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pH Effect on the Formation of Deep-Eutectic-Solvent-Based Aqueous Two-Phase Systems

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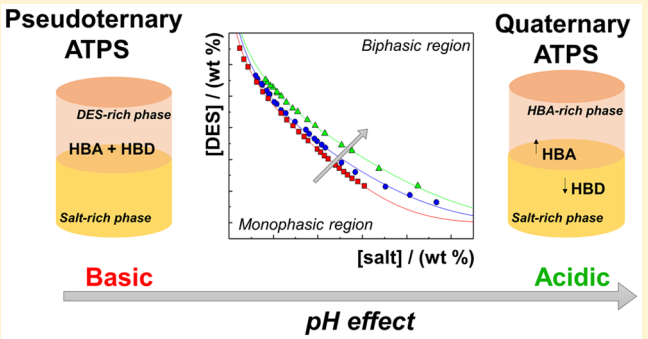
Supporting Information

ABSTRACT: Deep eutectic solvents (DES) are an emerging type of green solvents that have been used in aqueous two-phase systems (ATPS). However, its application in ATPS can present some limitations, due to the nonstoichiometric partition between the two aqueous phases of the hydrogen-bond acceptor (HBA) and the hydrogen-bond donor (HBD) that compose the DES. In this context, pseudoternary DES-based ATPS, in which DES' components stoichiometry is maintained, appear as potential liquid−liquid systems to the extraction and separation of biomolecules. Here the pH influence on the phase equilibrium and DES' components partition on ATPS composed of DES, constituted by tetrabutylammonium chloride + ethanol/ *n*-propanol, and potassium citrate/citrate buffer was evaluated at 298 K and pH 9, 7, and 5. In addition the partition coefficients of gallic acid, caffeine, and L-tryptophan were also determined, allowing inferences to be made on the potential application of these systems. The results suggest that the change on the pH impacts the chemical speciation of the potassium citrate salt, and consequently its ability to form an ATPS, allowing the stoichiometric partition of DES' components and the formation of a pseudoternary system and the tuning of biomolecules partition behavior to be controled.



INTRODUCTION

Deep eutectic solvents (DES), nowadays considered green solvents, result from hydrogen bonds that occur between a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD). DES share some of the ionic liquid's well-known characteristics, namely low volatility, high conductivity, wide liquid temperature range, and high solvation ability for a large number of compounds, and additionally present high biodegradability, easy preparation, and renewable character.[1−4] Due to these properties, DES have been applied in the formation of aqueous two-phase systems (ATPS)[4−12] allowing high extraction levels of proteins,[5−9] textile dyes,[10] phenolic compounds,[4,11,12] amino acids, and alkaloids to be obtained.[4,12] In these systems, the HBA and HBD are able to modulate the properties of the ATPS' phases, allowing high partition coefficients to be obtained for biomolecules of interest.[4,10,12]

It was previously demonstrated that there is a non-stoichiometric partition of the HBA and HBD between the coexisting phases of the ATPS, which results from a disruption of the hydrogen bonds between the DES' components.[10−12] Nevertheless, it was recently reported that, by using a DES composed of a HBA and a HBD of high hydrophilicity, such as the cholinium chloride (HBA) and the glucose (HBD), mixed with a more hydrophobic phase forming compound (the poly(propylene) glycol, PPG), it is possible to induce the formation of a pseudoternary system, by preserving the stoichiometry of the initial DES in the coexisting phases of the system, even though the hydrogen bonds complexes may no longer be present.[12] Furthermore, the authors demonstrated that the manipulation of the HBA and HBD partition between the ATPS' phases depends on the nature of the DES components, the nature of the other phase forming compound, as well as their concentration in the starting mixture.[12] As reported in the literature, these types of systems facilitate the recovery of DES components and allow the purification of the target biomolecules, making pseudoternary DES-based ATPS a potential downstream purification process for the extraction of value-added compounds.[12] However, this behavior was never previously demonstrated in DES/salt-based ATPS.

In our previous works the mechanism that rules the formation of DES-based ATPS was addressed.[4,11] These works suggest that DES/salt-based ATPS are induced by the competition between the high charge density salt and the HBA, usually a quaternary ammonium salt, for the formation of hydration complexes, with the HBD acting as an additive that allows manipulation of the equilibrium and the phases

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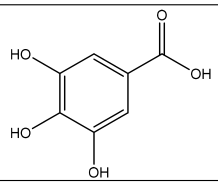
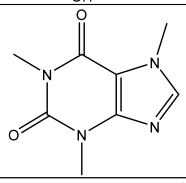
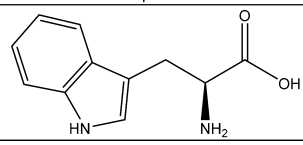
Table 1. Biomolecules Chemical Structure, Purity, Molecular Weight (M_w), Logarithm of Octanol–Water Partition Coefficient ($\log(K_{OW})$), and Acid Dissociation Constants (pK_a)[24]

Name	Chemical structure	Purity (wt %)	M_w (g mol^{-1})	$\log(K_{OW})$	pK_{a1}/pK_{a2}
Gallic Acid		99.5	170.1	0.72	3.9/9.0
Caffeine		99.0	194.2	-0.55	---[a]
L-Tryptophan		99.0	204.2	-1.09	2.5/9.4

[a]Caffeine does not suffer chemical speciation.

properties. These systems present some advantages such as the low phase viscosity and consequently a fast phase separation, low cost (usually polymers are more expensive than salts), and sharper separation of compounds, when compared with polymer-based ATPS. Furthermore, salt-based ATPS allows for work on a large range of pH values which could be of high interest for the separation of some specific compounds. Actually, it is known that external factors such as pH and temperature can be used to control the liquid–liquid equilibrium and tuning the biomolecules partition behavior.[13]

In this context, the goal of this work is to evaluate the pH effect on DES/salt-based ATPS equilibrium. ATPS constituted by potassium citrate salt ($K_3C_6H_5O_7$) and DES composed of tetrabutylammonium chloride ($[N_{4444}]Cl$) as HBA and the alcohols ethanol and *n*-propanol as HBD are evaluated. The use of citrate-based organic salt is desirable due to its high affinity with water and consequently strong salting-out effect, high biodegradability, and nontoxicity.[14−16] In addition, $K_3C_6H_5O_7$ can be used to prepare buffer solutions in a large range of pH values when mixed with citric acid ($C_6H_8O_7$).[14,15,17,18] The quaternary ammonium salt $[N_{4444}]Cl$, previously used as HBA of DES applied in solid–liquid extraction[19−21] and biomolecules partition in ATPS,[14,22,23] was selected considering its low hydrophilic character ($\log(K_{OW}) = 1.32$)[24] when compared with the conventionally used cholinium chloride ($[N_{111(2OH)}]Cl$, $\log(K_{OW}) = -4.66$).[24] To further add to the DES low hydrophilicity, the alcohols ethanol ($\log(K_{OW}) = -0.16$) and *n*-propanol ($\log(K_{OW}) = 0.36$) were used as HBD, and mixed with the HBA at three different molar ratios (2:1, 1:1, and 1:2). Finally, in order to evaluate the ability of these systems to be applied in separation processes, biomolecules of three different classes—a phenolic antioxidant (gallic acid), an amino acid (L-tryptophan), and an alkaloid (caffeine)—were selected as molecular probes to determine biomolecules partition and extraction behavior at different pH values.

MATERIAL AND METHODS

Materials. The liquid–liquid phase diagrams were prepared using aqueous solutions of potassium citrate tribasic monohydrate ($K_3C_6H_5O_7{\cdot}H_2O$, >99 wt % pure from Sigma-Aldrich) and citric acid ($C_6H_8O_7$, >99 wt % pure from Alphatec). The DES mixtures were obtained using the quaternary ammonium salt tetrabutylammonium chloride ($[N_{4444}]Cl$, 97 wt % pure from Sigma-Aldrich) and the alcohols ethanol, purchased from Fischer Scientific (99.8 wt % pure), and *n*-propanol from Lab-Scan (99.5 wt % pure). All chemicals were used without further purification. The biomolecules studied were the phenolic compound gallic acid, the alkaloid caffeine, and the amino acid L-tryptophan. The gallic acid was purchased from Merck. The caffeine and L-tryptophan from Fluka. The chemical structure and purity of these biomolecules and their properties are shown in Table 1.

METHODS

Determination of Phase Diagrams and Tie-Lines. As established in our previous works,[4,10−12] the liquid–liquid equilibria experimental data for DES-based ATPS can be obtained by the direct dissolution of the HBA and HBD in the aqueous solution, without previous preparation of DES. Thus, to determine the phase diagrams, aqueous solutions of $K_3C_6H_5O_7$ (for pH 9) or $K_3C_6H_5O_7/C_6H_8O_7$ buffer (for pH 7 and 5)[16] and aqueous solutions of $[N_{4444}]Cl$ and alcohols mixed at different molar ratios (2:1, 1:1, and 1:2) were prepared with $\pm 10^{-4}$ g of uncertainty. A Metrohm 831 Karl Fischer coulometer was used to measure the water content of the salt and DES constituents, which was considered during the solutions preparation. The cloud point titration method was used to obtain the solubility curves at (298 ± 1) K and atmospheric pressure as described in previous works.[4,12] Liquid–liquid equilibrium experimental data of the ternary systems composed of $[N_{4444}]Cl$ + $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + H_2O and ethanol or *n*-propanol + $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + H_2O were also determined.

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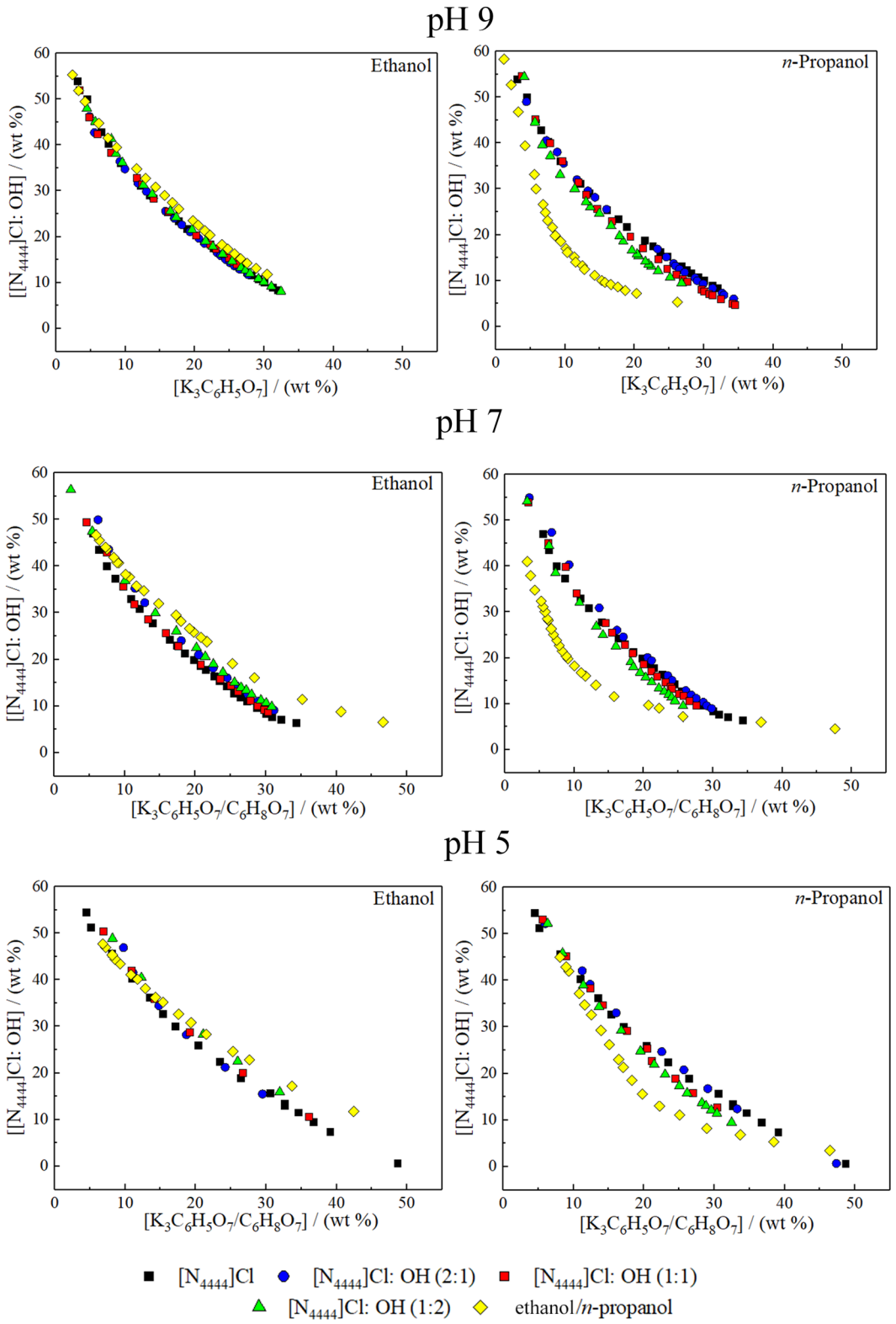

Figure 1. Phase diagrams at 298 K and atmospheric pressure of ATPS composed of $[N_{4444}]Cl{:}OH$ + $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + H_2O at different molar ratios of $[N_{4444}]Cl{:}OH$ and pH values.

One mixture point at the biphasic region of each ATPS was chose to determine the tie-line phases composition. The mixture point was gravimetrically prepared ($\pm 10^{-4}$ g), stirred, and centrifuged (3500 rpm during 30 min) at (298 ± 1) K to reach the equilibrium. After that, the bottom and top phases were separated and weighted. A combination of three analytical methods was used to determine the coexisting phases' composition: ^{1}H nuclear magnetic resonance (NMR) spectroscopy, thermogravimetric analysis (TGA), and Karl Fischer titration. The amount of $[N_{4444}]Cl$, ethanol, and *n*-propanol present in both phases were determined by ^{1}H NMR analysis using a Bruker Avance 400 at 400 MHz, with deuterated

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dimethyl sulfoxide and tetramethylsilane (TMS) as the solvent and internal reference, respectively.[4] The amount of $[N_{4444}]Cl$, $K_3C_6H_5O_7$ and the fraction of water + alcohol were determine by TGA, using a PerkinElmer TGA 400 according with the method proposed by Farias et al.[11] The Karl Fischer titration (Mettler Toledo V30, volumetric Karl Fischer titrator) was used to confirm the water content in each ATPS' phase. To confirm the quality of the obtained results, a mass balance was performed considering the initial mass of each component and the amounts quantified in both phases.

In quaternary systems, like DES-based ATPS, the tie-lines are in the space.[12] Thus, three components were taken into account for the calculation of the tie-line length (TLL), as described by eq 1:

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

where $[X]$, $[Y]$, and $[Z]$ are $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$, $[N_{4444}]Cl$, and alcohol weight fraction percentages on the top or bottom phases, respectively.

Biomolecules' Partition. The study on the partition of biomolecules was carried out at a fixed tie-line with an overall composition of 25 wt % of citrate-based salt +30 wt % of DES. For each biomolecule an aqueous solution was prepared at the following concentrations: 5.15×10^{-3} mol L^{-1} for caffeine, 4.90×10^{-3} mol L^{-1} for L-tryptophan, and 2.94×10^{-3} mol L^{-1} for gallic acid. These solutions were used in place of the water composition in each tie-line. The phase-forming components were properly weighed using an analytical balance ($\pm 10^{-4}$ g), vigorously stirred until complete dissolution of all components, and centrifuged at 3500 rpm for 30 min at (298 ± 1) K to achieve the complete partition of the biomolecules.[12] At least two individual experiments were carried out for each ATPS. An UV−vis spectrophotometer (UV-1800, Shimadzu) was used to measure the biomolecule concentration in both phases at the wavelength of 262, 273, and 279 nm for gallic acid, caffeine, and L-tryptophan, respectively, and using calibration curves previously established. The partition coefficient and the extraction efficiency percentage were determined according to eqs 2 and 3.

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

$$EE = \frac{w_{Biom}^{top}}{w_{Biom}^{top} + w_{Biom}^{bottom}} \times 100 \quad (3)$$

where $[Biom]_{top}$ and $[Biom]_{bottom}$ are the concentrations of the each extracted biomolecule in the top and bottom phases and w are the weights of the extracted biomolecules in the top or bottom phase.

RESULTS AND DISCUSSION

Phase Diagrams. The binodal curves of the systems composed of $[N_{4444}]Cl$, alcohol (OH), citrate salt or buffer, and water at different pH values (9, 7 and 5) are shown in Figure 1. The binodal curves experimental data and respective tie-lines are presented in the Supporting Information. Since DES-based ATPS are quaternary systems,[4,12] a three-dimensional representation is also provided in the Supporting Information.

Considering the formation mechanism of the ternary systems composed of $[N_{4444}]Cl$, $K_3C_6H_5O_7$, and water, there is a competition between the two salts for the formation of hydration complexes.[14,17] Due to the higher charge density of the citrate-based salt, this presents a higher ability to interact with water, inducing the salting-out of $[N_{4444}]Cl$ to the opposite aqueous phase. When an HBD is added to the system, namely ethanol or *n*-propanol, changes on the binodal curves are observed which seem to depend on the alcohol nature and concentration, cf. Figure 1. The presence of ethanol slightly reduces the biphasic region, while the *n*-propanol induces an increase of this region.

Despite both alcohols used in this work being completely miscible with water, it was previously demonstrated that addition of a salt to an alcohol solution induces the water molecules migration away from the ethanol/*n*-propanol molecules.[4,25,26] In fact, both alcohols are able to form ATPS in aqueous solutions of $K_3C_6H_5O_7$ salt, with no need of $[N_{4444}]Cl$. Wang et al.[27] used the effective excluded volume theory (EVV) to explain the ability of alcohols to induce ATPS formation. These authors[27] proposed that the boiling point temperature can be used to explain the strength of the acting forces (van der Waals and hydrogen-bond) between the alcohol molecules. Thus, molecules with higher boiling points (*n*-propanol boiling point: 370 K; ethanol boiling point: 351 K) present high acting forces between its molecules than those with lower boiling points, and thus can be easily excluded from a salt-rich phase in an ATPS.[27] In fact, the ternary system composed of *n*-propanol + $K_3C_6H_5O_7$ + H_2O presents the largest biphasic region of all of the systems here studied (Figure 1).

In what concerns the pH effect on the binodal curves and considering that both DES components—$[N_{4444}]Cl$ and alcohol—do not suffer chemical speciation in the range of pH values studied,[24] the changes observed are induced by the chemical speciation of the citrate salt; the speciation curve of $K_3C_6H_5O_7$ is presented in the Supporting Information. At pH 9 and 7, $K_3C_6H_5O_7$ is present in aqueous solution as $C_6H_5O_7^{3-}$. By the acidification of the system until pH 5, there is a reduction of the negative charge to $C_6H_5O_7^{2-}$ and $C_6H_5O_7^{-}$ and, consequently, the salt ability to form ATPS is reduced, as shown in Figure 2. At higher acidic pH, citric acid and monovalent dihydrogen citrate anion species are predominant, preventing the formation of ATPS at these conditions.[18]

Coutinho and co-workers[28] demonstrated that the entropy of hydration (ΔS_{hyd}) is the driving force in the formation of salt-based aqueous two-phase systems. However, entropy of hydration data for citrate anions were never reported. Still, the better ability of the triple charged citrate anion to induce phase separation and ATPS formation can be gauged from its Gibbs free energy of hydration (ΔG_{hyd}), where $C_6H_5O_7^{3-}$ (ΔG_{hyd} = −2793 kJ mol^{-1}) > $C_6H_5O_7^{2-}$ (ΔG_{hyd} = −968 kJ mol^{-1}) > $C_6H_5O_7^{-}$ (ΔG_{hyd} = −81 kJ mol^{-1}). Ions with more negative ΔG_{hyd} result in a more structured water "lattice" around the salt ions, and consequently, the water amount available to hydrate the $[N_{4444}]Cl$ decreases.[16]

So, at pH 9 the predominance of the more negative ΔG_{hyd} values of the citrate ions are more efficient to promote the phase separation, in agreement with the Hofmeister series,

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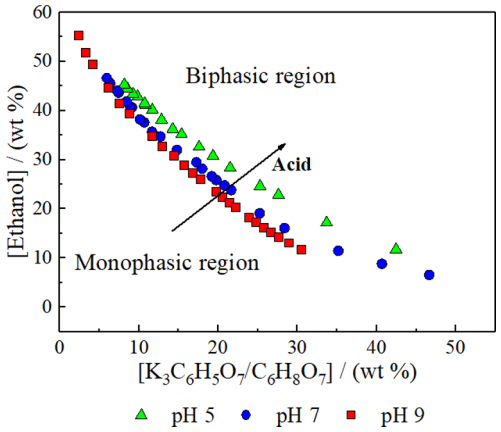

Figure 2. Effect of pH in the phase diagrams at 298 K and atmospheric pressure of ATPS composed of ethanol + $K_3C_6H_5O_7$/$C_6H_8O_7$ + H_2O at different pH values.

where anions with higher charge density are better salting-out agents.[16,29] The composition of top and bottom phases of each ATPS, at pH 9, 7, and 5, and respective TLL, are presented in Table 2.

In all of the systems evaluated, and independently of the pH, the top phases are mainly composed of $[N_{4444}]Cl$, while the bottom phases are richer in citrate-based salt. As expected and due to their low hydrophilicity, alcohols are present at higher concentration in the $[N_{4444}]Cl$-rich phase, the more hydrophobic phase of these systems. In order to evaluate the HBA and HBD final stoichiometry, the $[N_{4444}]Cl$:OH molar ratio was determined for both top and bottom phases, and the obtained results are presented in Figure 3.

Farias et al.[12] demonstrated that HBA:HBD initial stoichiometry can be maintained in the coexisting phases on ATPS composed of a polymer (PPG) and hydrophilic DES constituted by $[N_{111(2OH)}]Cl$ as HBA and glucose as HBD. This was possible considering the low solubility of the DES components in the polymer-rich phase and, consequently, preferential partition to the opposite phase. For the authors, to obtain a DES-based ATPS with pseudoternary characteristics, factors such as HBA and HBD nature and their concentration in the DES mixture need to be considered.[12] It was also postulated that the same could be achieved with the opposite polarities, i.e., in ATPS composed of a strong salting-out salt and DES of low hydrophilicity, creating pseudoternary salt/DES-based ATPS.[12] Through the data present in Figure 3, in the same way that was observed on the binodal curves, small differences were found between pH 9 and pH 7. At both pH values, 9 and 7, ethanol and *n*-propanol are mainly partitioned to the $[N_{4444}]Cl$-rich phase (top phase). As previously reported, in these pH values there are the predominance of the $C_6H_5O_7^{3-}$ ions, from $K_3C_6H_5O_7$ in aqueous solution, which present a high ability to salting-out the alcohol species to the opposite phase ($[N_{4444}]Cl$-rich phase). As a consequence, under these conditions, the HBA:HBD initial stoichiometry tends to be maintained in $[N_{4444}]Cl$-rich phase for both alcohols, proving what was previously postulated. However, due to the very low concentration of both HBA and HBD on the bottom phase the initial molar ratio is just kept for ethanol-based systems. For the propanol-based DES systems, distinct final molar ratios were observed, similar to those previously reported in polymer/DES-based ATPS.[12] On the other hand, at pH 5 a larger amount of alcohol is partitioned to the bottom phase, which is richer in salt, and consequently the HBA:HBD initial stoichiometry is not maintained. This behavior could be related with the predominant presence of the $C_6H_5O_7^{2-}$ and $C_6H_5O_7^{-}$ anion species at pH 5, which present a lower ability to induce the salting-out of both $[N_{4444}]Cl$ and alcohols to the top phase. These results suggest that the pH enables the HBA:HBD molar ratio of aqueous two-phase systems to be tuned. In order to evaluate the pH effect on the separation ability of these systems, the partition coefficients of gallic acid, L-tryptophan, and caffeine were also measured.

Table 2. Liquid−Liquid Equilibrium Experimental Data at 298 K and Atmospheric Pressure of $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ (1) + $[N_{4444}]Cl$ (2) + alcohol (3) + H_2O (4) Systems in the Mixture Point 25 wt % of Citrate-Based Salt +30 wt % of DES

	top phase (wt %)			bottom phase (wt %)			
alcohol molar fraction	*w*1	*w*2	*w*3	*w*1	*w*2	*w*3	TLL
pH 9							
0[a]	0.1	63.0		46.9	4.5		74.5
ethanol/$[N_{4444}]Cl$-based ATPS							
0.33	2.8	58.1	4.8	42.2	7.3	0.6	64.5
0.50	2.0	55.5	9.9	43.0	5.5	0.7	65.4
0.67	1.7	51.4	16.6	41.1	5.4	2.0	62.4
1.00[b]	2.4		63.9	40.4		10.4	65.6
n-propanol/$[N_{4444}]Cl$-based ATPS							
0.33	2.1	59.5	6.3	42.8	5.0	0.6	68.2
0.50	2.1	58.3	10.5	41.4	4.2	2.2	67.3
0.67	1.7	51.5	20.2	41.4	2.6	2.3	65.5
1.00[b]	1.5		81.9	37.1		7.7	82.3
pH 7							
0[a]	2.4	63.2		45.0	3.9		73.1
ethanol/$[N_{4444}]Cl$-based ATPS							
0.33	2.7	57.7	4.1	41;0	4.1	2.1	65.9
0.50	2.3	56.7	7.3	40.6	3.9	3.5	65.3
0.67	2.0	50.1	13.4	38.5	3.9	3.4	59.7
1.00[b]	2.0		63.1	44.3		9.8	68.1
n-propanol/$[N_{4444}]Cl$-based ATPS							
0.33	1.7	60.3	6.9	40.0	3.9	5.3	68.2
0.50	1.5	55.7	12.7	41.7	4.7	4.1	65.6
0.67	1.0	51.8	17.9	38.1	3.4	3.8	62.6
1.00[b]	0.5		76.0	40.7		10.0	77.3
pH 5							
0[a]	6.4	55.2		45.5	1.0		66.9
ethanol/$[N_{4444}]Cl$-based ATPS							
0.33	7.8	52.6	3.8	43.2	1.1	0.6	62.6
0.50	7.1	49.1	4.3	42.2	3.5	4.4	57.5
0.67	9.1	44.4	7.0	39.2	2.3	8.3	54.5
1.00[b]	6.7		51.4	41.0		11.2	52.7
n-propanol/$[N_{4444}]Cl$-based ATPS							
0.33	6.2	51.1	3.9	48.9	0.3	0.3	66.5
0.50	4.4	53.7	3.9	42.4	1.6	4.8	64.5
0.67	4.0	49.4	13.5	42.7	1.1	2.8	62.8
1.00[b]	1.5		71.7	36.5		11.4	69.7

[a]Ternary system composed of $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + $[N_{4444}]Cl$ + H_2O. [b]Ternary system composed of $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + alcohol (OH) + H_2O.

Biomolecules' Partition on Salt/DES-Based ATPS. Since small differences were observed between the binodal curves and HBA:HBD molar fraction at pH 7 and pH 9, only systems at pH 5 and pH 9 were chosen to evaluate the effect of

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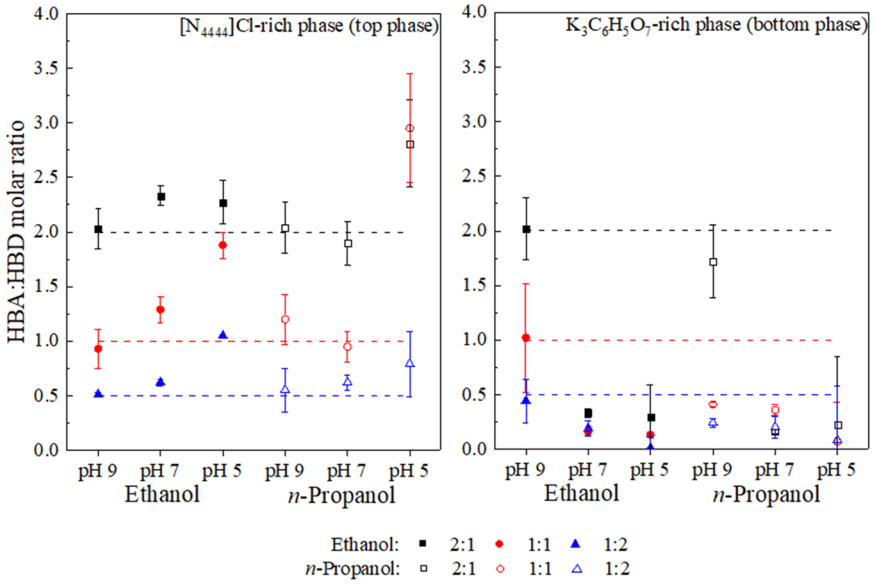

Figure 3. HBA ([N_{4444}]Cl) and the HBD (alcohols) molar ratio in the initial mixture composition (dashed lines) and the coexisting phases (symbols) of ATPS composed of $K_3C_6H_5O_7$ (pH 9) or $K_3C_6H_5O_7/C_6H_8O_7$ (pH 7 or 5) + [N_{4444}]Cl + alcohol + H_2O.

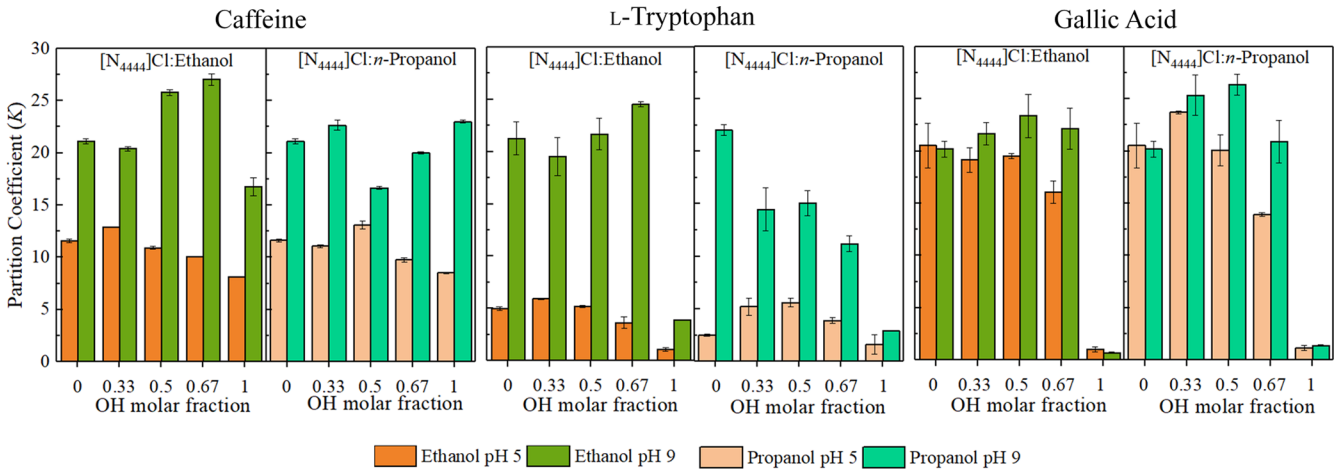

Figure 4. Partition coefficients (K) of gallic acid, caffeine, and L-tryptophan to the [N_{4444}]Cl-rich phase in systems composed of $K_3C_6H_5O_7$ or $K_3C_6H_5O_7/C_6H_8O_7$ + [N_{4444}]Cl + alcohol (OH) + H_2O at different [N_{4444}]Cl:OH molar fractions and pH values: pH 5 and pH 9.

nature and concentration of the alcohol as well as the pH of the systems in the partition of gallic acid, L-tryptophan, and caffeine. The partition coefficients (K) obtained for each biomolecule are presented in Figure 4.

Caffeine, an alkaloid, does not suffer chemical speciation with pH (as shown Figure S7, Supporting Information). So, in both pHs studied caffeine is found in a noncharged form, which means that the pH changes should not affect its partition behavior in function of caffeine's properties. As a consequence, any changes observed on the K values to systems with the same overall composition must be related to changes on the phase's properties. It means that the higher K values measured at pH 9 than at pH 5 are due to the stronger ability of the citrate ions at pH 9 to salt-out caffeine to the [N_{4444}]Cl-rich phase than citrate ions at pH 5, as previously discussed.

L-Tryptophan, at pH 5 is found in zwitterionic form (without net charge).[30,31] At pH 9, around of 70% of its species are found without net charge and 30% in a monovalent negative charged ion species (Figure S8, in the Supporting Information). At pH 9 the K values are higher than at pH 5, for all of the systems. This is a result of the stronger salting-out ability of the citrate salt in this condition, which favors the mass transfer of the L-tryptophan to [N_{4444}]Cl-rich phase. Moreover, is important to highlight the [N_{4444}]Cl influence on the L-tryptophan partition. The higher K values are clearly visible compared to the systems without [N_{4444}]Cl (ethanol/n-propanol + $K_3C_6H_5O$ + H_2O). A similar behavior was previously demonstrated with a different ammonium quaternary salt, [$N_{111(2OH)}$]Cl.[4,12] The authors[4,12] suggested that the high partition of L-tryptophan to the [$N_{111(2OH)}$]Cl-rich phase is a result of specific interactions that can occur between this biomolecule and [$N_{111(2OH)}$]Cl. These interactions must be essentially dispersive, though coloumbic interactions between the ammonium cation and the negatively charged amino acid cannot be discarded.

Finally, the partition behavior of gallic acid was evaluated, the biomolecule with the highest chemical speciation among those evaluated. The gallic acid partition coefficient values obtained are presented in Figure 4. According to gallic acid speciation curve (Figure S6, in the Supporting Information), in

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solutions with 3.9 < pH < 9.0, gallic acid is found negatively charged, with mono and divalent anions. At pH 5 the divalent negative charge is predominant (around of 90%), while at pH 9 mono and divalent species are found in almost equal quantities (50/50%). At pH 5 the predominance of the divalent charged species occurs, which contributes to a low partition of the gallic acid to the $[N_{4444}]Cl$-rich phase, and consequently an increase of the K values to the $K_3C_6H_5O_7$-rich phase was observed. At pH 9 a slight increase was observed in the K values which are related to the strong salting-out effect of the salt ($K_3C_6H_5O_7$) on this biomolecule. Moreover, similar to the L-tryptophan partition behavior, through the analysis of the ternary systems (ethanol/*n*-propanol + $K_3C_6H_5O$ + H_2O) and the quaternary systems at both pH values, is possible to realize that the $[N_{4444}]Cl$ presence seems to have a high effect on gallic acid partition. In the ternary systems ethanol/*n*-propanol + $K_3C_6H_5O_7$ + H_2O, without the quaternary ammonium salt, the top phase is richer in alcohol and predominantly noncharged, which induces the preferential gallic acid partition to the salt-rich phase. This behavior is in agreement with data previously reported in the literature, where it was demonstrated that noncharged gallic acid species tend to be partitioned to the more hydrophobic phase, while charged gallic acid species tends to partition to the hydrophilic phase.[32] This fact reinforce a high affinity between the gallic acid and the $[N_{4444}]Cl$, similar to the amino acid L-tryptophan.

Considering the partition behavior for all biomolecules in this study, at pH 9 the K values tend to increase with the ethanol presence, while with the *n*-propanol the K values tend to the opposite side (biomolecules partition to the salt-rich phase). An exception is the gallic acid partition at pH 9, in which the K values also increase with the *n*-propanol concentration. For all biomolecules the highest K values were found for the quaternary systems with $[N_{4444}]Cl$:OH molar ratio 1:1 and 1:2. Besides, the differences in the K values can be primarily attributed to the phase properties. As discussed before, in an acidic medium the ions with a lower charge density are predominant and consequently a minor salting-out effect occurs on the biomolecules under study. So, to manipulate the partition behavior a combination of hydrophobic/hydrophilic and electrostatic interactions, as well as the salting-out effect, need to be considered.

CONCLUSIONS

New DES/salt-based ATPS were here proposed, where the phase properties could be changed by alcohol nature and pH. It was shown for the first time a DES/salt-based ATPS where the initial HBA:HBD molar ratio can be maintained in ATPS coexisting phases. This behavior was achieved through the choice of less hydrophilic DES (based on $[N_{4444}]Cl$ and ethanol/*n*-propanol) which are easily salting-out to a new phase by the citrate salt. The pH effect results of the chemical speciation of the citrate salt, with reduction of its ability to form ATPS and the increase of the system acidity. Moreover, the phases properties can be also used to tune the partition of different biomolecules in this type of systems. It was observed that besides the nature of the biomolecules and the DES, the pH of the medium has also an important role in the partition mechanism, which is predominantly dominated by the hydrophobicity difference between the phases and the salting-out ability of the salt. The results of this work show the versatility of the DES-based ATPS, where external factors such as pH can be used to control the DES components partition, moving from a quaternary to a pseudo ternary system, and consequently to change the phases properties and tune the partition behavior of target biomolecules. The possibility to work in a large range of pH can be useful to the application of these ATPS to extract and purify compounds which are sensitive to pH changes. Moreover, these systems have been shown to have potential as downstream purification processes for extraction of value-added compounds.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.iecr.8b04256.

Detailed experimental data of binodal curves, biomolecules extraction efficiencies, partition coefficients, tie-lines representation, and 3D representation of the quaternary systems (PDF)

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Notes

The authors declare no competing financial interest.

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