

## Ionic-Liquid-Based Aqueous Biphasic Systems with Controlled pH: The Ionic Liquid Anion Effect

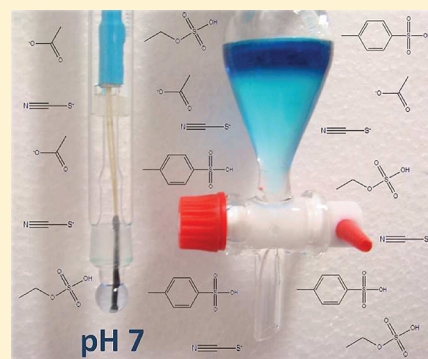
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**S** Supporting Information

**ABSTRACT:** This work addresses the effect of different anions in imidazolium-based ionic liquids (ILs) through the formation of aqueous biphasic systems (ABS) using a phosphate buffer solution ( $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  mixture of salts) aiming at controlling the pH value of the coexisting aqueous phases. Twelve ABS were investigated and the corresponding solubility curves were determined by the cloud point titration method at 298 K. In general, the IL anions aptitude to induce the formation of ABS increases with the decrease of the ability of the ions to form hydration complexes, i.e., with the decrease on the hydrogen-bond basicity of individual fluids. This trend is in close agreement to that observed in ABS with different inorganic salts, showing that the ILs rank is not dependent on the salting-out species used.



### INTRODUCTION

Liquid–liquid extraction approaches that make use of aqueous biphasic systems (ABS) are advantageous choices for the development of more environmentally benign and biocompatible separation processes, due to the use of nonvolatile compounds and a dominant aqueous media.<sup>1–3</sup> ABS are typically formed by the concomitant addition of aqueous solutions of two-water-soluble polymers or by the addition of a salting-out inorganic salt to a polymer aqueous solution. However, polymer–polymer ABS have a relatively high cost, are highly viscous, and require longer periods for phase separation.<sup>4</sup> For electrolyte–polymer systems, the high ionic strength of the aqueous media strongly limits their applications in the biotechnological field.

In the past decade, ionic liquids (ILs) have shown great potential as possible replacements for conventional organic solvents, and also as phase tunable additives. The large number of potential anion/cation combinations allows the fine-tuning of the ILs' physical properties,<sup>5</sup> and the design of ILs with an adjustable solubility in water and controllable biocompatibility.<sup>6–8</sup> Those factors are crucial in the ABS formation ability and on the use of ILs in the biotechnological field as separation/purification agents.<sup>9–13</sup>

Since the first work reporting on ABS composed of ILs and inorganic salts, by Rogers and co-workers,<sup>14</sup> the number of publications dealing with IL-based ABS (IL-ABS) has been rising in the past few years. In most of these works, the number of ILs studied is indeed quite limited, with the study of the inorganic salt effect as the major source of interest. Concerning the employed ILs, most works have focused on imidazolium-

based ILs<sup>4,15–19</sup> and reported the effect of the cation side alkyl chain length,<sup>11,20–23</sup> with the chloride<sup>15,23–26</sup> and bromide counterions.<sup>15,23,26–29</sup> Additional works have addressed a wide range of IL cations and anions conjugated with the kosmotropic salt  $\text{K}_3\text{PO}_4$ ,<sup>21–24,30–32</sup> and more recently, with  $\text{Na}_2\text{SO}_4$  and  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ .<sup>12,33</sup>

The  $\text{K}_3\text{PO}_4$  is the inorganic salt most studied as an IL-ABS promoter, followed by hydrogenophosphates,<sup>15,20,23,24,27,34</sup> carbonates,<sup>9,15,20,23–25,34</sup> citrates,<sup>26,28,29,35</sup> or other compounds (weaker salting-out species), such as carbohydrates<sup>19,30</sup> and aminoacids.<sup>36,37</sup> Nevertheless, in most of these systems, there is not an adequate control of the pH value of the aqueous phases which is a crucial issue regarding the separation/extraction of particular biomolecules. Only recently, the inorganic phosphate buffer solution composed of  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$  has found some interest among researchers within the topic of biomolecules purification.<sup>8,38</sup> This work, and its companion article in ref 39, are focused on the capability of forming IL-ABS making use of a phosphate buffer (PB),  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$  mixture. The major advantage of this salt, over other salting-out inducing salts, relies on the possibility of controlling the pH values of the aqueous phases, what is essential when dealing with biomolecules. Imidazolium-based ILs combined with a wide series of hydrophilic anions were investigated, and the effect of the IL anion on the formation of ABS is here presented and discussed.

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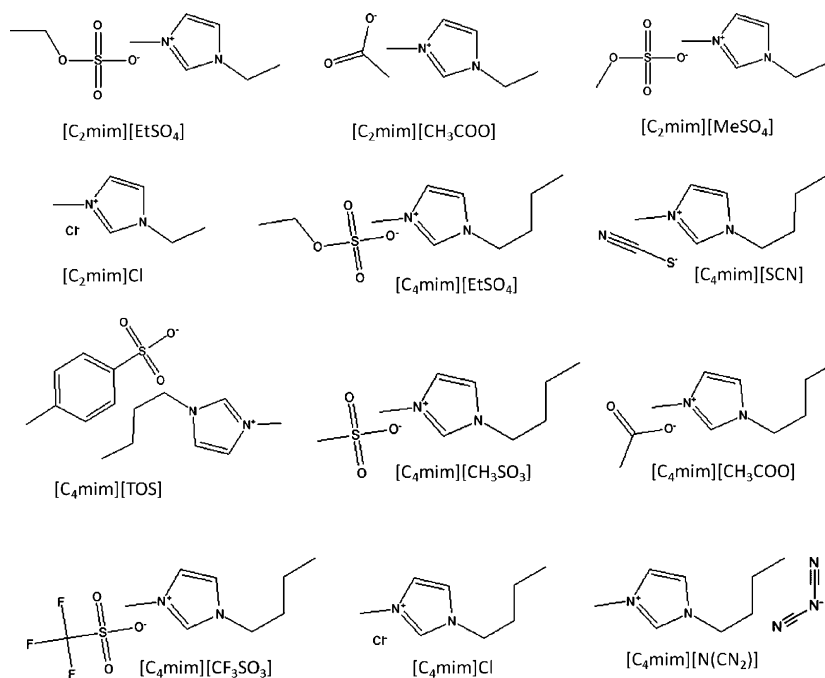


Figure 1. Chemical structure of the ILs studied.

## EXPERIMENTAL SECTION

**Materials.** The present study was carried out using aqueous solutions of  $K_2HPO_4$  and  $KH_2PO_4$ . The salts have purities higher than 98 wt % and were purchased from Sigma-Aldrich. The ILs used were 1-ethyl-3-methylimidazolium chloride,  $[C_2mim]Cl$ ; 1-butyl-3-methylimidazolium chloride,  $[C_4mim]Cl$ ; 1-butyl-3-methylimidazolium dicyanamide,  $[C_4mim][N(CN)_2]$ ; 1-butyl-3-methylimidazolium methanesulfonate,  $[C_4mim][CH_3SO_3]$ ; 1-butyl-3-methylimidazolium triflate,  $[C_4mim][CF_3SO_3]$ ; 1-butyl-3-methylimidazolium acetate,  $[C_4mim][CH_3COO]$ ; 1-butyl-3-methylimidazolium ethylsulfate  $[C_4mim][EtSO_4]$ ; 1-butyl-3-methylimidazolium tosylate,  $[C_4mim][TOS]$ ; 1-butyl-3-methylimidazolium thiocyanate,  $[C_4mim][SCN]$ ; 1-ethyl-3-methylimidazolium methylsulfate,  $[C_2mim][MeSO_4]$ ; 1-ethyl-3-methylimidazolium ethylsulfate,  $[C_2mim][EtSO_4]$ ; and 1-ethyl-3-methylimidazolium acetate,  $[C_2mim][CH_3COO]$ . All ILs were purchased at IoLiTec (Ionic Liquid Technologies, Germany), and presented mass fraction purities higher than 99 %, latter confirmed by us by  $^1H$  NMR and  $^{13}C$  NMR. Their ionic structures and respective abbreviations are presented in Figure 1.

Ultrapure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus, was used.

**Phase Diagrams and Tie-Lines.** The solubility curves data of the systems composed of IL, water, and the phosphate buffer were determined using the cloud point titration method at 298 K ( $\pm 1$  K). The experimental procedure adopted in this work follows the method already validated by us for ABS constituted by other ILs and inorganic salts.<sup>22,32,39</sup> Aqueous solutions of a phosphate buffer (PB) composed with the two inorganic salts  $K_2HPO_4$  and  $KH_2PO_4$  (pH = 7.0; Henderson–Hasselbalch equation equivalents = 1.087) at 40 wt % and of each IL, at ca. 60 wt %, were previously prepared. Repetitive dropwise addition of the aqueous inorganic salts solution to each aqueous solution of IL was carried out until the detection of a cloudy solution, followed by the dropwise addition of ultrapure

water until the detection of a limp and monophasic phase. The whole procedure was performed under constant stirring. The ternary system compositions were determined by the weight quantification of all components added within  $\pm 10^{-4}$  g.

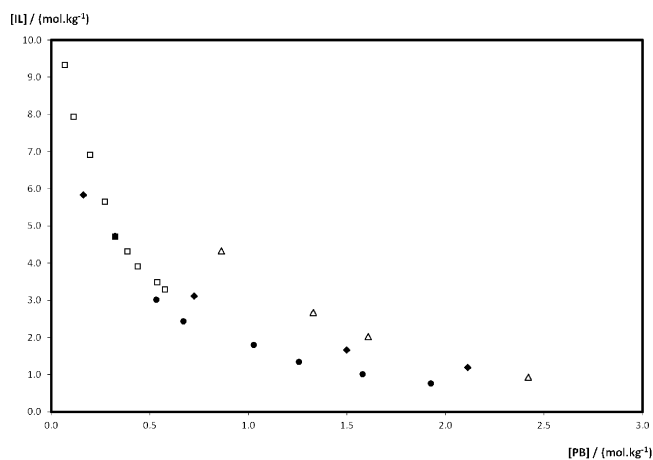
The tie-lines (TLs) associated to each phase diagram were determined by a gravimetric method previously used by us<sup>12,22,32</sup> and originally proposed by Merchuck et al.<sup>40</sup> A mixture at the biphasic region was prepared, vigorously stirred, and allowed to reach the equilibrium by the phases separation for at least 24 h and at 298 K, using small ampules (10 cm<sup>3</sup>) especially designed for this step. After the separation of the coexisting phases, they were carefully and individually weighed. Each TL was determined by application of the lever-arm rule.<sup>40</sup> For that purpose, the experimental solubility curves were correlated using the following equation:<sup>40</sup>

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

where  $Y$  and  $X$  represent, respectively, the IL and salt mass fraction percentages, and  $A$ ,  $B$ , and  $C$  are the regression constants.

## RESULTS AND DISCUSSION

The present study addresses the capacity of twelve ILs for the formation of ABS using a phosphate buffer aiming at controlling a neutral pH at the coexisting phases. All phase diagrams were determined at 298 K ( $\pm 1$  K) and at atmospheric pressure. The solubility curves obtained are depicted in Figure 2 (for  $[C_2mim]$ -based ILs) and Figure 3 (for  $[C_4mim]$ -based ILs). The mass fraction solubility data for all the investigated systems are presented in the Supporting Information. The experimental solubility curves for the investigated systems were correlated using eq 1. The obtained regression parameters are reported in Table 1. The respective tie-lines, and corresponding tie-line lengths (TLLs), are presented in Table 2. The graphical representation of the TLs for each system is depicted in the Supporting Information.



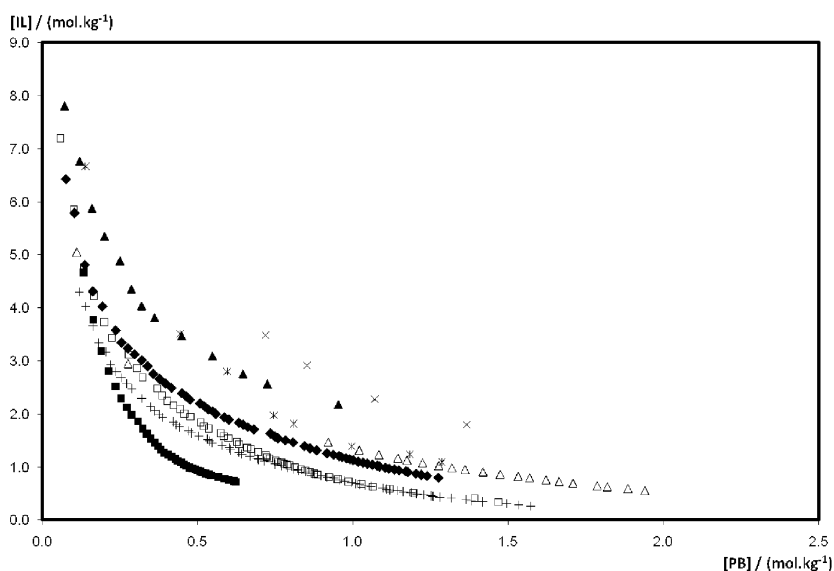
**Figure 2.** Phase diagrams for  $[\text{C}_2\text{mim}]$ -based ILs and the potassium phosphate buffer solution at 298 K:  $\blacklozenge$ ,  $[\text{C}_2\text{mim}][\text{MeSO}_4]$ ;  $\bullet$ ,  $[\text{C}_2\text{mim}][\text{EtSO}_4]$ ;  $\triangle$ ,  $[\text{C}_2\text{mim}][\text{CH}_3\text{COO}]$ ;  $\square$ ,  $[\text{C}_2\text{mim}]\text{Cl}$ .

The effect of different IL anions toward the ABS formation was evaluated making use of the common cation  $[\text{C}_2\text{mim}]^+$  and is presented in Figure 2. In molality units, the ability of the IL anion to induce the formation of ABS follows the order  $[\text{C}_2\text{mim}][\text{CH}_3\text{COO}] < [\text{C}_2\text{mim}][\text{MeSO}_4] < [\text{C}_2\text{mim}]\text{Cl} < [\text{C}_2\text{mim}][\text{EtSO}_4]$ . In addition, Figure 3 depicts the solubility curves for  $[\text{C}_4\text{mim}]$ -based ILs. Accordingly to the obtained trends, the sequence for the ability of the various ILs for phase separation, for instance at  $0.8 \text{ mol}\cdot\text{kg}^{-1}$ , is as follows:  $[\text{C}_4\text{mim}][\text{CH}_3\text{SO}_3] < [\text{C}_4\text{mim}]\text{Cl} < [\text{C}_4\text{mim}][\text{CH}_3\text{COO}] < [\text{C}_4\text{mim}][\text{EtSO}_4] < [\text{C}_4\text{mim}][\text{N}(\text{CN})_2] < [\text{C}_4\text{mim}][\text{SCN}] < [\text{C}_4\text{mim}][\text{TOS}] < [\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ . The anion rank observed for the  $[\text{C}_4\text{mim}]$ -based series studied here is in close agreement with the results previously obtained by our group for  $[\text{C}_2\text{mim}]$ - and  $[\text{C}_4\text{mim}]$ -based ILs with  $\text{K}_3\text{PO}_4$  and  $\text{Na}_2\text{SO}_4$  as the main salting-out species.<sup>33,34</sup> Although the anions rank is more pronounced with  $[\text{C}_4\text{mim}]$ -based ILs, in general, the cation alkyl chain length does not affect the

sequence on the anion ability in promoting ABS, and the inorganic salt used does not change the IL trend. Indeed, comparing with the previous results,<sup>33,39</sup> and although the sequence of the ILs is not altered, the main differences among different inorganic salts rely on their ability to salt-out the IL and on the pH of the aqueous media. Both  $\text{K}_3\text{PO}_4$ <sup>22,32</sup> and the  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$  mixture are able to salt-out all the ILs analyzed from aqueous solutions. On the contrary, this effect is more pertinent when dealing with  $\text{Na}_2\text{SO}_4$ , that being a neutral salt in aqueous solution, leads to acidic solutions for most of the imidazolium-based ILs investigated.<sup>33</sup> As a result, this salt was found to be quite limited in the promotion of IL-ABS indicating that the pH of the aqueous solution has a significant impact on the liquid–liquid demixing. Indeed, only imidazolium-based ILs with long alkyl chains and/or with anions with low hydrogen bond basicity are able to undergo the formation of ABS in the presence of  $\text{Na}_2\text{SO}_4$ .<sup>33</sup>

In general, in complex mixtures, as those containing high charge density salts with an enhanced capacity for creating ion–water hydration complexes (such as  $\text{K}_3\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ , and  $\text{Na}_2\text{SO}_4$ ), the capability of the inorganic salt to induce ABS increases with the decreasing on the ionic liquid affinity for water because the ionic liquid is salted-out by the inorganic ions.<sup>33,39</sup> Therefore, hydrophobic ILs are easily salted-out due to their poorer affinity for water. These ILs are those composed of long aliphatic side chains at the cation (or the anion) and/or composed of anions with lower hydrogen-bond basicity values (anions with a low hydrogen bond acceptor strength). In this context, the ability of the IL anions to induce ABS closely correlates with the decrease on the hydrogen basicity ( $\beta$ ) values of ionic fluids with common cations,<sup>32,33</sup> and as previously discussed by us.<sup>32,33</sup> Therefore, IL anions that tend to preferentially interact with the protons of water (and thus, to preferentially create hydration complexes) require more quantities of inorganic salt to undergo liquid–liquid demixing.

Previously, we have demonstrated that the salting-out effect of ILs is ruled by entropic contributions that are a direct result of the formation of water–ion hydration complexes and of the



**Figure 3.** Phase diagrams for the  $[\text{C}_4\text{mim}]$ -based ILs and the potassium phosphate buffer solution (PB) at 298 K:  $\times$ ,  $[\text{C}_4\text{mim}][\text{CH}_3\text{SO}_3]$ ;  $\blacksquare$ ,  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ ;  $\blacktriangle$ ,  $[\text{C}_4\text{mim}]\text{Cl}$ ;  $*$ ,  $[\text{C}_4\text{mim}][\text{CH}_3\text{COO}]$ ;  $\triangle$ ,  $[\text{C}_4\text{mim}][\text{EtSO}_4]$ ;  $+$ ,  $[\text{C}_4\text{mim}][\text{TOS}]$ ;  $\square$ ,  $[\text{C}_4\text{mim}][\text{SCN}]$ ;  $\blacklozenge$ ,  $[\text{C}_4\text{mim}][\text{N}(\text{CN})_2]$ .

**Table 1. Adjusted Parameters and Respective Standard Deviations (std) Obtained by the Regression of Eq 1 at 298 K and Atmospheric Pressure**

ionic liquid	A ± std	B ± std	C ± std	R <sup>2</sup>
[C <sub>2</sub> mim]Cl	76.3 ± 3.2	-0.260 ± 0.028	2.2·10 <sup>-4</sup> ± 1.0·10 <sup>-4</sup>	0.9880
[C <sub>4</sub> mim]Cl	79.4 ± 1.2	-0.291 ± 0.009	8.5·10 ± 1.4·10 <sup>-5</sup>	0.9970
[C <sub>2</sub> mim][MeSO <sub>4</sub> ]	77.2 ± 4.5	-0.196 ± 0.026	2.3·10 <sup>-5</sup> ± 7.6·10 <sup>-6</sup>	0.9976
[C <sub>2</sub> mim][EtSO <sub>4</sub> ]	117.1 ± 8.2	-0.366 ± 0.027	2.2·10 <sup>-5</sup> ± 7.6·10 <sup>-6</sup>	0.9971
[C <sub>2</sub> mim][CH <sub>3</sub> COO]	113.8 ± 3.7	-0.270 ± 0.010	3.5·10 <sup>-5</sup> ± 1.1·10 <sup>-6</sup>	1.0000
[C <sub>4</sub> mim][CH <sub>3</sub> COO]	91.4 ± 8.7	-0.320 ± 0.051	9.0·10 <sup>-5</sup> ± 4.4·10 <sup>-5</sup>	0.9931
[C <sub>4</sub> mim][N(CN) <sub>2</sub> ]	83.8 ± 0.4	-0.359 ± 0.002	7.1·10 <sup>-5</sup> ± 1.8·10 <sup>-6</sup>	0.9996
[C <sub>4</sub> mim][CF <sub>3</sub> SO <sub>3</sub> ]	167.9 ± 4.8	-0.739 ± 0.016	2.0·10 <sup>-4</sup> ± 4.5·10 <sup>-5</sup>	0.9972
[C <sub>4</sub> mim][CH <sub>3</sub> SO <sub>3</sub> ]	166.3 ± 17.0	-0.412 ± 0.034	1.9·10 <sup>-15</sup> ± 8.1·10 <sup>-6</sup>	0.9998
[C <sub>4</sub> mim][TOS]	96.5 ± 0.4	-0.382 ± 0.002	1.0·10 <sup>-4</sup> ± 1.4·10 <sup>-6</sup>	0.9998
[C <sub>4</sub> mim][SCN]	90.0 ± 1.0	-0.429 ± 0.006	2.0·10 <sup>-4</sup> ± 7.5·10 <sup>-6</sup>	0.9980
[C <sub>4</sub> mim][EtSO <sub>4</sub> ]	82.2 ± 1.2	-0.291 ± 0.006	3.5·10 <sup>-5</sup> ± 2.4·10 <sup>-6</sup>	0.9982

**Table 2. Mass Fraction Compositions and Respective Standard Deviations (std) for the Initial Mixture (M) and TL Data, and Respective TLLs, for the Top (T) and Bottom (B) Phases, Composed of IL (Y) and Inorganic Salt (X) at 298 K and Atmospheric Pressure**

ionic liquid	100 × mass fraction composition						TLL
	Y <sub>M</sub> ± std	X <sub>M</sub> ± std	Y <sub>T</sub> ± std	X <sub>T</sub> ± std	Y <sub>B</sub> ± std	X <sub>B</sub> ± std	
[C <sub>2</sub> mim]Cl	25.06 ± 0.04	29.98 ± 0.03	46.21 ± 0.02	3.70 ± 0.27	0.04 ± 0.01	61.06 ± 0.02	73.64
[C <sub>2</sub> mim][CH <sub>3</sub> COO]	25.01 ± 0.04	23.00 ± 0.04	36.76 ± 0.03	14.44 ± 0.07	2.49 ± 0.40	39.42 ± 0.03	42.41
[C <sub>2</sub> mim][MeSO <sub>4</sub> ]	24.99 ± 0.04	22.99 ± 0.04	41.66 ± 0.02	9.29 ± 0.11	6.10 ± 0.16	38.52 ± 0.03	46.03
[C <sub>2</sub> mim][EtSO <sub>4</sub> ]	25.04 ± 0.04	22.99 ± 0.04	45.66 ± 0.02	6.54 ± 0.15	2.58 ± 0.39	40.89 ± 0.03	55.10
[C <sub>4</sub> mim]Cl	25.11 ± 0.04	29.95 ± 0.03	46.79 ± 0.02	3.29 ± 0.30	1.48 ± 0.68	59.03 ± 0.02	71.83
[C <sub>4</sub> mim][CH <sub>3</sub> COO]	25.22 ± 0.04	22.92 ± 0.04	35.73 ± 0.03	7.84 ± 0.13	0.00 ± 0.01	59.12 ± 0.02	65.50
[C <sub>4</sub> mim][N(CN) <sub>2</sub> ]	25.12 ± 0.04	29.95 ± 0.03	69.23 ± 0.02	0.28 ± 3.52	0.00 ± 0.01	46.84 ± 0.02	83.43
[C <sub>4</sub> mim][CF <sub>3</sub> SO <sub>3</sub> ]	25.08 ± 0.04	29.97 ± 0.03	82.88 ± 0.01	0.91 ± 1.10	0.00 ± 0.01	42.57 ± 0.02	92.77
[C <sub>4</sub> mim][CH <sub>3</sub> SO <sub>3</sub> ]	25.00 ± 0.04	30.00 ± 0.03	40.50 ± 0.03	11.74 ± 0.09	9.47 ± 0.11	48.31 ± 0.02	47.96
[C <sub>4</sub> mim][TOS]	29.58 ± 0.03	15.01 ± 0.07	55.51 ± 0.02	2.01 ± 0.50	0.96 ± 0.01	29.36 ± 0.03	61.03
[C <sub>4</sub> mim][SCN]	19.89 ± 0.05	15.37 ± 0.07	57.76 ± 0.02	1.07 ± 0.94	1.24 ± 0.80	22.41 ± 0.05	60.41
[C <sub>4</sub> mim][EtSO <sub>4</sub> ]	17.84 ± 0.06	25.03 ± 0.04	37.27 ± 0.03	7.15 ± 0.14	1.34 ± 0.75	40.21 ± 0.03	48.82

increase of the surface tension of water.<sup>41,42</sup> This tendency was previously confirmed with the cation side alkyl chain length and with the cation core influence on the ABS formation.<sup>22,39</sup> In general, the larger the molar volume of the cations, the higher the ability of the IL to form ABS. In fact, when comparing ILs with aromatic and aliphatic cores, no major differences were observed demonstrating that the hydrogen-bonding of the cations with water is not the major driving force in creating ABS.<sup>39</sup> However, when analyzing the effect of the IL anion, a more complex scenario is at work. Anions are typically more polarizable than cations, due to their more diffuse valence electronic configuration. Hence, the anions' hydration is usually stronger than that of cations.<sup>41</sup> In this context, the facility of the anions to hydrogen-bond with water, and hence to form hydration complexes, plays a significant role and is more relevant than that observed with previous works where the effect of the IL cation was investigated.<sup>22,39</sup> Indeed, this is the main reason behind the strong correlation between the ILs ability to induce ABS and that of the hydrogen-bond basicity values. When comparing different ionic liquid anions, both enthalpic and entropic effects occur and cannot be discarded.

All the interpretations made with the somewhat more complex ternary systems (IL + water + inorganic salt) are in close agreement with our previous results on binary systems composed of IL + water.<sup>43-46</sup> Although the increase on the solubilization of an IL cation in water is a direct result of the decrease of the molar entropy of solution (and where no major

changes on the molar enthalpy of solution are observed), on the contrary, the solubility of an IL anion in water leads to significant changes in both the molar enthalpy and entropy of solution.<sup>43</sup> The lower the change in the molar enthalpy of solution, the larger the IL solubility in water, indicating that it is the solvation of the IL at the IL-rich phase that rules the overall liquid-liquid phase behavior in ABS.<sup>43</sup>

## CONCLUSIONS

Novel phase diagrams of twelve ABS with a controlled pH were determined by the cloud point titration method at 298 K. The ability of different IL anions on the formation of ABS in the presence of a common phosphate buffer was presented and discussed. On the basis of the phase diagram behavior, it was observed that the sequence of the ILs, regarding their aptitude for creating ABS, is in close agreement to that obtained previously with the K<sub>3</sub>PO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> inorganic salts, and demonstrating that the ILs rank is not dependent on the salting-out species used.

In general, the lower the hydrogen bond basicity of the IL (i.e., less affinity of the IL anion to interact with the protons of water), the higher the ability of the ionic fluid to undergo liquid-liquid demixing in the presence of the phosphate buffer. Contrarily to the IL cation effect on the formation of ABS, which is mostly governed by steric and entropic contributions, the IL anion influence toward the formation of biphasic systems

is a main result of favorable (or nonfavorable) hydrogen-bond interactions.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Experimental binodal mass fraction data and phase diagrams for the ATPS composed by each system. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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