Study of the pseudo-ternary aqueous two-phase systems of deep eutectic solvent (choline chloride:sugars) + K$_2$HPO$_4$ + water

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ABSTRACT

Deep eutectic solvents (DES) are a new type of solvents that share some characteristics with Ionic Liquids while being cheaper, easier to prepare and, in some case, more readily biodegradable. This work reports a study on the Liquid-liquid Equilibria (LLE) of aqueous two-phase systems composed by DES + K$_2$HPO$_4$ + water at 298.15 K. Various DES using choline chloride as hydrogen bond acceptor (HBA), and sugars as hydrogen bond donor (HBD), were prepared at several different proportions. An approach based on thermogravimetric analysis is here proposed to quantify the phase's components. The results show that the hydrogen bonds of DES are disrupted in aqueous solutions, leading to a differential partition of the HBA and HBD between the two phases, and to be coherent a quaternary ATPS must be considered. The NRTL model was used to describe the experimental LLE data with a low mean deviation. These systems were applied to Gallic Acid partition showing good extraction levels.

1. Introduction

Aqueous two-phase systems (ATPS) were introduced by Albertson [1] for protein purification. The phases of an ATPS are composed by immiscible aqueous solutions of polymer-polymer, salt-salt, or polymer-salt [2—4]. Conventional systems formed by the polymer poly(ethylene)glycol and salt present high biocompatibility with a broad range of molecules, low cost and toxicity [5,6]. However, these systems also have disadvantages such as emulsion formation and high viscosity. To minimize these effects and increase the separation efficiency, ATPS formed by organic solvents such as acetone [7], alcohols [8], and ionic liquids (ILs) [9,10] have been proposed for extraction and purification of biomolecules.

ILs are the result of the combination of organic cations with dispersed charge, and a wide range of anions. Rogers and co-workers showed that they could be used to prepare ATPS [9] with a good extraction capacity for several organic and inorganic molecules [9—11]. Despite the ILs advantages, they are expensive, often

poorly biodegradable [12,13], and some studies relate possible toxicity [12,14].

Deep eutectic solvents (DES) are a new type of solvents with similar physical characteristics to IL's, yet they are easier to prepare in a pure state and are characterized by a greater biodegradability. The DES are formed by an association of a hydrogen bond acceptor, like quaternary salts of ammonium [15] and others (e.g. phytic acid sodium, proline, and betaine) [16], and hydrogen donors (alcohols, sugars, metallic salts, and others) with strong hydrogen bonds [15—17]. These solvents can be made from natural composites, and then they are called natural deep eutectic solvents (NADES) [18]. In general, the component structure of these eutectic solvents results in a wide range of physical properties [19] and, due to similar properties with ILs, they have been proposed as a substitute of IL's in ATPS [20—24].

Xu and coworkers [20—23] were the first to apply DES in ATPS formation. The authors suggest that when adding DES to a salt solution of a strongly hydrophilic salt (e.g. K$_2$HPO$_4$), there is a water competition between salt and DES, and, due to the higher affinity of the salt for the water two immiscible aqueous phases are formed: The top phase is rich in DES while the bottom phase is rich in salt [23]. They report results of purification and extraction of proteins in ATPS formed by different DES and salt (K$_2$HPO$_4$) but made no considerations about the DES stability in aqueous solution [20—23].

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Passos et al. [24] questioned for the first time the stability of DES in ATPS formed by DES (composed of choline chloride and organic acids) and poly(propylene)glycol. According to these authors, there are no real DES-based ATPS since the DES integrity is destroyed in aqueous solutions. This hypothesis was proven by ¹H NMR technique, showing that the ATPS formation resulted in a non-stoichiometric partition of choline chloride and the hydrogen bonds donors between the two phases. Nevertheless, the authors concluded that, although these ATPS were actually quaternary systems they still held a great potential to be used in the extraction process. No such study was so far carried for DES + salt + H₂O ATPS.

In this way, the aim of this study is to measure liquid-liquid phase equilibria (LLE) of pseudo-ternary systems composed of DES + K_2HPO_4 + H₂O and compare them with the ternary system composed by Choline Chloride (ChCl) + K_2HPO_4 + H₂O. A new analytical technique based on thermogravimetric analysis is proposed for the quantification of the phases composition, and the results should shed light about the stability of the DES on these ATPS. The NRTL model will be applied for the correlation of the experimental LLE data. Additionally, a study about gallic acid partition in these systems will be carried.

2. Experimental

2.1. Materials

Choline Chloride was purchased from Merck Millipore (Darmstadt, Germany). The sugars fructose, glucose, and saccharose were obtained from Neon Commercial (São Paulo, SP, Brazil) and dibasic potassium phosphate from Alphatech LTDA (São Bernardo do Campo, SP, Brazil). The water used in all experiments passed by a reverse osmosis system purification apparatus (Vexer, Smart VOS 106). The detailed information about the materials mentioned above is listed in Table 1.

2.2. DES preparation

On this study, three DESs were prepared in three different ratios of quaternary ammonium salts (choline chloride) to hydrogen bond donors (D-glucose, D-fructose, saccharose), as is shown in Table 2. The eutectic mixtures were prepared using mild heating, combined with stirring at 323.15 K until a homogeneous transparent liquid was formed [16]. The materials used for the DES preparation were previously dried at 398.15 K for at least 2 h and maintained at a desiccator with silica gel until the use. Given that a thermogravimetric analysis was adopted for the quantification of the phases composition an HBA:HBD mass ratio, instead of the conventional mol ratio, was here adopted. Both mass and molar proportions of the systems studied are reported in Table 2.

2.3. Determination of the phase diagrams

Binodal curves were determined by the cloud point titration method [25] at 298.15 ± 0.5 K and atmospheric pressure (around 91 kPa). Firstly, aqueous solutions of K_2HPO_4 at 60% (m/m) and aqueous solutions of the nine DES (Table 2) at 75% (m/m) were prepared. In this method, DESs or potassium phosphate solution was added drop-wise to each other, under agitation, until the cloud point was visually detected. Thus, water was added drop-wise until a clear and limpid solution is detected again, that corresponds to the monophasic region. Mixture composition was calculated by weight quantification (AUX320, Shimadzu) with an uncertainty of ±0.0001 g. This procedure was repeated until enough points were obtained in the binodal curve and the composition of the mixture was registered at each cloud point [24,25]. The binodal curve was also obtained for the ternary system choline chloride (ChCl) + K_2HPO_4 + water.

2.4. Determination of tie-lines (TLs)

For each system composed of three different DES (ChClGlu_1, ChClFru_1, and ChClSac_1) and a ternary system (Choline Chloride + K_2HPO_4 + water) six points of overall composition situated within biphasic were chosen. The systems were prepared adding gravimetrically choline chloride or DES, salt, and water in centrifuge tubes (15 mL), to obtain approximately 10 g of total mass with a 0.0001 g uncertainty. Thus, these compositions were stirred by a vortex mixer (MX-S, Gomixer) until the full dissolution of the compounds, and allowed to reach the equilibrium at 298.15 K in a thermostatic water bath (RW– 1025G, Lab Companion) for approximately 24 h [26]. The top and bottom phases are carefully separated and weighted. The density and pH measurements (Data at Supporting Information) were made in each phase using a densimeter (DMA-35, Anton Paar) and a pH meter (pH 11 series, Oakton™), respectively. The pH meter was previously calibrated, with three buffers (pH values of 4.00, 7.00 and 10.00). The determinations of phase's composition of each system are shown in the Sections 2.4.1 and 2.4.2. For this, the top phase of each tie-line was carefully separate from the bottom phase with a glass Pasteur pipette, and only after that, the bottom phase was collected to avoid any contamination between the phases.

2.4.1. System ChCl + K_2HPO_4 + water

For the ternary system formed by choline chloride, K_2HPO_4 and water the TLs were determined by a gravimetric method described by Merchuk et al. [27]. Considering the relationship between the phases and overall system composition, using a lever rule was possible to obtain each TL. For this, the experimental binodal curves were fitted using Eq. (1) [278]:

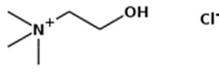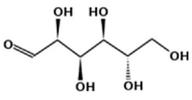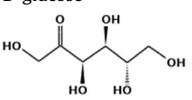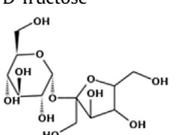$$Y = A \exp\left[\left(BX^{0.5}\right) - \left(CX^3\right)\right] \tag{1}$$

where Y and X are, respectively, the choline chloride and salt weight percentages. A, B and C are constants obtained by regression of the experimental binodal data.

To obtain the components concentration in the top and bottom phases and determined each tie-line a mass balance, Eqs. (2)–(5) were solved.

Table 1
Sources and purity of the materials.

Chemical name	Source	Purity (mass fraction)	Purification method
Choline Chloride	Merck Millipore	≥0.9800	None
Glucose	Neon Commercial	≥0.9995	None
Fructose	Neon Commercial	≥0.9995	None
Saccharose	Neon Commercial	≥0.9995	None
Potassium phosphate dibasic	Alphatec	≥0.9800	None
Water	—		Reverse osmosis

Table 2
Structures and compositions of the studied DESs.

Quaternary ammonium salt (HBA)	Hydrogen-bond donors (HBD)	HBA:HBD (mol ratio)	HBA:HBD (mass ratio)	Abbreviation
Choline chloride	D-glucose	2.5:1	2:1	ChClGlu_1
		1.3:1	1:1	ChClGlu_2
		1:1.5	1:2	ChClGlu_3
	D-fructose	2.5:1	2:1	ChClFru_1
		1.3:1	1:1	ChClFru_2
		1:1.5	1:2	ChClFru_3
	Saccharose	5:1	2:1	ChClSac_1
		2.5:1	1:1	ChClSac_2
		1:0.8	1:2	ChClSac_3

$$[ChCl]_{top} = A \times \exp\left(B \times [salt]_{top}^{0.5} - C \times [salt]_{top}^3\right) \tag{2}$$

$$[ChCl]_{bottom} = A \times \exp\left(B \times [salt]_{bottom}^{0.5} - C \times [salt]_{bottom}^3\right) \tag{3}$$

$$[ChCl]_{top} = \left(\frac{[ChCl]_M}{\propto}\right) - \left[\left(\frac{1-\propto}{\propto}\right) \times [ChCl]_{bottom}\right] \tag{4}$$

$$[salt]_{top} = \left(\frac{[salt]_M}{\propto}\right) - \left[\left(\frac{1-\propto}{\propto}\right) \times [salt]_{bottom}\right] \tag{5}$$

where the subscripts top and bottom designate, respectively, the top (choline chloride rich-phase) and bottom (salt rich-phase) phases, and M represents the mixture composition. The parameter α is the ratio between the weight of the top phase and the overall mixture.

2.4.2. Systems DES + K_2HPO_4 + water

Considering that those systems composed of DES (ChCl + HBD) + K_2HPO_4 + water can become quaternary systems [24] the quantification of the phase's components by a gravimetric method as described by Merchuk et al. [27] is not suitable. A thermogravimetric analysis using a Perkin Elmer TGA 400, was here developed. This technique quantifies each of the four components (ChCl, HBD, salt, and water) that may be present in the ATPS systems.

For this purpose, samples of each phase, around 10 mg, were weight in a platinum pan and submitted to a synthetic air atmospheric (50 mL/min) and scanned from 303.15 to 923.15 K at a heating rate of 10 K/min.

To evaluate the accuracy of this technique in quantifying the phase's components, mixtures of known concentrations were made to represent top and bottom phases of each system; these samples were analyzed in triplicate. More details about TGA analysis validation were in Supporting Information. The phase's samples were analyzed in duplicate.

2.4.3. Ion exchange analysis

To evaluate the ion exchange between the phase's systems, two tie-lines of each system are chosen and the phosphorus and potassium ions were determined by atomic emission spectroscopy using an inductively coupled plasma spectrometer (ICP-AES,

Varian, model 720-ES) with an axial torch configuration. For this analysis, 1 mL of the phase's samples were digested in 5 mL of nitric acid 4 of hydrogen peroxide for 30 min at 398 K. The samples were also filtered and properly diluted previously the analysis on the ICP.

2.5. Gallic acid partition

Two points of overall composition (corresponding to tie-line 1 and tie-line 6, Table 3), at the biphasic region, were prepared for each system composed of DES (ChClGlu_1, ChClFru_1, and ChClSac_1) and choline chloride. Three gallic acid concentrations were evaluated (250, 500 and 750 mg/L). All mixtures were prepared with a 0.0001 g uncertainty in centrifuge tubes, properly stirred by a vortex mixer (MX-S, Gomixer) and maintained at 298.15 K in a thermostatic bath (Lab Companion, RW - 1025G) for at least 18 h [28]. After separation of the phases, the gallic acid concentration in each phase was determined using a UV-VIS spectrophotometer (UV-1800, Shimadzu) at 262 nm. Gallic acid partition coefficient (K_{GA}) was determined as the ratio between the concentration of gallic acid of the top phase (choline chloride/DES rich-phase) and in the bottom phase (salt rich-phase) (Eq. (6)).

$$K_{GA} = \frac{[GA]_{top}}{[GA]_{bottom}} \tag{6}$$

Extraction efficiency was calculated considering the equilibrium concentration of gallic acid in the top and bottom phases, according to Eq. (7):

$$E_{topo}(\%) = \frac{[GA]_{top} \times V_{top}}{[GA]_{top} \times V_{top} + [GA]_{bottom} \times V_{bottom}} \tag{7}$$

where V corresponds the volume of each phase.

3. Results and discussion

3.1. Binodal data

The binodal curves were obtained with DES composed by three different sugars as HBD at three different proportions with choline chloride (HBA). The binodal curve for the system with choline chloride (ChCl) without any HBD was also obtained. The salt used to obtain these ATPSs, K_2HPO_4, was chosen due to its high-water solubility (around 1600 g/L) and salting-out ability [20,29]. All

Table 3
Liquid-liquid equilibrium experimental data to ternary ChCl and quaternary systems at 298.15 K and atmospheric pressure, where: (1) K_2HPO_4; (2) ChCl; (3) Water; (4) HBD and (5) DES[a] (2:1, mass fraction).

TL	Overall Composition		Top phase				Bottom phase			
	$100W_1$	$100W_{2 \text{ or } 5}$	$100W_1$	$100W_2$	$100W_3$	$100W_4$	$100W_1$	$100W_2$	$100W_3$	$100W_4$
ChCl										
1	25.1	25.0	13.0	37.3	49.8	–	41.9	7.8	50.1	–
2	25.7	25.8	11.1	40.5	48.4	–	45.1	6.2	48.7	–
3	26.5	26.4	9.7	43.3	47.0	–	47.9	4.8	47.2	–
4	27.2	27.1	8.2	46.7	45.1	–	49.5	4.5	46.3	–
5	27.8	27.9	7.1	49.4	43.5	–	51.4	3.5	45.2	–
6	28.5	28.5	6.3	51.7	42.1	–	53.3	2.8	43.9	–
ChClGlu_1										
1	28.6	28.6	11.5	37.0	44.7	6.9	37.1	10.4	41.4	10.8
2	29.3	29.3	9.7	40.6	43.8	5.8	38.8	8.6	39.9	11.7
3	29.9	30.0	8.2	44.5	41.4	5.9	42.3	5.3	39.1	12.5
4	30.5	30.6	6.8	47.7	39.7	5.8	42.9	6.0	37.1	12.5
5	31.2	31.2	6.3	49.5	38.9	5.4	43.6	6.1	37.1	13.0
6	31.7	31.7	5.4	51.0	38.2	5.4	44.9	6.1	35.6	13.2
ChClFru_1										
1	28.6	28.5	16.7	32.2	43.5	7.7	34.9	12.7	42.3	10.4
2	29.3	29.2	12.4	38.0	42.9	7.2	38.1	9.7	40.7	11.1
3	29.9	29.9	10.4	41.5	42.0	6.1	40.3	8.6	39.0	12.1
4	30.5	30.6	8.9	45.4	39.6	6.1	42.5	6.6	37.2	12.5
5	31.2	31.2	8.2	47.8	38.1	5.9	43.8	5.8	37.0	12.9
6	31.7	31.8	8.1	48.5	38.1	5.2	45.8	4.6	35.7	13.8
ChClSac_1										
1	28.6	28.6	14.9	33.8	44.2	7.1	35.6	10.8	42.9	10.9
2	29.3	29.2	10.7	40.8	42.7	5.8	39.7	8.1	41.5	11.9
3	29.9	30.0	9.1	44.4	40.7	5.9	41.3	6.9	40.0	12.2
4	30.5	30.6	7.8	47.3	39.5	5.4	43.3	6.0	38.6	12.8
5	31.2	31.2	7.1	50.2	37.3	5.4	43.4	5.6	37.7	13.0
6	31.8	31.7	7.4	50.6	37.2	4.8	45.1	5.5	35.7	13.6

[a] Standard uncertainty u are u(w) = 0.002, u(T) = 0.2 K, and u(P) = 10 kPa.

binodal curves are represented in Fig. 1 in mass percentages (% m/m). Detailed data is reported at Supporting Information.

The binodal curves are shown in Fig. 1 indicates the phase boundary between the monophasic and biphasic regions. The greater the phase-formation ability of each DES, the closer the binodal curve is to the axis origin. Higher concentrations of HBD on the DES reduce the biphasic region in all systems. Furthermore, all binodal curves have a similar behavior to that of the choline chloride binodal curve without HBD. This can be seen in Fig. 2 where all binodal curves were plotted as a function of choline chloride concentration in the DES mixture. Here an almost complete superposition of all binodal curves on that formed only by choline chloride, salt, and water is observed. This is very similar to the results reported by Passos et al. [24] for DES-based ATPS with poly (propylene) glycol. According to the authors [24], this behavior indicates that the ATPS formation is dominated by the choline chloride with the HBD having a small effect on phase-forming ability. The results here reported show that the same to hold for DES-salt ATPS.

3.2. Tie-lines

Six tie-lines were obtained for each DES-based ATPS, with three different DES (ChClGlu_1; ChClFru_1 and ChClSac_1), and for the ChCl + K_2HPO_4 +water system. For this system, the phase's quantification was made using the gravimetric method proposed by Merchuk et al. [27]. In this method, the Eq. (1) was used to correlate the experimental binodal curve and the tie lines were obtained from a mass balance according to Eqs. (2)–(5). The tie-lines of these systems are reported in Fig. 3, in mass fractions. For the systems with DES, the HDB were excluding of the ternary representation, due to the trivial effect of these at phases formation.

For the systems composed by DES, TGA analysis was used to quantify the composition of the system's phases. The tie-lines determination of DES-based ATPS system considered the difference between melting/decomposition temperature of the four components of these ATPS. This technique was previously used with satisfactory results in water content determination at liquid-liquid equilibrium and solid-liquid equilibrium between water and ILs [36], quantification of DES in composites [37] and water content determination in DES [16]. For the purpose of verifying the accuracy of this technique, mixtures of known composition, with concentrations close to those expected for the top and bottom phases of each system were analyzed (Supporting Information). The maximum and mean deviations obtained in the phase compositions determination were of 1 and 0.4%, respectively. The experimental LLE data for ATPSs are presented in Table 3 and Fig. 3. In Table 3, the overall composition was shown in DES terms while the phase's compositions were represented considering each component.

As is shown by the results reported in Table 3, the HBD partitions preferentially to the bottom phase, while the HBA (choline chloride) is present in higher concentrations in the top phase, shown the hydrogen bonds of DES are disrupted in aqueous solutions addressed in this work.

The mass ratio between HBA and HBD in each phase is not the same as the original ratio of the DES formation showing a differential partition of the HBD and HBA between the two phases. If the structure of the DES were kept in aqueous solution, the relationship between HBA and HBD in both phases and for all tie-lines should be the same. Although the hypothesis that DES structure can be maintained in ATPS due to the hydrophilic salt and the salting-out effect has been previously reported [20–23], it is in disagreement with this work. For these DES-salt systems, there was a breaking of the hydrogen bonds responsible for DES formation, in agreement with the results for DES-based ATPS formed by DES and PPG
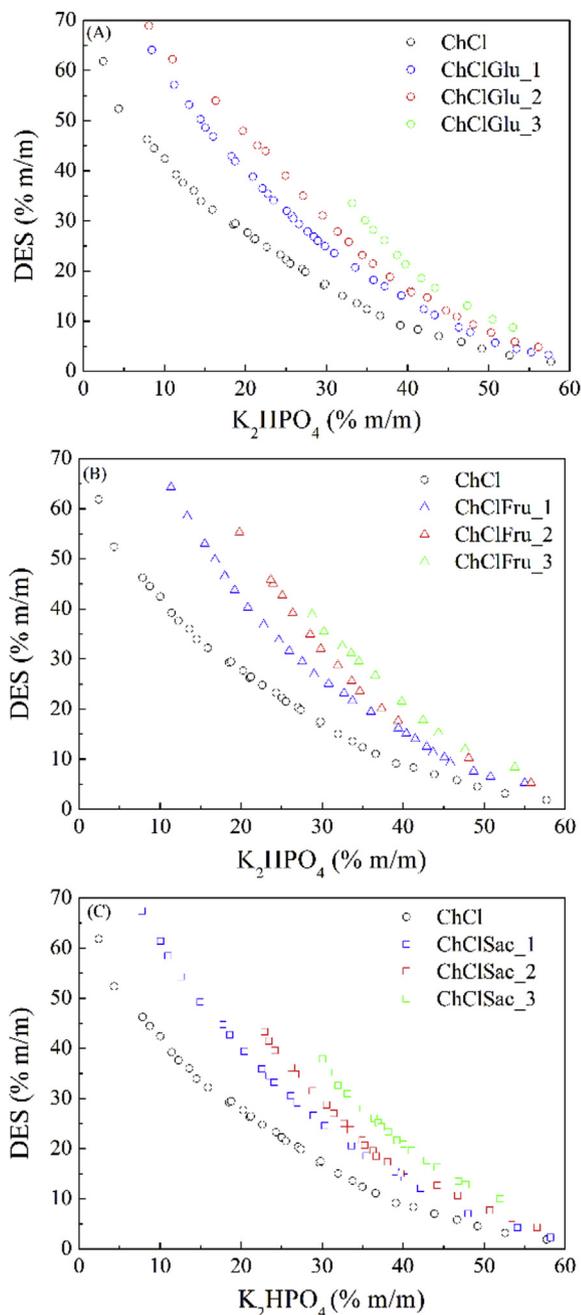
Fig. 1. Phase diagram of different DES-based ATPS at 298.15 K: (A) ChClGlu; (B) ChClFru and (C) ChClSac.

Fig. 2. Representation of binodal curves as a function of ChCl concentration in the different DES.

3.3. Ion exchange analysis

Although ion exchange may occur in salt-salt systems, as firstly observed by Gutowski et al. [9], its extension depends on the strength of the position of the ions along the Hofmeister series [30]. Kosmotropic ions, such as K^+, HPO_4^{2-}, Cl^- and also Ch^+, tend to be solvated by water (water-structuring salts). The high hydration tendency of these ions can also be evaluated regarding the $\Delta_{hyd}G$ (Gibbs energy of hydration) as shown by Marcus [31], in which the strong bond strength between the ions and the water is showed, making the ion exchange between the salts negligible. According to Dupont et al. [30], ion exchange in the system composed by alkyl-methylimidazolium chlorides and ion PO_3^{4-} is small, as already foreseen by Gutowski et al. [9]. Since, cation-anion interaction of Cholinium Chloride is stronger than for alkyl-methylimidazolium chlorides it is possibility to realize that ion exchange practically will not occur. Finally, Kurnia et al. [32] showed that strong electrolytes at the concentrations traditionally observed in ATPS are not entirely dissociated, which also contributes to non-ion exchange.

In this work, to confirm the non-ion exchange an inductively coupled plasma spectrometer (ICP) analysis was used to quantify the phosphorus and potassium ions at the phase's systems (Table 4).

Considering that for each phosphorus ion present in the salt molecule (K_2HPO_4) there are two ions of potassium, the molar ratio between the concentrations of potassium and phosphate ions in the phase's systems must be near to two. This relation was obtained for two tie-lines of each system studied, and the results are shown in the Table 4. This analysis confirmed that the ions exchange between the salts of this work is negligible, as previously reported in literature [9,26,32−35].

3.4. Liquid-liquid equilibrium modeling

To describe the liquid-liquid equilibrium phase diagrams here measured, the Non-Random Two-Liquid (NRTL) model was applied. The NRTL model proposed by Renon and Prausnitz [38] is based on the local composition concept, and it 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 [39]. The parameters for this model were estimated using TML-LLE 2.0 software developed by Stragevitch and D'Ávila [40] which estimate the parameters by the minimization of the

(polypropylene glycol) presented by Passos et al. [24]. Some tie-lines were further analyzed by ^1H NMR to confirm the data obtained by TGA analysis (Supporting Information). This behavior is observed in Fig. 4 where molar ratio HBA:HBD of each phase in equilibrium is presented for all tie-lines. The dashed line represents the original ratio HBA:HBD of the DES.

Since for such systems, the broke of the hydrogen bond has been proven, the DES synthesis step can be suppressed without any loss of the phase behavior of the system. However, DES stability should always be evaluated in works involving ATPS. In this work, the tie-line length (TLL) and the slope of the tie-line (STL) were not calculated as usual, because the binary representation is not a real representation of these systems, considering they are formed by four components and not only three.
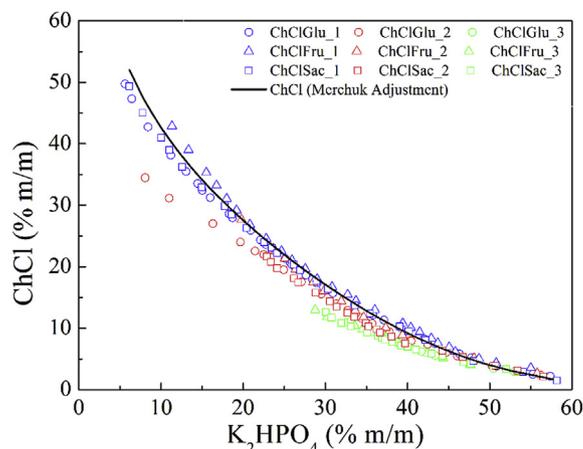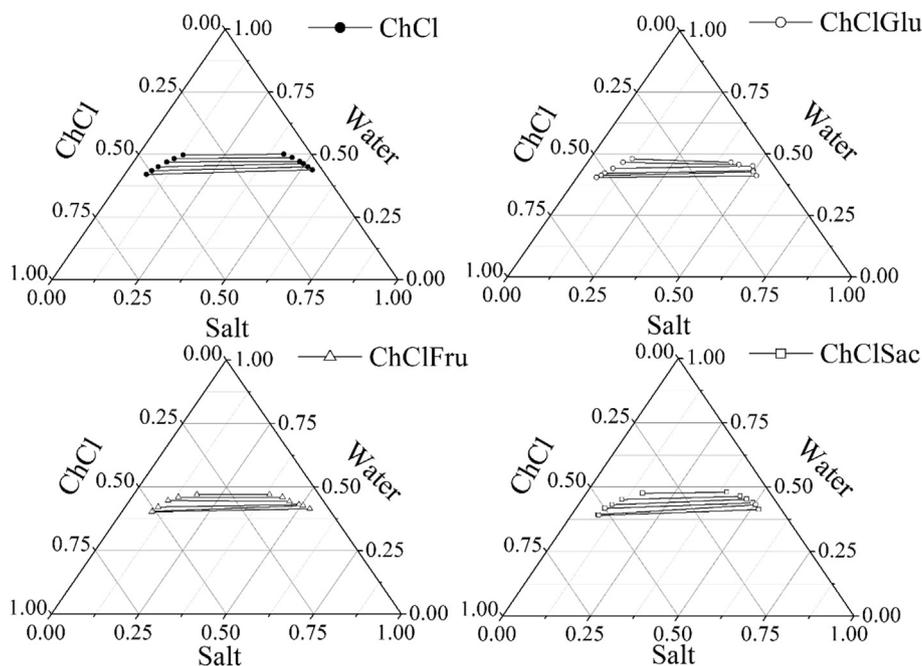
Fig. 3. Ternary representation of the tie-lines data to the systems: ChCl/DES + K₂HPO₄+ water. In the systems with DES, the component HBD was omitted.
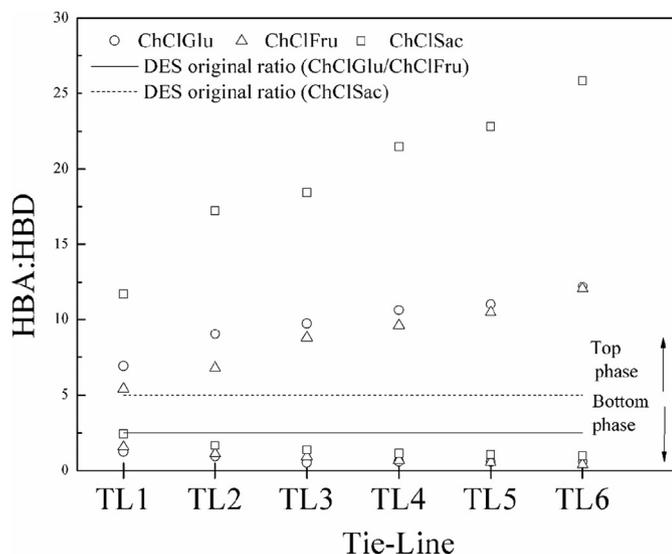

Fig. 4. The relation between the molar ratio of the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) in the DES original ratio (dashed line) and at the top and bottom phases of the tie-lines.

objective function described in Eq. (8), using the modified Simplex numeric method [41].

$$OF = \sum_{k}^{D} \sum_{j}^{M} \sum_{i}^{N-1} \left(w_{ijk}^{I.exp} - w_{ijk}^{I.calc} \right)^2 + \left(w_{ijk}^{II.exp} - w_{ijk}^{II.calc} \right)^2 \quad (8)$$

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

The Table 5 presents the binary interaction parameters for the NTRL model for the ternary system (ChCl + K₂HPO₄ + water) and

quaternary systems (ChCl + HBD + K₂HPO₄ + water) at 298.15 K.

The NRTL model provides a very good description of the experimental data for all the systems studied, with a mean deviation below 1.7%.

3.5. Gallic acid partition on DES-based ATPS

Firstly, three concentrations of gallic acid were partitioned in two tie-lines of each system (TL1 and TL6). The overall compositions of these tie-lines are shown in Table 3. The partition coefficient (K_{GA}), as well as the extraction efficiency at gallic acid (E_{GA} %) separation, are presented in Fig. 5.

The pH of the studied systems ranged from 10.3 to 11.0, and no significant differences between pH of the top and bottom phases were observed (details provided in Supporting Information).

In all cases, the K_{GA} values were bigger than 1, what indicates the gallic acid preference for the choline chloride rich phase (top phase) and higher values of K_{GA} result in higher E_{GA} % at the top phase. According to Almeida et al. [42], the gallic acid molecule has a good capacity to form hydrogen bonds, and thus preferentially partitions to the more hydrophilic phase. Here, however, a combined effect of the salting out by the K₂HPO₄ and favorable interactions with choline chloride, probably by hydrogen bonding with the hydroxyl (-OH) group present in a choline chloride molecule, leads to the gallic acid preference for the choline chloride rich phase.

Differences between HBD type on gallic acid partition were observed as shown in Fig. 5, on a display of the designer solvent ability of the DES and DES-based ATPS. The HBD hydrophilicity follows the order: saccharose > glucose > fructose and the K_{GA} decreases with the hydrophilicity of the HBD. Even if glucose and fructose are monomers of saccharose, these monosaccharides have different molecular conformations, i.e. different access to -OH groups to make hydrogen bonds, which in glucose are more favorable than in fructose [43,44]. Comparing the system with and without HBD, it seems to have a competition between the HBD and the gallic acid to establish hydrogen bonds thus allowing a modulation of the partition coefficient on these systems.
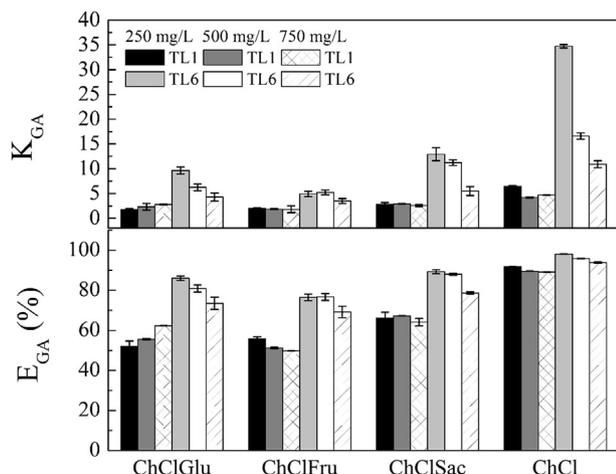
Table 4
Potassium and phosphorus concentration in the top and bottom phases obtained by TGA and ICP analysis.[a]

TL[b]	Potassium (C_K mol/Kg)				Phosphorus (C_P mol/kg)				C_K/C_P ICP	
	TGA[c] Top	ICP top	TGA[c] Bottom	ICP Bottom	TGA[c] Top	ICP top	TGA[c] Bottom	ICP Bottom	Top	Bottom
ChCl + K_2HPO_4+Water										
TL1	1.49	1.56	4.81	5.48	0.75	0.80	2.41	2.61	1.9	2.1
TL6	0.72	0.71	6.12	6.37	0.36	0.40	3.06	3.25	1.8	2.0
ChClGlu + K_2HPO_4+Water										
TL1	1.32	1.32	4.26	4.26	0.66	0.71	2.13	2.28	1.9	1.9
TL6	0.62	0.66	5.16	5.60	0.31	0.40	2.58	2.83	1.6	2.0
ChClFru + K_2HPO_4+Water										
TL1	1.92	2.00	4.01	4.29	0.96	1.09	2.00	2.13	2.0	1.8
TL6	0.93	0.69	5.26	5.60	0.47	0.40	2.63	2.65	1.7	2.1
ChClSac + K_2HPO_4+Water										
TL1	1.71	1.76	4.09	4.44	0.86	0.95	2.04	2.36	1.9	1.9
TL6	0.85	0.69	5.18	5.78	0.42	0.39	2.59	2.69	1.8	2.1

[a] Standard uncertainty u are $u(C_i) = 0.01$ mol/kg; $u(C_K/C_P) = 0.1$.
[b] The overall composition of each tie-line analyzed is in Table 3.
[c] Calculated from the salt concentration determined by TGA (Table 3).

Table 5
NRTL parameters to ternary and quaternary systems at 298.15 K.

System	Parameter	A_{ij} (J/mol)	A_{ji} (J/mol)	$Alfa_{ij}$	Rmsd[a] (%)
ChCl + K_2HPO_4+Water	$K_2HPO_4 - ChCl$	19297.6	16273.0	0.2091	0.7
	$K_2HPO_4 - $ water	−14851.3	4191.1	0.2200	
	$ChCl - $ water	−10507.2	11279.6	0.3432	
ChClGlu + K_2HPO_4+Water	$K_2HPO_4 - ChCl$	16732.8	19601.9	0.2000	0.9
	$K_2HPO_4 - HBD$	962.6	−518.0	0.3487	
	$K_2HPO_4 - $ water	6674.5	6501.5	0.3484	
	$ChCl - HBD$	300.1	1568.9	0.2209	
	$ChCl - $ water	−2806.8	10084.1	0.4346	
	$HBD - $ water	−11964.7	36269.0	0.2480	
ChClFru + K_2HPO_4+Water	$K_2HPO_4 - ChCl$	19128.0	10903.8	0.2000	1.7
	$K_2HPO_4 - HBD$	962.8	−518.0	0.3487	
	$K_2HPO_4 - $ water	13028.9	7114.3	0.3484	
	$ChCl - HBD$	−701.7	−1854.9	0.2000	
	$ChCl - $ water	−7229.0	19093.1	0.2000	
	$HBD - $ water	−12941.6	36062.8	0.2006	
ChClSac + K_2HPO_4+Water	$K_2HPO_4 - ChCl$	16323.7	12572.4	0.2000	1.3
	$K_2HPO_4 - HBD$	962.8	−517.0	0.3487	
	$K_2HPO_4 - $ water	6674.5	6501.5	0.3484	
	$ChCl - HBD$	−701.2	−1854.9	0.2504	
	$ChCl - $ water	−2806.8	10084.1	0.4598	
	$HBD - $ water	−12941.6	36062.8	0.2661	

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

Fig. 5. Partition coefficients (K_{GA}) and extraction efficiencies (%E_{GA}) of gallic acid at systems composed of DES and choline chloride.

Considering that the HBD preferentially partition to the bottom phase and that its presence reduces the gallic acid partition, gallic acid at 500 mg/L was partitioned at the six tie-lines of each system with DES to evaluate the relation between the K_{GA} e K_{HBD} (Fig. 6).

The Fig. 6 shows that K_{GA} increases with the tie-line length, while, K_{HBD} remains almost constant. Although there are no differences between the partition of the various HBD studied, there is a clear influence on the HBD on the gallic acid partition. The knowledge about the influence of the HBD of the phase's system is important to develop the ability to design novel ATPS with different phase's properties targeted to the biomolecule to be partitioned. This work shows that using DES-based ATPS, even when the DES forming bonds are broken, it is also possible to design a solvent capable of partitioning gallic acid in a similar or superior way to different systems formed by IL-salt and polymer-salt [28,42,45,46] previously proposed, since the HBDs (sugars) acts as a choline chloride rich phase modulator. K and E (%) values presented in Figs. 5 and 6 are shown in Table S9 (Supporting Information).
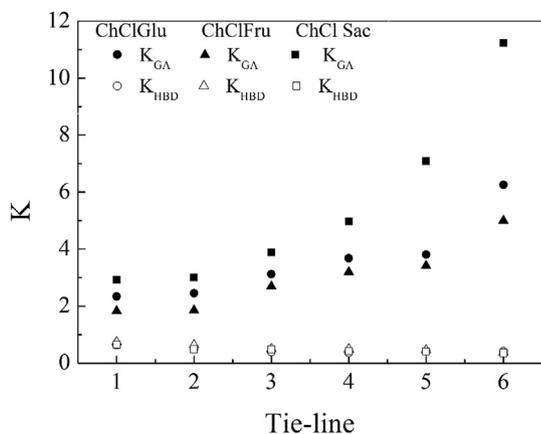
Fig. 6. K_{GA} and K_{HBD} at the different tie-lines for the systems composed by DES + K_2HPO_4 + water at 298.15 K.

4. Conclusions

Deep eutectic solvents formed by choline chloride (HBA) and various sugars (HBD) were prepared and used to form ATPS. Liquid-liquid equilibrium data at 298.15 K were measured, and it was shown that their formation was dominated by the HBA while the HBD act as additives reducing the biphasic region of all DES tested. A thermogravimetric analysis methodology was here proposed for the measurement of the phases composition with standard deviations below 1%. Using this technique, it was possible to verify that the DES hydrogen bond was broken when these solvents are used to prepare ATPS. Thereat, the top phase is rich in HBA (choline chloride) while the HBD preferentially partitions towards the bottom phase (K_2HPO_4 rich-phase). The experimental tie-lines here measured were correlated with the NRTL model which provides a good representation of the data (RMSD < 1.7%). Studies of gallic acid partition were further carried on theses ATPS, and the results highlight the designer solvent ability of the choline chloride (HBA) + sugar (HBD) where the HBD can be used to modulate the partition behavior of a target molecule.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fluid.2017.05.018.

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