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Ionic Liquid Based Aqueous Biphasic Systems with Controlled pH: The Ionic Liquid Cation Effect

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S *Supporting Information*

ABSTRACT: This work addresses the evaluation of the ability of ionic liquid cations on the formation of aqueous biphasic systems (ABS's), with K_2HPO_4 or a mixture of inorganic salts, K_2HPO_4/KH_2PO_4, aiming at controlling the pH values of the coexisting aqueous phases. Using chloride-based ionic liquids, the effects of the cation core, the length of the alkyl side chain, and the positional isomerism on ABS formation ability were investigated. All binodal curves were determined by the cloud-point titration method at 298 K. From the obtained phase diagrams it is shown that the biphasic area increases with the cation side chain length, from ethyl to hexyl chains, although for longer chains, an inversion on the binodal curves sequence appears due to the self-aggregation of the longer chain ionic liquids in aqueous solutions. The influence of the cation core and the positional isomerism of the ionic liquids on their ability to form ABS closely correlates with the ionic fluid affinity for water.

INTRODUCTION

Aqueous biphasic systems (ABS's) are formed when polymer/polymer, polymer/salt, and salt/salt mixtures are dissolved in water above critical concentrations.[1−3] Because both phases in ABS's are essentially composed of water, they offer a gentle and more biocompatible environment, better suited for the biomolecules' extraction than conventional approaches that make use of molecular organic solvents. The application of polymer-based ABS's is already well-documented.[1−5] However, most of the aqueous solutions of the phase-forming polymers present high viscosities, form opaque systems, and present a limited range of polarities at their coexisting phases.[6] For that reason, there has been a large effort to find new alternatives to polymers in the composition of ABS's.

Ionic liquids (ILs) are salts with a melting temperature below 100 °C[7,8] and that have been shown to be particularly valuable as extractive solvents for the most distinct solutes. They possess a large array of fascinating properties, such as a negligible vapor pressure, a high chemical and thermal stability, a large liquidus temperature range, and widely tunable thermophysical properties by the appropriate pairing of different cations with any of a growing number of anions.[9] Therefore, in recent years, ILs have received extensive attention as "designer solvents".[10] IL-based ABS's (IL-ABS's) were reported, for the first time, by Rogers and co-workers in 2003.[11] After this first impulse, the number of works describing different IL-ABS's has been growing in the past few years. Those works have addressed not only a wide variety of ionic liquids[12,13] but also the use of various salting-out inducing salts (mostly phosphates,[12−24] hydrogenophosphates,[15,17,19−21,25] citrates,[6,26−30] and carbonates[14,17,19−22,31−33]), as well as carbohydrates,[34,35] polymers,[36−38] amino acids,[40,41] and more recently, anionic surfactants.[39] Since ILs are used in aqueous solution, they are not constrained by the need for low melting temperatures, and even compounds that do not fit the low melting point definition of ionic liquids can be used for preparing IL-ABS's. These systems have been extensively studied as successfully separation/extraction methods of different compounds and molecules, such as alkaloids,[12,31,40,41] phenolic compounds,[42] antibiotics,[25,31,43−48] amino acids,[13,16,30,36,42,49] proteins,[23,50−54] enzymes,[23,55−58] and drugs.[59]

This work is focused on the study of the ability of IL cations to form ABS's with two salts, K_2HPO_4, and the phosphate buffer solution composed of K_2HPO_4/KH_2PO_4. These salts were chosen to obtain ABS's with controlled pH values. Aiming at evaluating the IL cation influence toward the formation of ABS's, with the two inorganic salts and/or inorganic salt mixture, this work will be divided in three major sections: the first part addresses the impact of the cation side alkyl chain length; the second part describes the influence of different cation cores (imidazolium, pyridinium, pyrrolidinium, and piperidinium), and the last part addresses the influence of the cation positional isomerism.

MATERIALS AND METHODS

Materials. K_2HPO_4 and KH_2PO_4, with mass fraction purities higher than 98 %, were purchased from Sigma-Aldrich. The ILs used are as described: 1-ethyl-3-methylimidazolium chloride, $[1\text{-}C_2\text{-}3\text{-}C_1\text{im}]$Cl (> 99 wt %); 1-butyl-3-methylimidazolium chloride, $[1\text{-}C_4\text{-}3\text{-}C_1\text{im}]$Cl (> 99 wt %); 1-hexyl-3-methylimidazolium

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chloride, [1-C_6-3-C_1im]Cl (> 98 wt %); 1-heptyl-3-methylimidazolium chloride, [1-C_7-3-C_1im]Cl (> 99 wt %); 1-benzyl-3-methylimidazolium chloride, [1-C_7H_7-3-C_1im]Cl (> 99 wt %), 1-octyl-3-methylimidazolium chloride, [1-C_8-3-C_1im]Cl (> 99 wt %); 1-butyl-2-methylpyridinium, [1-C_4-2-C_1py]Cl (> 98 wt %); 1-butyl-3-methylpyridinium chloride, [1-C_4-3-C_1py]Cl (> 98 wt %); 1-butyl-4-methylpyridinium chloride, [1-C_4-4-C_1py]Cl (> 98 wt %); 1-butyl-1-methylpyrrolidinium chloride, [1-C_4-1-C_1pyr]Cl (> 99 wt %); 1-butyl-1-methylpiperidinium chloride [1-C_4-1-C_1pip]Cl (> 99 wt %). All ILs were purchased at IoLiTec (Ionic Liquid Technologies, Germany), and their mass fraction purities were further confirmed by ^{1}H NMR and ^{13}C NMR. Their ionic structures with their respective abbreviations are reported 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.

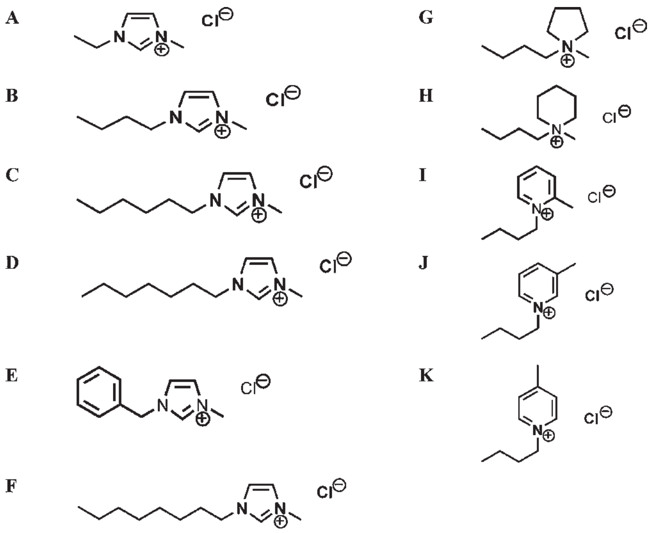

Figure 1. Chemical structure of the ILs studied: [1-C_2-3-C_1im]Cl (A), [1-C_4-3-C_1im]Cl (B), [1-C_6-3-C_1im]Cl (C), [1-C_7-3-C_1im]Cl (D), [1-C_7H_7-3-C_1im]Cl (E), [1-C_8-3-C_1im]Cl (F), [1-C_4-1-C_1pyr]Cl (G), [1-C_4-1-C_1pip]Cl (H), [1-C_4-2-C_1py]Cl (I), [1-C_4-3-C_1py]Cl (J), [1-C_4-4-C_1py]Cl (K).

Phase Diagrams and Tie Lines. The binodal data were determined for all of the ILs studied using the cloud-point titration method previously described by us[13,16] at 298 K (± 1 K). Aqueous solutions of the inorganic salts at ca. 40 wt % of K_2HPO_4 (with a pH value of 9.1) and K_2HPO_4/KH_2PO_4 (phosphate buffer solution with a pH value of 7.0), and aqueous solutions of each IL (≈ 60 wt %) were individually prepared. Repetitive dropwise addition of the aqueous inorganic salt solution to the 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 monophasic region. 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) were determined by a gravimetric method previously described by us[13,16,58] and originally proposed by Merchuck et al.[60] A mixture at the biphasic region was prepared, vigorously stirred, and allowed to reach equilibrium by the phase separation for 24 h at 298 K, using small ampules (10 cm^3) especially designed for this task. After this period, both phases were carefully separated and weighed. Each individual TL was determined by the application of the lever-arm rule.[60] For that purpose, the experimental binodal curves were correlated by the following eq 1,[60]

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

where Y/wt % and X/wt %, represent, respectively, the IL and salt weight fractions, and A, B, and C are adjusted parameters obtained by regression.

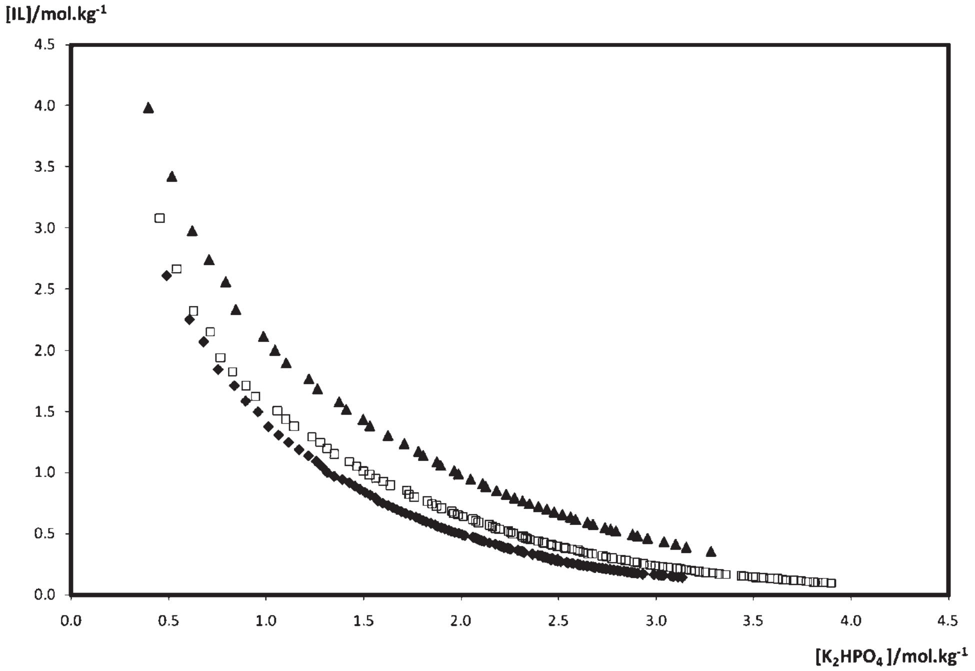

Figure 2. Phase diagrams for the ILs [1-C_n-3-C_1im]Cl and the salt K_2HPO_4 at 298 K: ▲, [1-C_2-3-C_1im]Cl; □, [1-C_4-3-C_1im]Cl; ◆, [1-C_6-3-C_1im]Cl.

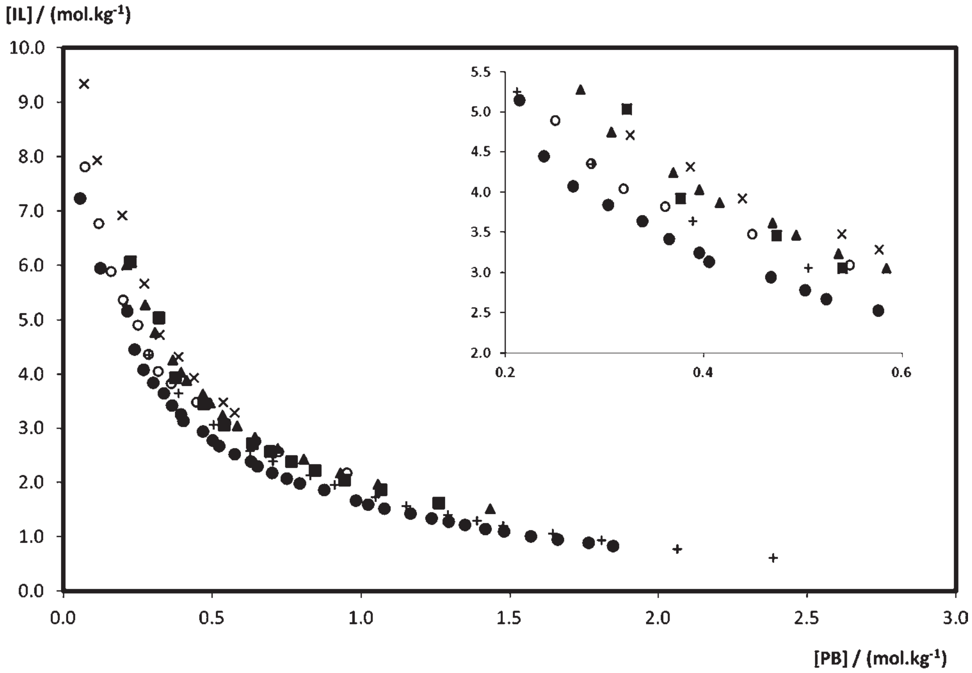

Figure 3. Phase diagrams for the ILs [1-C_n-3-C_1im]Cl and the phosphate buffer solution at 298 K: ×, [1-C_2-3-C_1im]Cl; ○, [1-C_4-3-C_1im]Cl; ●, [1-C_6-3-C_1im]Cl; ▲, [1-C_7-3-C_1im]Cl; +, [1-C_8-3-C_1im]Cl; ■, [1-C_7H_7-3-C_1im]Cl.

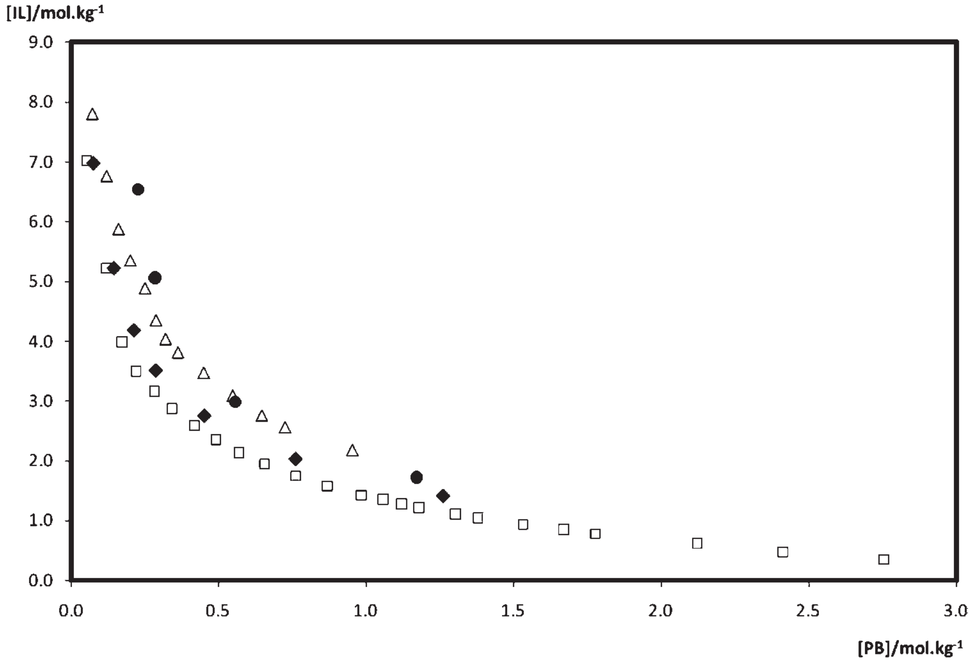

Figure 4. Phase diagrams for ILs constituted by different cation cores and the potassium phosphate buffer solution at 298 K: ●, [1-C_4-1-C_1pyr]Cl; △, [1-C_4-3-C_1im]Cl; ♦, [1-C_4-1-C_1pip]Cl; □, [1-C_4-3-C_1py]Cl.

■ RESULTS AND DISCUSSION

The phase diagrams, presented in Figures 2 and 3, depict the effect of different alkyl side chains at the IL cation in the formation of ABS. The binodal data of these systems (in mass fraction units) are presented in the Supporting Information, Tables S1 to S9.

Figure 2 reports the ABS's composed of three different imidazolium-based ILs and the inorganic salt K_2HPO_4. It is observed that the increase of the cation aliphatic chain from C_2 to C_6 leads to an increase of the biphasic region envelope due to the increasing hydrophobicity of the IL.[13,16,61] This behavior has been previously described for IL-ABS's containing other

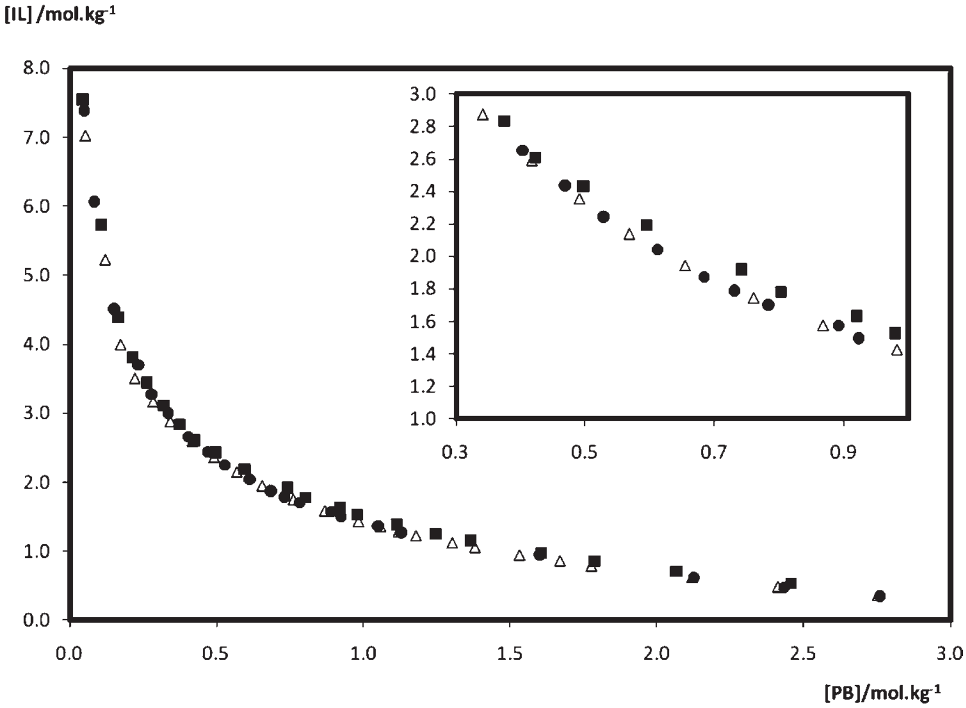

Figure 5. Phase diagrams for pyridinium-based ILs and the potassium phosphate buffer solution at 298 K: ■, $[1\text{-}C_4\text{-}2\text{-}C_1\text{py}]\text{Cl}$; △, $[1\text{-}C_4\text{-}3\text{-}C_1\text{py}]\text{Cl}$; ●, $[1\text{-}C_4\text{-}4\text{-}C_1\text{py}]\text{Cl}$.

salts as a characteristic of ILs with alkyl chains up to six carbon atoms.[13,32]

The study of the influence of the IL alkyl chain length through the ABS formation ability, conjugated with the phosphate buffer (PB) solution containing the salts K_2HPO_4/KH_2PO_4, is reported in Figure 3 for a larger number of ILs with a wider range of aliphatic chain lengths. The binodal curves for the systems with the PB, and with different imidazolium-based ILs, have an increasing tendency of phase separation that can be described by the series: $[1\text{-}C_2\text{-}3\text{-}C_1\text{im}]\text{Cl} < [1\text{-}C_7\text{-}3\text{-}C_1\text{im}]\text{Cl} \approx [1\text{-}C_7H_7\text{-}3\text{-}C_1\text{im}]\text{Cl} < [1\text{-}C_8\text{-}3\text{-}C_1\text{im}]\text{Cl} \approx [1\text{-}C_4\text{-}3\text{-}C_1\text{im}]\text{Cl} < [1\text{-}C_6\text{-}3\text{-}C_1\text{im}]\text{Cl}$.

The same tendency in the increasing ability of ABS formation with the cation side chain length, between C_2 and C_6, is observed in both salt solutions. However, as the number of carbons in the alkyl chain increases, above C_6, the ability for ABS formation changes and no longer follows the hydrophobicity of the IL. This trend was previously observed by us[18] using the salt K_3PO_4 and has also been described by others,[13,19,21,53,62−64] for other inorganic salts (K_2HPO_4 and K_2CO_3). Najdanovic-Visak et al.[62] suggested that when the number of carbons in the longer alkyl side chain of the imidazolium cation is superior to 6, the capacity of the ABS formation is altered due to the possibility of micelle formation in aqueous solutions. While the salting-out effect of the inorganic salt remains, the tendency of the IL to self-aggregate in aqueous solution reduces the ability of the liquid−liquid demixing and, thus, decreases the aptitude for ABS formation. Finally, the presence of a benzyl group ($[C_7H_7]$) in the imidazolium cation core has no major impact through the ABS formation capability. Only a marginal increase in the ability to form ABS was observed when compared with $[C_7\text{mim}]\text{Cl}$.

Figure 4 shows the influence of the IL cation core toward the ABS formation capacity using the phosphate buffer solution.

Table 1. Adjusted Parameters ($\pm\ 10^{-4}$) Obtained from the Regression of Equation 1 at 298 K and Atmospheric Pressure

ternary system		regression parameters		
salt	ionic liquid	*A*	*B*	*C*
K_2HPO_4	$[1\text{-}C_2\text{--}C_1\text{im}]\text{Cl}$	76.8955	−0.2896	$1.9880 \cdot 10^{-5}$
	$[1\text{-}C_4\text{-}3\text{-}C_1\text{im}]\text{Cl}$	71.3577	−0.2840	$2.9722 \cdot 10^{-5}$
	$[1\text{-}C_6\text{-}3\text{-}C_1\text{im}]\text{Cl}$	88.9897	−0.3298	$3.4743 \cdot 10^{-5}$
K_2HPO_4/KH_2PO_4	$[1\text{-}C_2\text{-}3\text{-}C_1\text{im}]\text{Cl}$	76.3424	−0.2604	$2.1623 \cdot 10^{-4}$
	$[1\text{-}C_4\text{-}3\text{-}C_1\text{im}]\text{Cl}$	79.4000	−0.2913	$8.4900 \cdot 10^{-6}$
	$[1\text{-}C_6\text{-}3\text{-}C_1\text{im}]\text{Cl}$	80.5229	−0.2934	$3.3300 \cdot 10^{-5}$
	$[1\text{-}C_7\text{-}3\text{-}C_1\text{im}]\text{Cl}$	100.4882	−0.3183	$6.8700 \cdot 10^{-6}$
	$[1\text{-}C_8\text{-}3\text{-}C_1\text{im}]\text{Cl}$	93.6696	−0.3003	$2.4360 \cdot 10^{-5}$
	$[1\text{-}C_7H_7\text{-}3\text{-}C_1\text{im}]\text{Cl}$	109.9598	−0.3722	$-1.1490 \cdot 10^{-5}$
	$[1\text{-}C_4\text{-}1\text{-}C_1\text{pyr}]\text{Cl}$	125.9078	−0.4682	$-4.3490 \cdot 10^{-5}$
	$[1\text{-}C_4\text{-}1\text{-}C_1\text{pip}]\text{Cl}$	82.6342	−0.3416	$8.1200 \cdot 10^{-6}$
	$[1\text{-}C_4\text{-}2\text{-}C_1\text{py}]\text{Cl}$	76.9970	−0.3348	$1.5875 \cdot 10^{-5}$
	$[1\text{-}C_4\text{-}3\text{-}C_1\text{py}]\text{Cl}$	76.1169	−0.3428	$1.9877 \cdot 10^{-5}$
	$[1\text{-}C_4\text{-}4\text{-}C_1\text{py}]\text{Cl}$	78.2432	−0.3495	$1.9715 \cdot 10^{-5}$

The mass fraction data for each binodal curve are reported in Supporting Information, Table S5 and Tables S10 to S12. According to these results, the sequence for the ability of phase formation for the various families is $[1\text{-}C_4\text{-}1\text{-}C_1\text{pyr}]\text{Cl} \approx [1\text{-}C_4\text{-}3\text{-}C_1\text{im}]\text{Cl} < [1\text{-}C_4\text{-}1\text{-}C_1\text{pip}]\text{Cl} < [1\text{-}C_4\text{-}3\text{-}C_1\text{py}]\text{Cl}$. This order is a reflection of the IL ability to be solvated by water. Although we have previously shown that the solubility of water in ILs largely depends on the availability of electrons at the aromatic cores for privileged hydrogen-bonding interactions; on the other hand, the solubility of ILs in water is mainly ruled by steric and entropic contributions.[61,65] As a result, the solubility of ILs in water

Table 2. Mass Fraction Compositions for the TLs and Respective TLLs, at the Top (T) and Bottom (B) Phases, and at the Initial Biphasic Composition of the Mixture (M), Composed of ILs (Y) and Inorganic Salts (X) at 298 K and Atmospheric Pressure

ABS		100·mass fraction/wt %						
salt	ionic liquid	Y_M ± std	X_M ± std	Y_T ± std	X_T ± std	Y_B ± std	X_B ± std	TLL
K_2HPO_4	[1-C_2-3-C_1im]Cl	19.79 ± 0.05	25.08 ± 0.04	2.30 ± 0.44	43.13 ± 0.02	38.53 ± 0.03	5.64 ± 0.18	52.21
	[1-C_4-3-C_1im]Cl	19.97 ± 0.05	24.88 ± 0.04	0.73 ± 1.37	44.83 ± 0.02	39.98 ± 0.03	4.13 ± 0.24	56.54
	[1-C_6-3-C_1im]Cl	20.11 ± 0.05	24.96 ± 0.04	43.26 ± 0.02	4.73 ± 0.21	0.83 ± 0.01	41.80 ± 0.02	56.34
K_2HPO_4/KH_2PO_4	[1-C_2-3-C_1im]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
	[1-C_4-3-C_1im]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
	[1-C_6-3-C_1im]Cl	25.08 ± 0.04	29.97 ± 0.03	55.31 ± 0.02	1.65 ± 0.61	0.06 ± 0.01	53.48 ± 0.02	75.68
	[1-C_7-3-C_1im]Cl	25.03 ± 0.04	29.99 ± 0.03	45.10 ± 0.02	6.31 ± 0.16	2.70 ± 0.37	56.33 ± 0.02	65.57
	[1-C_8-3-C_1im]Cl	30.11 ± 0.03	20.05 ± 0.05	49.01 ± 0.02	4.62 ± 0.22	1.84 ± 0.54	43.14 ± 0.02	60.91
		34.92 ± 0.03	20.08 ± 0.05	56.95 ± 0.02	2.74 ± 0.36	0.99 ± 1.01	46.78 ± 0.02	71.21
	[1-C_7H_7-3-C_1im]Cl	24.97 ± 0.04	30.01 ± 0.03	28.26 ± 0.04	13.95 ± 0.07	22.65 ± 0.04	41.37 ± 0.02	27.99
	[1-C_4-1-C_1pyr]Cl	30.07 ± 0.03	15.00 ± 0.07	38.81 ± 0.03	7.42 ± 0.13	23.33 ± 0.04	24.06 ± 0.04	20.87
	[1-C_4-1-C_1pip]Cl	25.09 ± 0.04	29.97 ± 0.03	26.98 ± 0.04	10.94 ± 0.09	22.81 ± 0.02	52.79 ± 0.02	42.06
	[1-C_4-2-C_1py]Cl	24.99 ± 0.04	23.02 ± 0.04	44.14 ± 0.02	2.76 ± 0.36	1.29 ± 0.01	48.10 ± 0.02	62.39
	[1-C_4-3-C_1py]Cl	24.98 ± 0.04	23.06 ± 0.04	45.23 ± 0.02	2.30 ± 0.43	0.81 ± 0.01	47.84 ± 0.02	63.62
	[1-C_4-4-C_1py]Cl	24.97 ± 0.04	23.05 ± 0.04	46.26 ± 0.02	2.26 ± 0.44	1.00 ± 0.01	46.45 ± 0.02	63.26

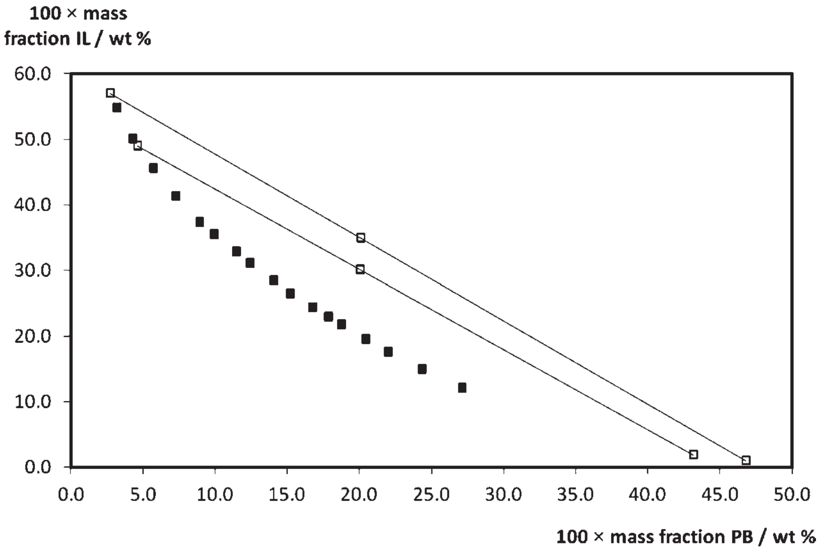

Figure 6. Phase diagram for IL [1-C_8-3-C_1im]Cl and the phosphate buffer solution at 298 K: ■, binodal curve data; □, TL data.

follows the IL cation molar volume,[66] and the trend obtained here closely agrees with our previous data.[65] Larger cations such as pyridinium and piperidinium are more able to induce ABS's when compared with the smaller 5-sided rings of imidazolium and pyrrolidinium. Albeit the pyridinium-based IL (with an aromatic core) is slightly more able to induce ABS's than the piperidinium-based IL (with an aliphatic core), we must be aware of steric effects that result from the position of their alkyl chains. The presence of a second aliphatic chain in pyridinium in a non-nitrogenated atom leads to a higher entropic contribution and, thus, to a higher aptitute of this IL to produce ABS's.

Figure 5 shows the influence of positional isomerism of the methyl moiety in a pyridinium cation through the formation of ABS's. For such a purpose, three pyridinium-based ILs were investigated, namely, [1-C_4-4-C_1py]Cl, [1-C_4-3-C_1py]Cl, and [1-C_4-2-C_1py]Cl. The mass fraction data of each binodal curve are reported in the Supporting Information, Tables S12 to S14. From the inspection of Figure 5, the sequence for the crescent ability of phase separation follows the rank: [1-C_4-2-C_1py]Cl < [1-C_4-3-C_1py]Cl ≈ [1-C_4-4-C_1py]Cl. Still, no major differences in the phase diagrams are observed among the three positional isomers. To the best of our knowledge, there are no references in literature regarding the formation of IL-ABS's using positional isomers, making thus a comparison with other systems impracticable.

The influence of the positional/structural isomerism on the mutual solubilities of ILs with water was discussed in a recent paper by Freire et al.[63] The authors[63] observed that a methyl substitution at the para position in a pyridinium ring contributes to a higher increment on the solubility of water in the IL when compared with the respective isomer with a methyl substitution at the meta position. This trend was explained based on the delocalization of the positive charge at the aromatic ring.[63] However, at the water-rich phase, the solubility of the corresponding isomers in water is less dependent on the ability of the aromatic protons to hydrogen bond with the oxygen of water.[63] In this context, both isomers present similar values of solubility in water with only a slightly higher ability of the para isomer to be solvated in aqueous media. This slight dependence on the ILs isomerism, and thus on the cation ability to hydrogen bond with water, in the solubility of the diverse ILs in water, closely correlates with the trends obtained here where no major divergences were observed. Nevertheless, we should be aware that the phosphate buffer is also composed of an inorganic salt (KH_2PO_4) tending for the salting-in regime, and not only by a strong salting-out inducing species (K_2HPO_4). Previously we have shown that low charge density salts lead to the salting-in of an IL in aqueous solution by the occurrence of specific interactions between the ions and the hydrophobic tails of the IL, while the salting-out phenomenon was a result of entropic contributions associated to the preferential formation of the ions' hydration complexes.[67,68] Therefore, in this work, we believe that a delicate mechanism on the salting-in/out balance associated to the steric hindrance of the IL is taking place, leading thus to a more complex scenario of dominant interactions.

The experimental binodal curves for the three IL-ABS's formed with K_2HPO_4 and the 11 systems composed of phosphate buffer were correlated using eq 1.The adjusted parameters are reported in Table 1.

The TLs and respective tie-line lengths (TLLs) determined for each system are presented in Table 2, while Figure 6 depicts an example on the TLs obtained for the system [1-C_8-3-C_1im]Cl + H_2O + PB. In Figure 6 it can be observed that a higher difference in the mass fraction of both solutes leads to higher TLLs.

■ CONCLUSIONS

In this work novel ABS phase diagrams for 11 ILs, based in piperidinium, pyridinium, pyrrolidinium, and imidazolium cores, were determined. Taking into account the phase diagram behavior, the effects of the IL cation core, the cation side alkyl chain length, and the positional isomerism at the cation toward the formation ability of ABS were presented and discussed. An increase in the length of the aliphatic chain at the cation leads to an increase on the IL ability to form ABS, when C_2 to C_6 alkyl chains are considered. However, when the number of carbons at the alkyl side chain is higher than 6, there is an inversion on the ILs sequence, due to the self-aggregation of ILs in aqueous phases. Finally, the influence of the cation core and the positional isomerism at the cation were also considered, and in general, the ability of ILs to induce ABS is more dependent on the size or molecular volume of the ions, and thus more dependent on steric contributions, than on the hydrogen-bonding ability of the diverse cations to interact with water.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental binodal mass fraction data for the studied systems (Tables S1 to S12). This material is available free of charge via the Internet at http://pubs.acs.org.

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