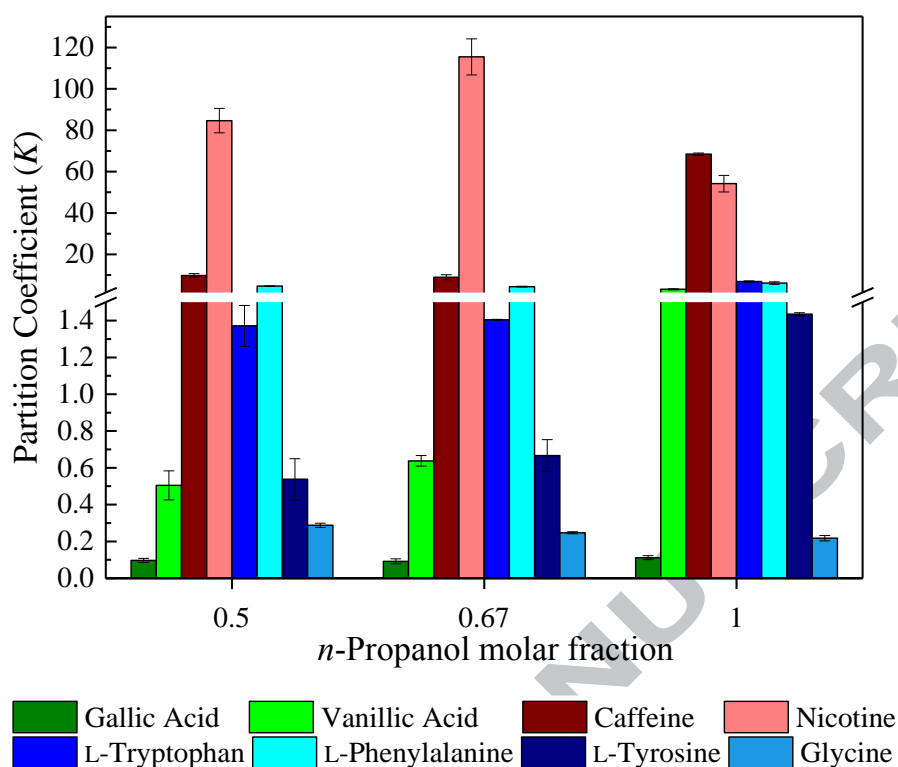


Figure 7. Biomolecules partition coefficients (K) to the top phase of ABS composed of $[N_{111}(2OH)]Cl + n$ -propanol + $K_2HPO_4 + H_2O$ at different $[N_{111}(2OH)]Cl$:propanol molar fraction.

With the exception of nicotine, all the biomolecules studied present a much lower partition to the top phase of n -propanol-based systems when compared with the quaternary mixtures composed of the remain alcohols. In fact, a top phase composed of ~80 wt % of n -propanol (*cf.* Table 2) has a high hydrophobic character, which seems to be not favorable to the extraction of hydrophilic or slightly hydrophobic biomolecules as those studied here – *cf.* Table 1. Nevertheless, in general the increase of n -propanol concentration in the quaternary mixture improves the partition of these biomolecules, showing that their partition is the result of a balance between different types of interactions. For example, contrarily to what was observed previously for ethanol-, 1,2-propanediol- and ethylene-glycol-based systems, L-tryptophan partition is significantly lower, due to the preferential migration of this amino acid to the bottom phase where most of $[N_{111}(2OH)]Cl$ remains. Furthermore, despite nicotine higher values of partition coefficient, there is a decrease of its partition in the absence of $[N_{111}(2OH)]Cl$, suggesting that also this biomolecule has specific interactions with this salt, which influences nicotine partition in this type of systems.

Beyond the good selectivities observed in $[N_{111}(2OH)]Cl$:alcohol-based systems, the possibility to form a three-phase system, and to separate more than two biomolecules in a single-step is also of interest. Thus, the extraction efficiency percentages ($EE\%$) of the biomolecules previously studied, along with β -carotene were determined in the *n*-propanol-based MuPS composed of 20 wt % of $[N_{111}(2OH)]Cl$:propanol (at 1:1 molar ratio) + 30 wt % of K_2HPO_4 + 50 wt % of H_2O . The obtained results are presented in Figure 8. The β -carotene was chosen for this study due to its high hydrophobic character ($\log(K_{ow}) = 11.12$).

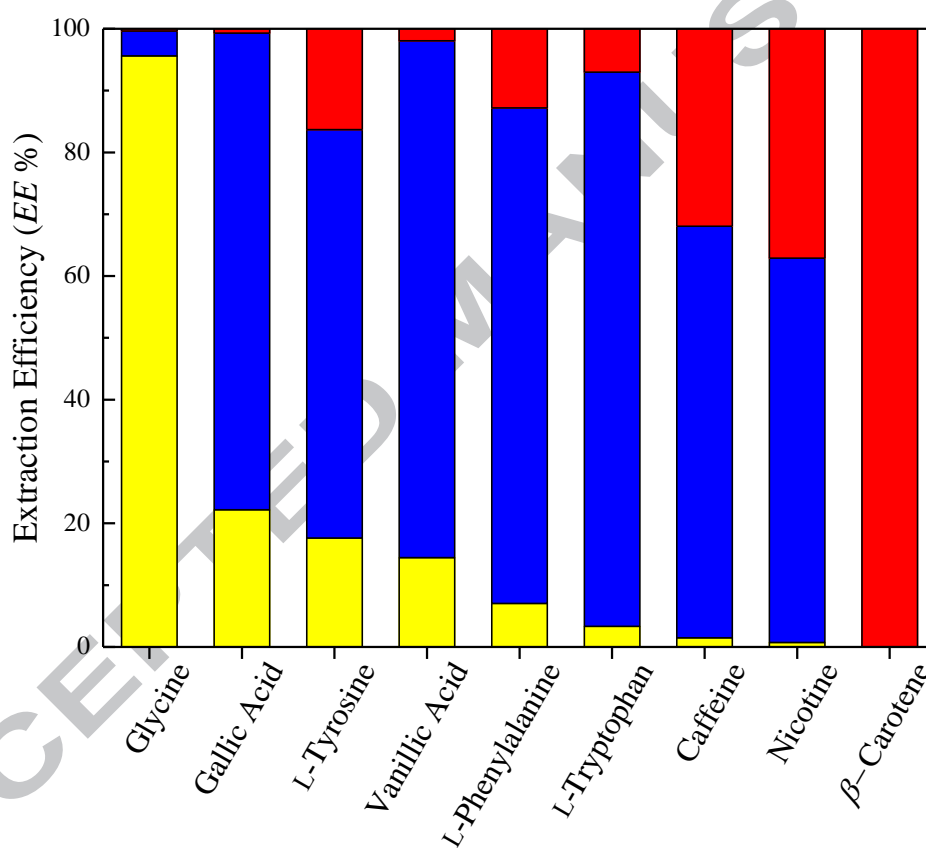


Figure 8. Biomolecules extraction efficiency percentage ($EE\%$) in the three-phases system constituted by 20 wt % of $[N_{111}(2OH)]Cl$:propanol (at 1:1 molar ratio) + 30 wt % of K_2HPO_4 + 50 wt % of H_2O : bottom phase (yellow bars), middle phase (blue bars), and top phase (red bars).

As previously discussed, the *n*-propanol-based three-phases system is constituted by a *n*-propanol-rich top phase, $[N_{111}(2OH)]Cl$ -rich middle phase, and a K_2HPO_4 -rich bottom phase. Thus, and similarly to what was observed in the two-phases systems, all the biomolecules are mainly partitioned to the $[N_{111}(2OH)]Cl$ -rich phase (middle phase), with

the exception of glycine that presents a *EE* % of 95.6 % to the bottom phase, and the β -carotene, which is completely extracted (*EE* % = 100 %) to the *n*-propanol-rich phase. These results show that the hydrophilic/hydrophobic character of the phases in equilibrium is the main factor that rules the partition of the target compounds, allowing the almost complete separation of three biomolecules (for example, glycine, L-tryptophan and β -carotene) in a single step.

In summary, the results here reported suggest that the alcohol presence in the quaternary mixtures composed of $[N_{111(2OH)}]Cl + \text{alcohol} + K_2HPO_4 + H_2O$ can change the chemical properties of the phases of the ABS. The change of the nature and concentration of the alcohol allows the tuning of the system phases properties, and consequently the partition and system selectivity can be manipulated according to the target biomolecules.

4. CONCLUSION

DES-based ABS composed of $[N_{111(2OH)}]Cl$ and alcohols at different molar fractions, K_2HPO_4 , and water were here studied for the first time. The alcohols effect in the DES-based ABS was evaluated. It was shown that, due to their higher hydrophobicity, the mono-alcohols (ethanol and *n*-propanol) induce an increase of the biphasic region, while the di-alcohols (1,2-propanediol and ethylene glycol) presence decreases the biphasic region of the $[N_{111(2OH)}]Cl-K_2HPO_4$ -based ABS, allowing the system phases properties manipulation. Furthermore, both the nature and concentration of the alcohols also interfere in the final HBA:HBD molar ratio obtained in the phases in equilibrium, being possible to keep this ratio constant only in the $[N_{111(2OH)}]Cl$ -rich phases of ethanol- and 1,2-propanediol-based systems. Remarkably, systems composed of *n*-propanol presented a much more complex behavior, with the ability to form aqueous multiphase systems with three aqueous phases in equilibrium in specific regions of the phase diagram. The NRTL model presented good correlation with the experimental data. The Finally, it was demonstrated that the studied systems are an alternative to selectively separate biomolecules of the same family, or separate more than two biomolecules in a single-step. The results show the high versatility of $[N_{111(2OH)}]Cl$:alcohols-based ABS, in which is possible to manipulate the phases properties and tune the partition of different biomolecules by the change of the HBD nature and concentration.

ACKNOWLEDGEMENTS

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. F.O. Farias is grateful for the scholarship provided by CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*). M. Mafra is grateful for Brazilian National Council for Scientific and Technological Development (CNPq - Grant 310905/2015-0). The authors also acknowledge *Fundação da Ciência e da Tecnologia* for the financial support through the project SAICTPAC/0040/2015.

REFERENCES

- [1] P. A. Albertsson, A. Cajarville, D. E. Brooks, F. Tjerneld, Partition of proteins in aqueous polymer two-phase systems and the effect of molecular weight of the polymer., *Biochim. Biophys. Acta.* 926 (1987), pp. 87–93.
- [2] J. Benavides, M. Rito-Palomares, J. A. Asenjo, Aqueous Two-Phase Systems, *Compr. Biotechnol.* (2011), pp. 697–713.
- [3] J. A. Asenjo, B. A. Andrews, Aqueous two-phase systems for protein separation: A perspective, *J. Chromatogr. A.* 1218 (2011), pp. 8826–8835.
- [4] A. M. Azevedo, A. G. Gomes, P. A. J. Rosa, I. F. Ferreira, A. M. M. O. Pisco, M.R. Aires-Barros, Partitioning of human antibodies in polyethylene glycol-sodium citrate aqueous two-phase systems, *Sep. Purif. Technol.* 65 (2009), pp. 14–21.
- [5] G. A. Gomes, A. M. Azevedo, M. R. Aires-Barros, D. M. F. Prazeres, Purification of plasmid DNA with aqueous two phase systems of PEG 600 and sodium citrate/ammonium sulfate, *Sep. Purif. Technol.* 65 (2009), pp. 22–30.
- [6] Q. Zeng, Y. Wang, Y. Huang, X. Ding, J. Chen, K. Xu, Deep eutectic solvents as novel extraction media for protein partitioning., *Analyst.* 139 (2014), pp. 2565–73.
- [7] C. X. Zeng, R. P. Xin, S. J. Qi, B. Yang, Y. H. Wang, Aqueous two-phase system based on natural quaternary ammonium compounds for the extraction of proteins, *J. Sep. Sci.* 39 (2016), pp. 648–654.

- [8] K. Xu, Y. Wang, Y. Huang, N. Li, Q. Wen, A green deep eutectic solvent-based aqueous two-phase system for protein extracting, *Anal. Chim. Acta.* 864 (2015) pp. 9–20.
- [9] N. Li, Y. Wang, K. Xu, Y. Huang, Q. Wen, X. Ding, Development of green betaine-based deep eutectic solvent aqueous two-phase system for the extraction of protein, *Talanta.* 152 (2016), pp. 23–32.
- [10] H. Zhang, Y. Wang, K. Xu, N. Li, Q. Wen, Q. Yang, Y. Zhou, I. Wen, Q. Yang, Y. Zhou, Ternary and binary deep eutectic solvents as a Novel Extraction Media for Protein Partitioning, *Anal. Methods.* (2016), pp. 8196–8207.
- [11] H. Passos, D. J. P. Tavares, A. M. Ferreira, M. G. Freire, J. A. P. Coutinho, Are Aqueous Biphasic Systems Composed of Deep Eutectic Solvents Ternary or Quaternary Systems?, *ACS Sustain. Chem. Eng.* 4 (2016) pp. 2881–2886.
- [12] F.O. Farias, F. H. B. Sosa, L. Igarashi-Mafra, J. A. P. Coutinho, M. R. Mafra, Study of the pseudo-ternary aqueous two-phase systems of deep eutectic solvent (choline chloride:sugars) + K_2HPO_4 + water, *Fluid Phase Equilib.* 448 (2017), pp. 143–151.
- [13] F. O. Farias, H. Passos, Á. S. Lima, M. R. Mafra, J. A. P. Coutinho, Is it possible to create ternary-like aqueous biphasic systems with deep eutectic solvents?, *ACS Sustain. Chem. Eng.* 5 (2017), pp. 9402–9411.
- [14] Y. Dai, J. Van Spronsen, G. J. Witkamp, R. Verpoorte, Y. H. Choi, Natural deep eutectic solvents as new potential media for green technology, *Anal. Chim. Acta.* 766 (2013), pp. 61–68.
- [15] A. P. Abbott, R. C. Harris, K. S. Ryder, C. D'Agostino, L. F. Gladden, M. D. Mantle, Glycerol eutectics as sustainable solvent systems, *Green Chem.* 13 (2011), pp. 82–90.
- [16] E. L. Smith, A. P. Abbott, K. S. Ryder, Deep Eutectic Solvents (DESs) and Their Applications, *Chem. Rev.* 114 (2014), pp. 11060–11082.
- [17] J. F. B. Pereira, Á. S. Lima, M. G. Freire, J. A. P. Coutinho, Ionic liquids as adjuvants for the tailored extraction of biomolecules in aqueous biphasic systems, *Green Chem.* 12 (2010), pp. 1661–1669.
- [18] H. Passos, A. Luís, J. A. P. Coutinho, M. G. Freire, Thermoreversible (Ionic-Liquid-Based) Aqueous Biphasic Systems, *Sci. Rep.* 6 (2016).
- [19] A. M. Ferreira, A. F. M. Cláudio, M. Válega, F. M. J. Domingues, A. J. D. Silvestre, R. D. Rogers, J. A. P. Coutinho, M. G. Freire, Switchable (pH-driven)

- aqueous biphasic systems formed by ionic liquids as integrated production–separation platforms, *Green Chem.* 19 (2017), pp. 2768–2773.
- [20] ChemSpider - Search and Share Chemistry. <http://www.chemspider.com> (Accessed November 2017).
- [21] D. Jones, A. G. Owen, J. F. Farrar, Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts, *Soil Biol. Biochem.* 34 (2002), pp. 1893–1902.
- [22] S. Moore, W. H. Stein, A modified ninhydrin reagent for the photometric determination of amino acids and related compounds, *J. Biol. Chem.* 211 (1954) pp. 907–903.
- [23] M. G. Freire, A. F. M. Cláudio, J. M. M. Araújo, J. A. P. Coutinho, I. M. Marrucho, J. N. C. Lopes, L. P. N. Rebelo, Aqueous biphasic systems: a boost brought about by using ionic liquids, *Chem. Soc. Rev.* 41 (2012) 4966–4995.
- [24] M. Hu, M. Wang, S. Li, Y. Jiang, H. Guo, Liquid–liquid equilibria for water+1-propanol/2-propanol+potassium chloride+cesium chloride quaternary systems at 298.1 ± 0.1 K, *Fluid Phase Equilib.* 263 (2008), pp. 109–114.
- [25] Y. Li, S. Yang, W. Zhang, X. Lu, H. Yang, J. Hu, Phase equilibria and salt effect on the aqueous two-phase system of ethanol + 2-propanol + salt $[(\text{NH}_4)_2\text{SO}_4/\text{Na}_2\text{SO}_4]$ + water, *J. Therm. Anal. Calorim.* 127 (2017), pp. 2473–2487.
- [26] R. L. Souza, S. P. M. Ventura, C. M. F. Soares, J. A. P. Coutinho, Á. S. Lima, Lipase purification using ionic liquids as adjuvants in aqueous two-phase systems, *Green Chem.* 17 (2015), pp. 3026–3034.
- [27] Y. Li, Y. Zhao, R. Huang, Q. Cui, X. Lu, H. Guo, A thermodynamic study on the phase behaviour of ethanol and 2-propanol in aqueous ammonium sulphate/sodium sulphate solution, *J. Mol. Liq.* 211 (2015), pp. 924–933.
- [28] Y. Wang, Y. Yan, S. Hu, J. Han, X. Xu, Phase diagrams of ammonium sulfate+ ethanol/1-propanol/2-propanol+ water aqueous two-phase systems at 298.15 K and correlation, *J. Chem. Eng. Data*, 55 (2009), pp. 876–881.
- [29] H. Passos, S.H. Costa, A.M. Fernandes, M.G. Freire, R.D. Rogers, J.A.P. Coutinho, A triple salting-out effect is required for the formation of ionic-liquid-based aqueous multiphase systems, *Angew. Chemie Int. Ed.* 56 (2017), pp. 15058–15062.
- [30] H. Renon, J. M. Prausnitz, Local compositions in thermodynamics excess

- functions for liquids mixtures, *AIChE J.* 14 (1968), pp. 116–128.
- [31] R. A. G. Sé, M. Aznar, Thermodynamic modelling of phase equilibrium for water + poly(ethylene glycol) + salt aqueous two-phase systems, *Brazilian J. Chem. Eng.* 19 (2002), pp. 255–266.
- [32] L. Stragevitch, S. G. DÁvila, Application of a Generalized Maximum Likelihood method in the reduction of multicomponent liquid-liquid equilibrium data, *Brazilian J. Chem. Eng.* 14 (1997).
- [33] J. A. Nelder, R. Mead, A simplex method for function minimization, *Comput. J.* 7 (1964), pp. 308–313.

Highlights

- > ABS composed of $[N_{111}(2OH)]Cl$ + alcohols + K_2HPO_4 + H_2O were studied.
- > Alcohols nature and concentration effect were evaluated.
- > The alcohol presence changes the liquid-liquid equilibrium.
- > *n*-Propanol-based systems present a triphasic region.
- > Alcohols act as designer solvents in the partition and selectivity of biomolecules.