

# Understanding the role of the hydrogen bond donor of the deep eutectic solvents in the formation of the aqueous biphasic systems

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## ABSTRACT

Deep eutectic solvents (DES) have been proposed as phase-forming compounds of aqueous biphasic systems (ABS). However, due to DES nature and the high water content of the ABS, their nature and behavior remains controversial. To foster the understanding of DES-based ABS, the present work uses the relative hydrophilicity of the hydrogen bond donor (HBD) as a descriptor to clarify its role on ABS formation and phase properties. Dipotassium phosphate ( $K_2HPO_4$ )-based ABS phase diagrams composed of cholinium chloride ( $[N_{111(20H)}]Cl$ ), as hydrogen bond acceptor (HBA), and several HBD (alcohols and sugars) were compared in molality units and the saturation solubility point for each binodal curve determined. The results here reported establish the HBD role as function of its relative hydrophilicity: very hydrophilic HBD act only as an adjuvant in the formation of ABS; HBD of intermediate hydrophilicity influence the ABS formation; while the most hydrophobic HBD tend to form organic-aqueous two-phase systems, where the HBA acts as an adjuvant to the system.

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## 1. Introduction

Aqueous biphasic systems (ABS) are a type of biphasic system that can be used in liquid-liquid extraction processes, first proposed by Albertson (1958) as alternative platforms for the partition of biomolecules [1]. They are characterized by two aqueous solutions that are immiscible above certain concentrations, being usually aqueous solutions of two salts, two polymers or a polymer and a salt [2,3]. Due to the search for more effective and more biocompatible, biodegradable and ecofriendly processes, the traditional ABS phase-forming compounds have been replaced by a wide range of compounds, from which it should be highlighted the Ionic Liquids (ILs) [3–5], and, more recently, by a new class of solvents called deep eutectic solvents (DES) [6,7], mainly because of DES nature, which share several characteristics with ILs, e.g. low volatility, thermal stability, liquid at room temperature, high solvation ability, high conductivity, among others [8–10].

The DES concept was proposed by Abbot et al. [8] to designate a

liquid resulting from the hydrogen bond interaction of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). The HBA most used is the cholinium chloride ( $[N_{111(20H)}]Cl$ ), while the HBD can be any molecule able to do hydrogen bond with it. The main characteristic of these solvents is the low melting point of these mixtures when compared with the individual components [8,11]. However, the temperature depression should present significant negative deviations from the ideality, otherwise the mixture is just a simple eutectic mixture and not a deep eutectic mixture [12].

The first use of DES as ABS phase-forming compounds was proposed by Zeng and co-workers [13], which created a system composed of cholinium chloride:urea and  $K_2HPO_4$  and tested its aptitude to partition bovine serum albumin (BSA). Thenceforward, DES-based ABS were applied to partition of proteins [7,14–16], dyes [17,18], phenolic compounds [19–21], amino acids [20–22] and alkaloids [20–22]. Considering that DES are formed by hydrogen bonds, which can be destroyed in water presence, Coutinho and co-workers studied the stability of DES composed of  $[N_{111(20H)}]Cl$ :sugars and  $[N_{111(20H)}]Cl$ :organic acids in ABS with salts [19] and poly(propylene)glycol polymer [17], respectively, demonstrating the non-stoichiometric partition of the HBA and HBD between the ABS coexisting phases. This fact indicates the break of the hydrogen

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bond between HBA and HBD of the DES, thus, this ABS should be regarded as quaternary systems. In this case, the ABS formation are induced by the competition between the salt and the  $[N_{111}(20H)]Cl$  for the formation of hydration complexes, with the HBD acting as an additive that allows the manipulation of the equilibrium and the phases polarities [19,20]. Nevertheless, it was noted that using a HBA and a HBD of high hydrophilicity mixed with a more hydrophobic phase former, or *vice-versa*, it is possible to preserve, to a certain extent, the stoichiometry of the initial DES forming a pseudoternary system [21,22].

Since the first work using DES in the formation of ABS proposed by Zeng and co-workers [13], others researchers dedicate attention to study the DES-based ABS. To make an overall picture about the state of art of DES-based ABS, in Table 1 is shown a chronologically

list of all the works from 2014 up to 2018. The works presented in Table 1 used quaternary ammonium salts (mainly cholinium chloride) or betaine as HBA and several molecules as HBD (e. g. alcohols, sugars or urea). Moreover, the most part of these works was mainly developed to apply the DES-based ABS in the biomolecules partition.

Regarding the DES behavior in the ABS, some authors defend the idea that when DES are mixed in water with an inorganic salt there is a competition between the salt and the DES for the water molecules, which was won by the salt due to its higher ability to form hydration complexes. From this, these authors considered that DES-based ABS in equilibrium would be constituted by a phase very rich in DES and other rich in salt and water [7,16,23,24]. However, it was very well established that in DES/salt and DES/polymer

**Table 1**  
Current literature data about DES-based ABS.

HBA	HBD	Phase Former	LLE Data	DES stability evaluation	Reference
Cholinium chloride	Urea	$K_2HPO_4$	X	X	[13]
Tetramethylammonium chloride	Urea		X	X	
Tetrapropylammonium bromide	Urea		X	X	
Cholinium chloride	Methylurea		X	X	
Cholinium chloride	Ethylene glycol	$K_2HPO_4$	Binodal curve	X	[7]
	Glycerol			X	
	Glucose			X	
	Sorbitol			X	
Tetramethylammonium chloride	Urea	$K_2HPO_4$	Binodal curve	X	[14]
	Ethylene glycol			X	
	Glycerol			X	
	Glucose			X	
	Sorbitol			X	
Betaine	Urea	$K_2HPO_4$	Binodal curve	X	[25]
	Methylurea			X	
	Ethylene glycol			X	
	Glycerol			X	
	Glucose			X	
	Sorbitol			X	
Cholinium chloride	Urea	PPG 400	Binodal curve	✓	[17]
	Acetic Acid			✓	
	Glycolic Acid			✓	
	Lactic Acid			✓	
	Citric Acid			✓	
Tetrabutylammonium chloride	PEG 200/400/600/1000/4000	$Na_2CO_3$	Binodal curve	X	[23]
Tetrabutylammonium bromide	PEG 200/600			X	
Tetraethylammonium chloride	PEG 200			X	
Tetraethylammonium bromide	PEG 200			X	
Cholinium chloride	Glucose	$K_2HPO_4$	Binodal curve and tie-lines	✓	[19]
	Fructose			✓	
	Saccharose			✓	
Cholinium chloride	PEG 2000	$NaH_2PO_4$	Binodal curve	X	[26]
		$Na_2CO_3$		X	
		$Na_3C_6H_5O_7$		X	
Cholinium chloride	Glucose	PPG 400	Binodal curve and tie-lines	✓	[22]
Cholinium chloride	Urea	PPG 400	Binodal curve	✓	[28]
	Ethylene glycol			✓	
	1,2-Propanodiol			✓	
Cholinium chloride	n-Propanol	$K_2HPO_4$	Binodal curve and tie-lines	✓	[20]
	Ethanol			✓	
	1,2-Propanodiol			✓	
	Ethylene glycol			✓	
Tetrabutylammonium bromide	PPG 400	Ionic Liquids	Binodal curve	X	[16]
Betaine	Glucose			X	
	Saccharose			X	
	Sorbitol			X	
	Xylitol			X	
Tetrabutylammonium Chloride	n-Propanol	$K_3C_6H_5O_7$	Binodal curve and tie-lines	✓	[18]
	Ethanol			✓	
Cholinium chloride	Urea	$K_3PO_4$	Binodal curve	X	[24]
Betaine	Lactic Acid			X	
	Sorbitol			X	
	Glycerol			X	
	Urea			X	
	Glucose			X	

systems there are no "DES-rich phase", since the breaking of the hydrogen bond from the DES dissolved in water is inevitable. In some specific conditions, using a HBA and a HBD of high hydrophilicity mixed with a more hydrophobic phase former or on the contrary, can occur a presence of a "pseudo-ternary ABS" where the DES maintain its composition in a certain range [21,22]. This makes the DES-based ABS a potential downstream purification processes, since it allows the purification of the target biomolecules and the recovery of DES-based components [17,19–22].

On most of the literature data there is a lack of information about the liquid-liquid equilibrium, particularly for the DES hydrogen bond stability [7,13,14,16,23–26]. Even after the increase of studies focused on the understanding of DES nature, some emphasizing the DES bonds disruption and indicating the necessity to evaluate the HBA and HBD stoichiometry, there are some works that still affirm that in DES-based ABS exists a DES-rich phase [16,24,26].

Considering the importance and usefulness of the DES-based ABS for several processes and the innumerable possibilities of mixtures to obtain DES, it is important to understand the role of each compound (HBA, HBD and phase former) to avoid wrong or precipitated conclusions about ABS properties and/or biomolecules partition behavior. Understanding how each compound of the quaternary system acts on the ABS makes possible the design of each system according some desired properties which may correspond to the most attractive properties of the DES-based ABS.

Thus, the aim of this work is to understand the HBD role of the DES, as a function of its relative hydrophilicity, and to predict how it can affect the liquid-liquid equilibrium of ABS. For this, binodal curves of ABS composed of dipotassium phosphate and DES with different HBD, from previous literature (glucose, fructose, saccharose [19], *n*-propanol, ethanol, 1,2-propanodiol and ethylene glycol [20]) and obtained in this work (xylose, sorbitol and glycerol) were used to provide a full picture of the HBD effect on the formation of ABS.

## 2. Material and methods

### 2.1. Materials

The new liquid–liquid phase diagrams were determined using aqueous solutions dipotassium phosphate and cholinium chloride. The HBD used in the DES formation were: glycerol, sorbitol and xylose. More details about the chemicals are in Table 2. All the chemicals were used without further purification. The water used in all experiments was purified by a reverse osmosis system purification apparatus (Vexer, Smart VOS 106).

### 2.2. Determination of phase diagrams

Aqueous solutions of each  $[N_{111(20H)}]Cl$ : HBD mixture (1:1 M fraction, 75 wt % aqueous solution) and  $K_2HPO_4$  aqueous solutions (60 wt%) were prepared gravimetrically (with a uncertainty of  $\pm 0.0001$  g) and used to determine the phase diagrams. The HBA: HBD molar ratio (1:1) was chosen to standardize the HBD

concentration and to evaluate only its nature effect. The binodal curves for the quaternary systems composed of  $K_2HPO_4$ ,  $[N_{111(20H)}]Cl$  (as HBA), HBD, and water were obtained by the cloud point titration method at  $(298 \pm 1)$  K and atmospheric pressure (around 91 kPa), according standard procedures [19,27].

The new binodal curves determined in this study are those composed of with the follow HBD: glycerol; xylose; sorbitol. The binodal curves of the quaternary systems composed of  $K_2HPO_4$ ,  $[N_{111(20H)}]Cl$  (as HBA), water, and other HBD were collected from the previous literature, namely: glucose, fructose, saccharose [19], *n*-propanol, ethanol, 1,2-propanodiol, ethylene glycol [20].

## 3. Results and discussion

### 3.1. Phase diagram and the hydrogen bond donor (HBD) effect

As previous reported, systems composed of DES, salt and water are in fact quaternary systems and the main responsible for the phases formation are the  $[N_{111(20H)}]Cl$  and the salt. In general, the hydrogen bond donor (HBD) act as an additive on the ABS and may, or may not, participate in the ABS formation. Independently of this, the HBD presence will influence the phases properties and the solutes partition in these ABS [17,19,20,22].

To evaluate the HBD effect in the liquid-liquid equilibrium, binodal curves obtained in our previous work for DES composed of cholinium chloride, as HBA, and different HBD (1:1) were plotted considering the  $[N_{111(20H)}]Cl$  and salt fractions in molality. The HBD evaluated were: glucose, fructose, saccharose [19], ethanol, *n*-propanol, 1,2-propanodiol and ethylene glycol [20]. Binodal curves with glycerol, sorbitol and xylose as HBD were obtained in this work. All binodal curves are presented in Fig. 1 as a function of the  $[N_{111(20H)}]Cl$  and  $K_2HPO_4$  concentrations, as well as the ternary system composed of  $[N_{111(20H)}]Cl + K_2HPO_4 + H_2O$ .

Fig. 1(A) shows the systems that used alcohols as HBD while, Fig. 1(B) presents all the systems that used sugar as HBD. The representation of all binodal curves together was provide at Supplementary material (Fig. S1). The presence of the alcohols (Fig. 1(A)), except for glycerol and ethylene glycol, enlarged the biphasic region in comparison with the ternary system composed of  $[N_{111(20H)}]Cl$  and salt. This effect was more pronounced when *n*-propanol was used as HBD. On the other hand, ABS with glycerol (Fig. 1(A)) and other sugars (Fig. 1(B)) as DES-based HBD did not affect significantly the phase equilibria, when compared with the ternary system, i.e. the biphasic region for each system was equal or slightly increased.

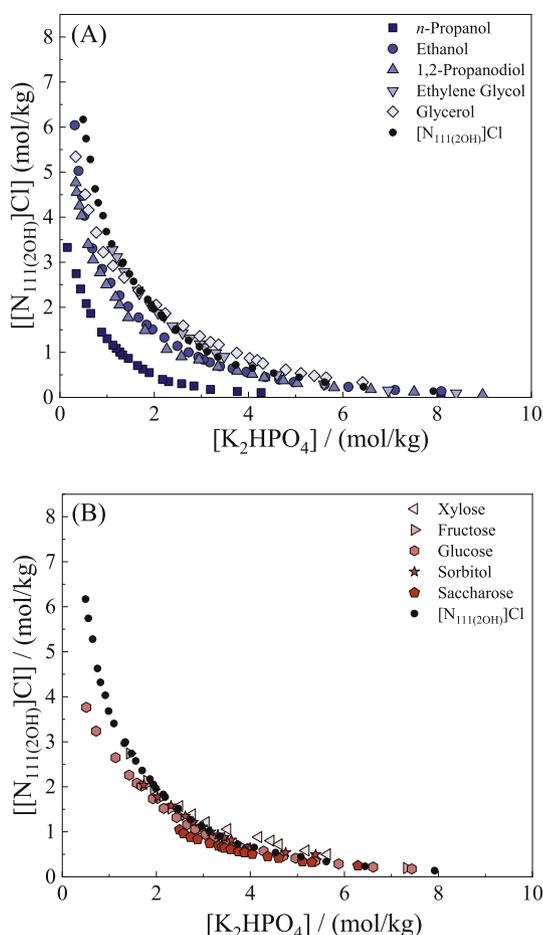
To obtain a further understanding of the different HBD in the liquid-liquid equilibrium, these are correlated with each chemical structure and the respective logarithm of octanol-water partition coefficient ( $\log(K_{OW})$ ), which are given in Fig. 2. The  $\log(K_{OW})$  corresponds to a relative measurement of the hydrophilicity/hydrophobicity of the molecules, in which the lower is this parameter the more hydrophilic is the target compound.

From Fig. 1 it was possible to determine the saturation solubility point which correspond to the point where the  $[N_{111(20H)}]Cl$  molality equals the molality of salt in the binodal curve [30]. This

**Table 2**

Compounds description: chemical name, molecular formula, source, purity and CAS number.

Chemical name	Molecular Formula	Source	Purity (wt %)	CAS number
Dipotassium phosphate	$K_2HPO_4$	Alphatech LTDA	$\geq 98.0$	7758-11-4
Cholinium chloride	$[N_{111(20H)}]Cl$	Merck Millipore	$\geq 98.0$	67-48-1
Glycerol	$C_3H_8O_3$	Synth	$\geq 99.5$	56-81-5
Sorbitol	$C_6H_{14}O_6$	Sigma-Aldrich	$\geq 98.0$	50-70-4
Xylose	$C_5H_{10}O_5$	Sigma-Aldrich	$\geq 99.0$	58-86-6



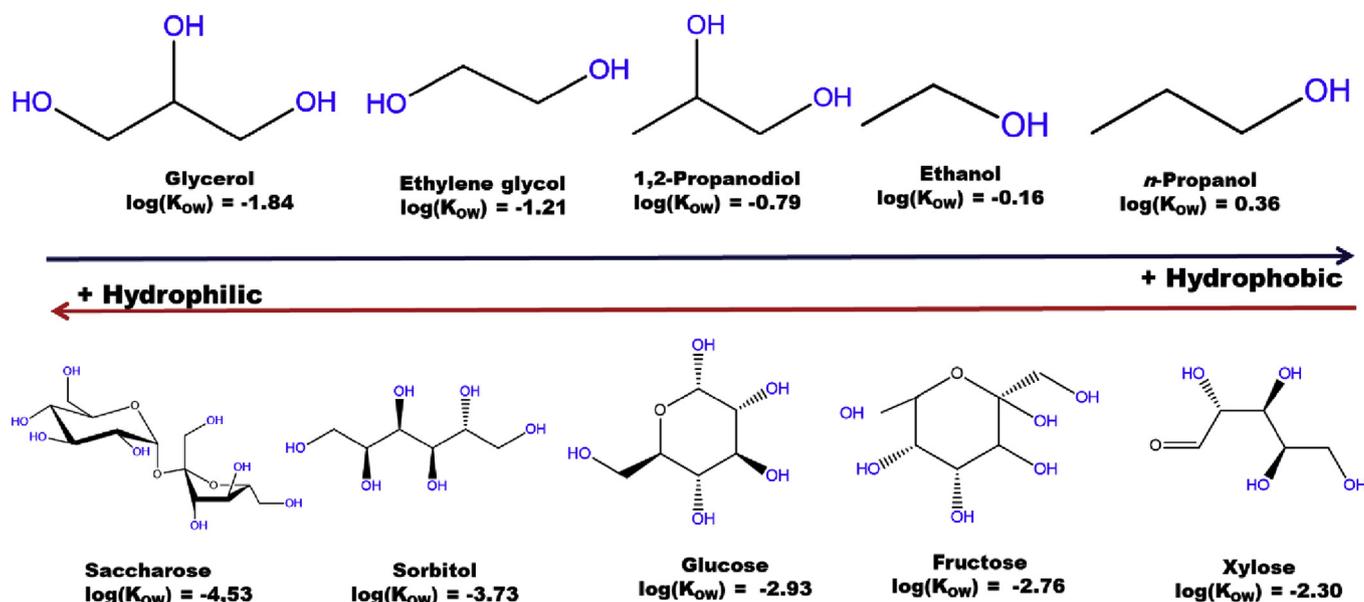
**Fig. 1.** Phase diagrams at 298 K and atmospheric pressure in function of  $[N_{111(20H)}]Cl$  concentration for ABS composed of  $[N_{111(20H)}]Cl$  + Alcohols +  $K_2HPO_4$  +  $H_2O$  (A) and  $[N_{111(20H)}]Cl$  + Sugars +  $K_2HPO_4$  +  $H_2O$ , using the follow HBD: *n*-propanol, ethanol, 1,2-propanediol and ethylene glycol [20]; fructose, glucose e saccharose [19] and glycerol, xylose and sorbitol (this work) at 1:1 M ratio.

point was graphically represented at Fig. S1 (Supplementary material). This parameter dependency on the  $\log(K_{OW})$  for each HBD is presented at Fig. 3. The saturation solubility ( $X = Y = 1.96$ ) for the ternary system composed of  $[N_{111(20H)}]Cl$ ,  $K_2HPO_4$  and water was represented with a black dashed line to provide a quick visual analysis of the HBD effect of the quaternary systems in comparison with the ternary one (without HBD). Lower values of the saturation solubility point correspond a larger biphasic region.

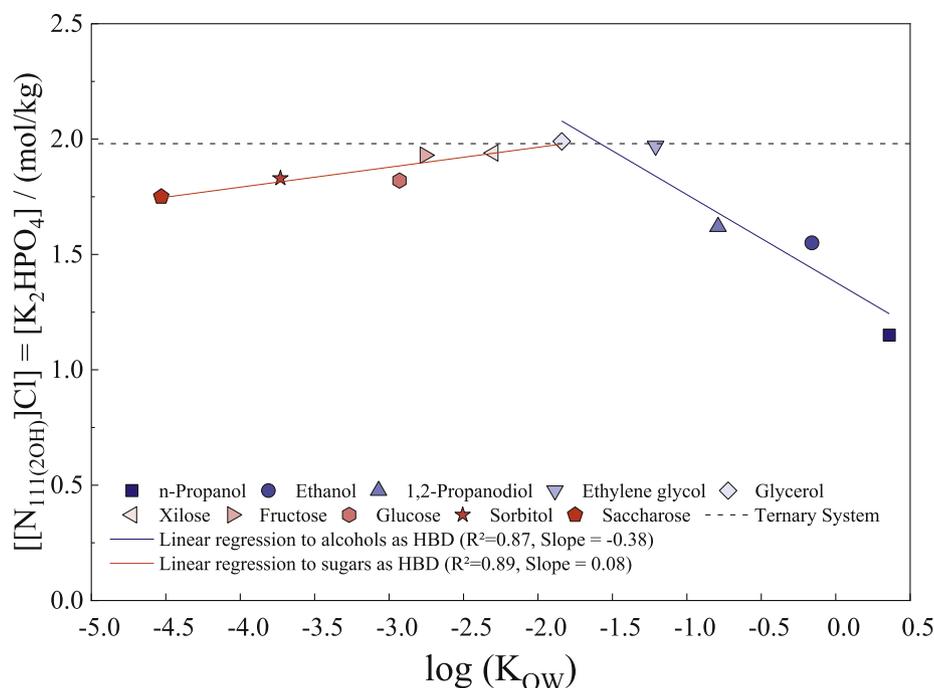
Fig. 3 shows the dependency of the ABS phase formation aptitude with the HBD hydrophilicity, where the less hydrophilic is the HBD the more it contributes to increase the biphasic region. The HBD with a lower relative hydrophilicity present a significant effect on the ABS formation, while the more hydrophilic HBDs ( $\log(K_{OW}) < -1.0$ ) have a minor effect on the ABS formation and their role is more significant in the tailoring of the phase's properties. Interestingly, as demonstrated in previous studies [20], the three HBDs that influence the phase equilibria are those able to form ABS with the  $K_2HPO_4$  without the addition of the HBA ( $[N_{111(20H)}]Cl$ ).

From Fig. 3 one can see a relationship between the ABS' phase formation and the number of hydroxyl groups present on the HBD. The evaluated sugars present from four to eight hydroxyl groups, while the alcohols present from one to three. The hydroxyl groups can form hydrogen bonds with water and, increasing, thus, the affinity between the water and the HBD and decreasing the effect over the phase demixing. Therefore, it was evidenced that the HBD only favors the ABS formation if the water affinity is low, being easily salted out by  $K_2HPO_4$  for the  $[N_{111(20H)}]Cl$ -rich phase [20].

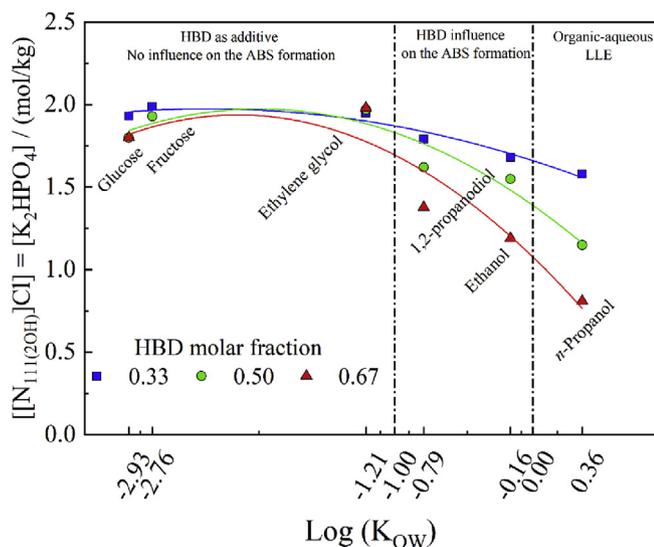
To confirm the pattern between each group of compounds, the linear least-squares regression of the both sugars and alcohols critical point values were plotted against the  $\log(K_{OW})$  of each HBD. The negative slope of the alcohols linear regression confirms the increase of the biphasic region, while the lower slope for the sugars agrees with the low influence of these HBD in the phase demixing. Interestingly, glycerol appeared as an intermediate compound between the alcohol and sugars series. The glycerol molecule has three available hydroxyl groups and seems to constitute a transition between the alcohols and sugar behavior.



**Fig. 2.** HBDs chemical structure and logarithm of octanol-water partition coefficient ( $\log(K_{OW})$ ) [29].



**Fig. 3.** Saturation solubility point for  $[N_{111(20H)}]Cl/K_2HPO_4$ -based aqueous biphasic systems in presence of several HBDs: *n*-propanol, ethanol, 1,2-propanediol and ethylene glycol [20]; fructose, glucose e saccharose [19] and glycerol, xylose and sorbitol (this work) at 1:1 M ratio.



**Fig. 4.** Saturation solubility point for cholinium chloride-based ABS using as HBD: glucose, fructose, ethylene glycol, 1,2-propanediol, ethanol and *n*-propanol in different molar fraction.

If the sugars seem to have only a minor influence on the ABS formation anymore, for the alcohols, the lower is the number of hydroxyl groups and larger the alkyl chain, the higher its contribution to the ABS formation until a point that these liquid-liquid systems start to act as common organic-aqueous immiscible systems. It is important to note that alcohols with very hydrophobic character ( $\log(K_{OW}) > 0$ ), such as the *n*-butanol, do not form ABS but conventional LLE systems. In the case of *n*-propanol, the bottom phase will be rich in water, salt and  $[N_{111(20H)}]Cl$ , while the top phase will be rich in *n*-Propanol (around 80 % wt). So, as previous reported in literature, the formation of ABS is a result of the alcohol

and salt nature, and the HBA,  $[N_{111(20H)}]Cl$ , acts solely as an adjuvant [20].

### 3.2. Effect of the HBA:HBD ratio in ABS formation

The patterns elucidated in previous section reinforce the importance to know the HBD nature before using them in the formation of ABS, and to select them in function of the ABS application. In general, the DES properties are a result from the chemical nature and molar fraction of the HBA and HBD used to obtain it, and they will influence the ABS formation. The influence of the HBA:HBD ratio for the systems containing glucose, fructose [19], ethylene glycol, 1,2-propanediol, ethanol and *n*-propanol [20] are presented at Fig. 4. The experimental data, in molality, for all of these systems are detailed in the Supplementary Material.

The results presented in Fig. 4 reinforce the importance of the HBD nature, *i.e.* its relative hydrophilicity, in the way it acts on the ABS. The most hydrophilic molecules will act exclusively as adjuvants to adjust the phases properties and characteristics, since independently of the HBD concentration (glucose, fructose and ethylene glycol) it is minor the influence of these compounds on the ABS formation. In these systems, the ABS formation is essentially the result of the  $[N_{111(20H)}]Cl$  and  $K_2HPO_4$  salting out effect. The HBD compounds of intermediate hydrophilicity, such as 1,2-propanediol and ethanol, are able to induce the phases separation, affecting the equilibrium of the  $[N_{111(20H)}]Cl/K_2HPO_4$  ABS and the biphasic region is slightly increased. Finally, the most hydrophobic molecules change the overall identity of the biphasic system, and in the limit the aqueous biphasic system can even become a conventional liquid-liquid system composed of an organic-rich phase and a water-rich phase.

The set of results studied in this work demonstrate that even the DES-based ABS with pseudoternary behavior are actually quaternary systems. On the basis of the results from this work, we proposed the measurement of the relative hydrophilicity of the HBD, through the  $\log(K_{OW})$  parameter, as a simple parameter to choose

the most adequate DES/quaternary system according to its ability to form ABSs and the characteristics and properties of the coexisting phases. Anyway, despite resembling or not a pseudoternary system, all the phase formers are quite important when applied DES-based ABS in the partition of different solutes. When applied to biomolecules partition the presence of a fourth compound can change or adjust the phases properties (e. g. density, hydrophobicity, and others) tune the partition behavior, increasing thus the separation and selectivity indexes [20,22]. These facts reinforced the importance of understanding how each HBD acts on the ABS formation, and consequently, designing its application as separation platform.

#### 4. Conclusions

The results of this work provide a better understanding about DES component effect on ABS formation. Considering the DES instability, it was demonstrated the need to know how HBA and HBD act on the system and how they will influence the phases equilibrium, and consequently, to obtain a further picture about the partition behavior of target compounds. The DES-based HBD, the focus on this work, can be used as an additive to the ABS or have direct influence on the formation of phases, in function of its relative hydrophilicity. Moreover, considering the wide number of HBD possibilities, which with different hydrophilicities and influences on the biphasic equilibria, makes this kind of ABS very interesting and versatile, where a delicate tuning of the phases properties can be achieved.

The results reported in the previous sections reinforce the necessity of understanding the HBA and HBD nature and how it will influence the ABS formation and preventing wrong interpretations. For example, systems that using sugars as HBD as reported as ternary systems [7,14,16,24,25] and all data here demonstrated lead to believe that they are in fact quaternary systems and there are no DES-rich phase. Summing up, there are no doubts about the versatility of the "DES"-based ABS, but we would like to emphasize that it is important to correctly address them by considering that one is working with a quaternary system prevent incorrect interpretations of the data.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fluid.2019.112319>.

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