



Phase diagrams of ionic liquids-based aqueous biphasic systems as a platform for extraction processes



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ABSTRACT

In the past few years, ionic liquid-based aqueous biphasic systems have become the subject of considerable interest as a promising technique for the extraction and purification of several macro/biomolecules. Aiming at developing guidelines for more benign and efficient extraction processes, phase diagrams for aqueous biphasic systems composed of ionic liquids and inorganic/organic salts are here reported. Several combinations of ionic liquid families (imidazolium, pyridinium, phosphonium, quaternary ammonium and cholinium) and salts [potassium phosphate buffer ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ at pH 7), potassium citrate buffer ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 5, 6, 7 and 8) and potassium carbonate (K_2CO_3 at pH ~ 13)] were evaluated to highlight the influence of the ionic liquid structure (cation core, anion and alkyl chain length), the pH and the salt nature on the formation of aqueous biphasic systems. The binodal curves and respective tie-lines reported for these systems were experimentally determined at (298 ± 1) K. In general, the ability to promote the aqueous biphasic systems formation increases with the pH and alkyl chain length. While the influence of the cation core and anion nature of the ionic liquids on their ability to form aqueous biphasic systems closely correlates with ionic liquids capacity to be hydrated by water, the effect of the different salts depends of the ionic liquid nature and salt valency.

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1. Introduction

Aqueous biphasic systems (ABS) are usually formed as a result of the mutual incompatibility in aqueous solution of two polymers, one polymer and one salt, or two salts above a certain concentration [1]. These systems are mainly composed by water and thereby capable to provide an excellent environment for a large set of cells and biologically active substances. In this way, ABS are commonly investigated for the extraction and purification of distinct (bio)molecules, such as proteins [2–6] and amino-acids [7–12]. They allow a high effectiveness, high yield, improved purity degree, a proper selectivity, technological simplicity and low cost [13]. Furthermore, ABS are considered as environmentally friendly techniques, since volatile organic solvents are not employed in the whole process [14]. The application of polymer-based ABS is well-documented [15–18], nevertheless, most of these aqueous systems present high viscosities, form opaque systems and are considered as limited techniques in terms of the range of polarities of the coexisting phases [19]. There has been, therefore, an effort to find new liquid–liquid alternatives, mainly in the application of distinct and non-polymeric ABS.

In the last decade, the use of ionic liquids (ILs) as a new agent in the formation of ABS has attracted a considerable interest as promising “greener” alternatives to volatile and hazardous organic solvents. This increasing attention is largely justified by their unique properties, such as their negligible vapor pressure, high chemical and thermal stability, their non-flammability, high ionic conductivity, wide electrochemical potential window and high solvation ability. In fact, these ionic compounds are normally known by their tunable properties, which mean that they can be designed for a specific purpose by the selection of the adequate cation/anion combination. Moreover, and allied with their tunable character, the use of ILs in the formation of ABS allows the possibility of tailoring their polarities and affinities by the organization of the ions in the bulk [20].

The use of ILs in the formation of ABS grew steadily [21,22] since in 2003 Rogers and co-workers [23] reported, for the first time, that hydrophilic ILs had the capacity to form ABS in the presence of some inorganic salts. Different lines of research were considered when studying the use of ILs as ABS promoting agents [21,22] namely, the study of the salt type, where phosphate [8–10,23–33], hydrogenophosphate [8,25,26,28,34,35], sulfate [25,27,30,36] and carbonate [8,25,27,32,37,38] and a range of other salts [21,39] were applied, as well as carbohydrates [7,40], polymers [41,42], amino-acids [43,44], and more recently anionic surfactants [45]. Furthermore, recent studies have introduced

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citrate-based salts in the formulation of IL-based ABS because they are more biodegradable and less toxic than most other salts [46–48]. Nevertheless, on most of these liquid–liquid extraction systems, the pH is not adequately controlled, which is a crucial issue regarding the separation or purification of some particular biomolecules, namely proteins [49] and enzymes [50]. Only recently, the inorganic potassium phosphate buffer (K_2HPO_4/KH_2PO_4) and the organic potassium citrate buffer ($C_6H_5K_3O_7/C_6H_8O_7$) solutions (both at pH 7), have been focus of interest among researchers considering not only their ability to form ABS when conjugated with ILs, but also their applicability while extraction and purification systems for several biomolecules [2,6,34,49–54].

Another aspect of major importance when considering the IL-based ABS formation is the study of the impact of the different ILs, not only in what concerns the use of several cations families [21], the imidazolium being one of the most investigated [41,55],

but also considering the effect of the cation alkyl side chain length [8,10,56] and finally, different anions [24,51]. More recently, and since the number of new IL families is increasing, some works concerning the use of new families, such as the acyclic phosphonium, quaternary ammonium and, more recently, the cholinium-based ILs, were reported [9,57–59].

Taking into account the significant extraction efficiencies achieved recently for paracetamol [53] and natural dyes [49] in presence of ammonium compounds, a study on the expansion of the range of ionic liquid-based aqueous biphasic systems (IL-ABS) as a platform for extraction processes is here reported. For that purpose imidazolium-, pyridinium-, phosphonium-, ammonium- and cholinium-based ILs were evaluate toward their ability to form ABS in presence of potassium phosphate buffer (KH_2PO_4/K_2HPO_4), potassium citrate buffer ($C_6H_5K_3O_7/C_6H_8O_7$) and potassium carbonate (K_2CO_3) solutions.

This work is divided into four main sections: (i) the first addresses the pH influence; (ii) the second reports the impact of different cation cores, (iii) the third describes the influence of the cation side alkyl chain length and anion effect, and the (iv) addresses the salt effect. In this context, it was chosen to evaluate one inorganic salt (K_2CO_3 at pH ~ 13) and two buffers with different ionic strength: the organic and benign potassium citrate buffer ($C_6H_5K_3O_7/C_6H_8O_7$ at pH 5, 6, 7 and 8) and the inorganic potassium phosphate buffer (KH_2PO_4/K_2HPO_4 at pH 7).

TABLE 1
Identification and purity of all ILs studied.

Ionic liquids		
Name	Acronym	Purity (wt%)
Tetraethylammonium chloride	[N _{2,2,2,2}]Cl	98
Tetrapropylammonium chloride	[N _{3,3,3,3}]Cl	98
Tetrapropylammonium bromide	[N _{3,3,3,3}]Br	98
Tetrabutylammonium chloride	[N _{4,4,4,4}]Cl	97
Tetrabutylammonium bromide	[N _{4,4,4,4}]Br	98
Choline salicylate	[Ch][Sal]	95
Benzyltrimethyl(2-hydroxyethyl)ammonium chloride	[BCh]Cl	97
Tetrabutylphosphonium chloride	[P _{4,4,4,4}]Cl	96.8
Tetrabutylphosphonium bromide	[P _{4,4,4,4}]Br	95.2
Tributyl(methyl)phosphoniummethylsulfate	[P _{4,4,4,1}][CH ₃ SO ₄]	98.6
Triisobutyl(methyl)phosphoniumtosylate	[P _{(4,4,4),1}][Tos]	99
1-Butyl-3-methylimidazolium chloride	[C ₄ mim]Cl	99
1-Butyl-3-methylpyridinium chloride	[C ₄ mpy]Cl	98

2. Experimental section

2.1. Materials

In this work, thirteen distinct ILs were investigated in terms of their capacity for ABS formation in conjugation with three salts. Their full name, acronym and purities are presented in table 1, being their respective ionic structures depicted in figure 1.

Phosphonium- and cholinium ILs were purchased from Cytec and Fluka, respectively and the ammonium-based ILs were acquired at Sigma–Aldrich. The [C₄mim]Cl and [C₄mpy]Cl were from

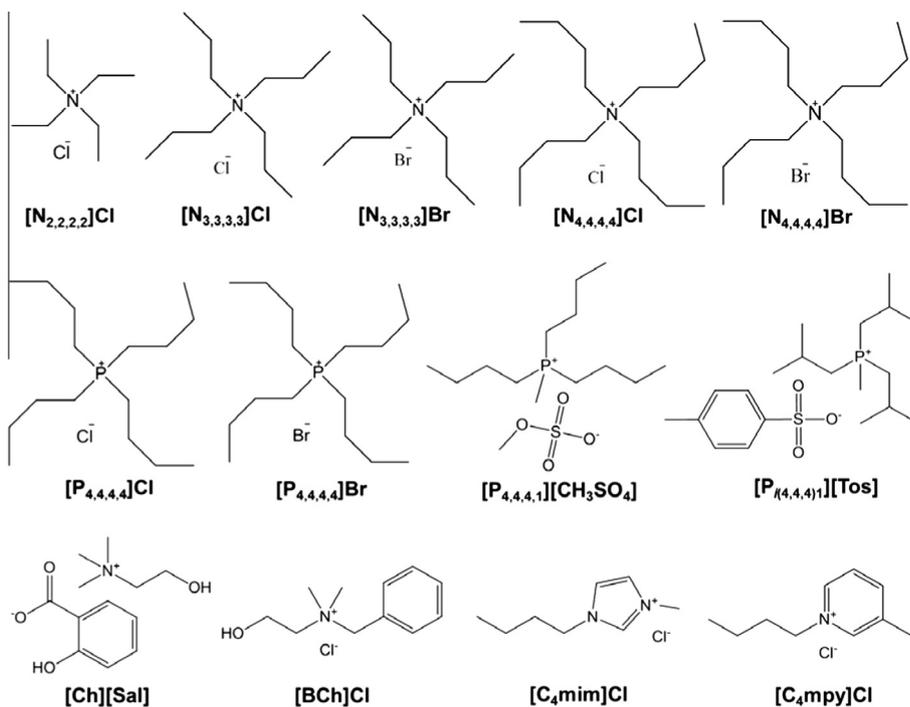


FIGURE 1. Chemical structure of the ILs studied.

Iolitec (Ionic Liquid Technologies, Heilbronn, Germany). The purity of each IL was further checked by ^1H and ^{13}C NMR spectra and found to be in accordance with the stated purity level provided by the suppliers.

Potassium phosphate monobasic, KH_2PO_4 (99.5 wt% of purity), K_2CO_3 (99 wt% of purity), and potassium citrate tribasic monohydrate, $\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$ (≥ 99 wt% of purity), were from Sigma–Aldrich, potassium phosphate dibasic, (K_2HPO_4) ($>98\%$ of purity) was from JMVP and citric acid monohydrate, $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ (100 wt% of purity) was from Fisher Scientific.

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

2.2. Experimental procedure

The binodal data were determined through the cloud point titration method at $T = (298 \pm 1)$ K and atmospheric pressure. The experimental procedure adopted in this work follows the method previously validated and described by us [10,24,34]. Thus, the salt [$\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH 7.0 [51]) at 40 wt%; $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ (pH 5, 6, 7 and 8 [48]) at 50 wt% (see table S1 at Supporting Information); K_2CO_3 (pH ~ 13) at 50 wt%] solution and aqueous solutions of the different ILs, with concentrations ranging from 40 wt% to 80 wt%, were prepared and used for the determination of the binodal

curves. The cloud point titration method consists in the repetitive dropwise addition of the salt solution into the aqueous solutions of each IL until the detection of a cloudy (biphasic) solution, followed by the dropwise addition of ultrapure water until the observation of a limpid solution (monophasic region). All the additions were made under constant and controlled stirring. The ternary system compositions were determined by the weight quantification of all components added within $\pm 10^{-4}$ g. From this experimental procedure, the weight mass fractions read at the cloud point correspond to the binodal curve. Aiming to determine with accuracy the monophasic/biphasic regions, the experimental solubility curves were correlated using the Merchuk equation (equation (1)) [60]:

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

where Y and X represent the IL and salt mass fraction percentages, respectively, and A , B , and C are constants obtained by the regression of the experimental binodal data. The Merchuk equation was adopted in this work since it is the most used and makes use of a lower number of adjustable parameters to correlate the data, when compared with the remaining alternatives [21]. Besides that, the Merchuk equation is the only one that allows the direct determination of the tie-lines through a mathematical approach (lever-arm rule). The tie-lines (TLs) associated to each mutual coexistence curve were determined by the gravimetric method proposed by Merchuk *et al.* [60]. The composition of the ternary mixtures

TABLE 2
Adjusted parameters and respective standard deviations (std) obtained from the regression of equation (1), at $T = (298 \pm 1)$ K and atmospheric pressure.

Salt component	IL	$A \pm \text{std}$	$B \pm \text{std}$	$C \pm \text{std}$	R^2
$\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 5	[N _{3,3,3,3}]Br	85.2 ± 0.9	-0.191 ± 0.005	$1.6 \cdot 10^{-5} \pm 2.2 \cdot 10^{-6}$	0.9972
	[N _{4,4,4,4}]Br	159.7 ± 7.5	-0.407 ± 0.011	$1.9 \cdot 10^{-5} \pm 5.6 \cdot 10^{-7}$	0.9997
$\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 6	[N _{3,3,3,3}]Br	86.9 ± 0.5	-0.238 ± 0.003	$1.5 \cdot 10^{-5} \pm 5.2 \cdot 10^{-7}$	0.9993
	[N _{4,4,4,4}]Br	164.9 ± 5.4	-0.466 ± 0.009	$2.7 \cdot 10^{-5} \pm 6.1 \cdot 10^{-7}$	0.9992
	[C ₄ mpy]Cl	94.2 ± 0.3	-0.213 ± 0.001	$5.0 \cdot 10^{-6} \pm 1.6 \cdot 10^{-7}$	0.9998
	[C ₄ mim]Cl	89.3 ± 0.2	-0.177 ± 0.001	$5.7 \cdot 10^{-6} \pm 1.2 \cdot 10^{-7}$	0.9998
$\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 7	[C ₄ mpy]Cl	94.4 ± 0.6	-0.221 ± 0.002	$6.9 \cdot 10^{-6} \pm 5.4 \cdot 10^{-7}$	0.9991
	[C ₄ mim]Cl	89.1 ± 0.3	-0.189 ± 0.001	$7.1 \cdot 10^{-6} \pm 1.1 \cdot 10^{-7}$	0.9997
	[P _{4,4,4,1}][CH ₃ SO ₄]	60.4 ± 0.8	-0.230 ± 0.005	$4.5 \cdot 10^{-5} \pm 7.5 \cdot 10^{-7}$	0.9977
	[P _{1(4,4,4)1}][Tos]	1016.4 ± 122.6	-0.945 ± 0.035	$8.0 \cdot 10^{-5} \pm 4.6 \cdot 10^{-6}$	0.9978
	[P _{4,4,4,4}]Cl	98.7 ± 0.6	-0.312 ± 0.002	$3.4 \cdot 10^{-5} \pm 3.4 \cdot 10^{-7}$	0.9997
	[P _{4,4,4,4}]Br	193.9 ± 7.5	-0.615 ± 0.013	$5.4 \cdot 10^{-5} \pm 2.6 \cdot 10^{-6}$	0.9976
	[Ch][Sal]	150.3 ± 3.9	-0.259 ± 0.007	$1.3 \cdot 10^{-5} \pm 4.0 \cdot 10^{-7}$	0.9993
	[BCh]Cl	98.8 ± 0.4	-0.189 ± 0.001	$6.1 \cdot 10^{-6} \pm 1.2 \cdot 10^{-7}$	0.9997
$\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 8	[N _{3,3,3,3}]Br	89.6 ± 0.3	-0.267 ± 0.002	$2.1 \cdot 10^{-5} \pm 2.7 \cdot 10^{-7}$	0.9997
	[N _{4,4,4,4}]Br	176.4 ± 5.7	-0.531 ± 0.009	$3.5 \cdot 10^{-5} \pm 8.8 \cdot 10^{-7}$	0.9984
	[C ₄ mpy]Cl	92.4 ± 0.4	-0.219 ± 0.001	$8.6 \cdot 10^{-6} \pm 2.2 \cdot 10^{-7}$	0.9996
	[C ₄ mim]Cl	92.1 ± 0.2	-0.200 ± 0.001	$7.6 \cdot 10^{-6} \pm 9.8 \cdot 10^{-8}$	0.9999
$\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ at pH 7	[N _{3,3,3,3}]Br	74.7 ± 0.6	-0.345 ± 0.003	$2.9 \cdot 10^{-5} \pm 9.1 \cdot 10^{-7}$	0.9993
	[N _{4,4,4,4}]Br	88.4 ± 0.4	-0.423 ± 0.002	$6.4 \cdot 10^{-5} \pm 1.5 \cdot 10^{-6}$	0.9994
	[N _{2,2,2,2}]Cl	69.0 ± 0.9	-0.286 ± 0.004	$1.3 \cdot 10^{-5} \pm 3.3 \cdot 10^{-7}$	0.9996
	[N _{3,3,3,3}]Cl	66.2 ± 0.8	-0.346 ± 0.005	$3.8 \cdot 10^{-5} \pm 1.9 \cdot 10^{-6}$	0.9991
	[N _{4,4,4,4}]Cl	73.2 ± 0.3	-0.397 ± 0.002	$5.5 \cdot 10^{-5} \pm 5.8 \cdot 10^{-7}$	0.9997
	[P _{4,4,4,1}][CH ₃ SO ₄]	76.3 ± 0.4	-0.306 ± 0.003	$7.6 \cdot 10^{-5} \pm 1.1 \cdot 10^{-6}$	0.9984
	[P _{1(4,4,4)1}][Tos]	221.2 ± 4.7	-0.652 ± 0.008	$1.4 \cdot 10^{-4} \pm 2.9 \cdot 10^{-6}$	0.9973
	[P _{4,4,4,4}]Cl	86.9 ± 0.4	-0.394 ± 0.002	$3.8 \cdot 10^{-5} \pm 6.4 \cdot 10^{-7}$	0.9994
	[P _{4,4,4,4}]Br	103.5 ± 2.2	-0.478 ± 0.008	$1.3 \cdot 10^{-4} \pm 3.5 \cdot 10^{-6}$	0.9984
	[Ch][Sal]	106.4 ± 1.2	-0.302 ± 0.005	$3.8 \cdot 10^{-5} \pm 2.2 \cdot 10^{-6}$	0.9988
	[BCh]Cl	87.9 ± 1.5	-0.269 ± 0.008	$4.4 \cdot 10^{-5} \pm 6.3 \cdot 10^{-6}$	0.9990
	K_2CO_3 at pH ~ 13	[N _{3,3,3,3}]Br	88.1 ± 0.4	-0.403 ± 0.002	$1.0 \cdot 10^{-4} \pm 1.0 \cdot 10^{-6}$
[N _{4,4,4,4}]Br		123.8 ± 1.0	-0.568 ± 0.004	$2.0 \cdot 10^{-4} \pm 3.8 \cdot 10^{-6}$	0.9994
[N _{2,2,2,2}]Cl		86.9 ± 0.6	-0.332 ± 0.003	$3.2 \cdot 10^{-5} \pm 6.5 \cdot 10^{-7}$	0.9991
[N _{3,3,3,3}]Cl		83.4 ± 1.3	-0.365 ± 0.005	$7.1 \cdot 10^{-5} \pm 9.3 \cdot 10^{-7}$	0.9995
[N _{4,4,4,4}]Cl		96.3 ± 0.8	-0.444 ± 0.003	$1.2 \cdot 10^{-4} \pm 1.2 \cdot 10^{-6}$	0.9996
[P _{4,4,4,1}][CH ₃ SO ₄]		102.0 ± 0.6	-0.432 ± 0.003	$2.3 \cdot 10^{-4} \pm 2.2 \cdot 10^{-6}$	0.9910
[P _{1(4,4,4)1}][Tos]		123.4 ± 2.4	-0.423 ± 0.009	$8.0 \cdot 10^{-4} \pm 9.5 \cdot 10^{-6}$	0.9995
[P _{4,4,4,4}]Cl		109.2 ± 0.6	-0.480 ± 0.002	$1.5 \cdot 10^{-4} \pm 1.4 \cdot 10^{-6}$	0.9997
[P _{4,4,4,4}]Br		187.5 ± 10.1	-0.748 ± 0.023	$2.6 \cdot 10^{-4} \pm 1.6 \cdot 10^{-5}$	0.9972
[Ch][Sal]		149.2 ± 2.6	-0.353 ± 0.006	$4.2 \cdot 10^{-5} \pm 1.0 \cdot 10^{-6}$	0.9983
[BCh]Cl		131.8 ± 2.1	-0.365 ± 0.006	$3.9 \cdot 10^{-5} \pm 9.6 \cdot 10^{-7}$	0.9978

TABLE 3

Mass fraction compositions and respective standard deviations (std) for the TLs and respective Tie-Line Lengths (TLLs), at the Top (*T*) and Bottom (*B*) phases, and at the initial biphasic composition of the mixture (*M*), composed of ILs (*Y*) and salts (*X*), at $T = (298 \pm 1)$ K and atmospheric pressure.

Salt component	IL	100 × Mass fraction composition/wt%							
		$Y_M \pm \text{std}$	$X_M \pm \text{std}$	$Y_T \pm \text{std}$	$X_T \pm \text{std}$	$Y_B \pm \text{std}$	$X_B \pm \text{std}$	TLL	
C ₆ H ₅ K ₃ O ₇ /C ₆ H ₈ O ₇ at pH 5	[N _{3,3,3,3}]Br	39.90 ± 0.03	22.04 ± 0.05	64.18 ± 0.02	2.20 ± 0.46	1.96 ± 0.51	53.06 ± 0.02	80.37	
		45.00 ± 0.02	19.99 ± 0.05	68.07 ± 0.02	1.38 ± 0.72	1.42 ± 0.70	55.15 ± 0.02	85.63	
	[N _{4,4,4,4}]Br	12.58 ± 0.08	34.58 ± 0.03	55.03 ± 0.02	6.77 ± 0.15	3.22 ± 0.31	40.71 ± 0.03	61.93	
		14.94 ± 0.07	34.69 ± 0.03	63.63 ± 0.02	5.08 ± 0.20	2.67 ± 0.37	42.15 ± 0.02	71.35	
C ₆ H ₅ K ₃ O ₇ /C ₆ H ₈ O ₇ at pH 6	[N _{3,3,3,3}]Br	45.02 ± 0.02	15.84 ± 0.06	57.67 ± 0.02	2.96 ± 0.34	0.45 ± 0.01	61.21 ± 0.02	81.65	
		40.92 ± 0.03	14.94 ± 0.07	52.69 ± 0.02	4.39 ± 0.23	2.85 ± 0.35	49.08 ± 0.02	66.94	
	[N _{4,4,4,4}]Br	15.00 ± 0.07	26.57 ± 0.04	61.79 ± 0.02	4.41 ± 0.23	5.49 ± 0.18	31.07 ± 0.03	62.29	
		14.97 ± 0.07	29.81 ± 0.03	63.80 ± 0.02	4.13 ± 0.24	2.79 ± 0.36	36.22 ± 0.03	68.93	
	[C ₄ mpy]Cl	42.93 ± 0.02	17.07 ± 0.06	49.75 ± 0.02	8.89 ± 0.11	5.10 ± 0.20	62.52 ± 0.02	66.79	
		[C ₄ mim]Cl	22.05 ± 0.05	39.09 ± 0.03	39.26 ± 0.03	19.43 ± 0.05	12.64 ± 0.08	49.82 ± 0.02	40.40
C ₆ H ₅ K ₃ O ₇ /C ₆ H ₈ O ₇ at pH 7	[C ₄ mpy]Cl	39.74 ± 0.03	26.58 ± 0.04	62.15 ± 0.02	3.56 ± 0.28	2.38 ± 0.42	64.97 ± 0.02	85.69	
	[C ₄ mim]Cl	37.00 ± 0.03	24.40 ± 0.04	52.90 ± 0.02	7.54 ± 0.13	5.33 ± 0.19	57.97 ± 0.02	69.32	
	[P _{4,4,4,1}][CH ₃ SO ₄]	15.18 ± 0.07	24.60 ± 0.04	57.22 ± 0.02	0.06 ± 0.01	4.46 ± 0.22	30.86 ± 0.03	61.09	
		40.22 ± 0.03	24.40 ± 0.04	76.47 ± 0.02	7.31 ± 0.14	0.00 ± 0.01	43.35 ± 0.02	84.54	
		40.13 ± 0.03	25.15 ± 0.04	78.36 ± 0.02	7.18 ± 0.14	0.00 ± 0.01	44.01 ± 0.02	86.58	
	[P _{4,4,4,4}]Cl	24.12 ± 0.04	19.09 ± 0.05	42.61 ± 0.02	7.03 ± 0.14	6.69 ± 0.15	30.47 ± 0.03	42.89	
	[P _{4,4,4,4}]Br	16.30 ± 0.06	16.05 ± 0.06	55.70 ± 0.02	4.08 ± 0.24	10.72 ± 0.09	17.74 ± 0.06	47.01	
	[Ch][Sal]	40.36 ± 0.03	24.26 ± 0.04	58.35 ± 0.02	12.62 ± 0.08	8.54 ± 0.12	44.84 ± 0.02	59.33	
	[BCh]Cl	39.67 ± 0.03	24.59 ± 0.04	56.30 ± 0.02	8.77 ± 0.11	10.40 ± 0.10	52.45 ± 0.02	63.36	
	C ₆ H ₅ K ₃ O ₇ /C ₆ H ₈ O ₇ at pH 8	[N _{3,3,3,3}]Br	44.76 ± 0.02	16.07 ± 0.06	62.40 ± 0.02	1.84 ± 0.54	0.70 ± 0.01	51.63 ± 0.02	79.29
			41.02 ± 0.03	14.82 ± 0.07	54.59 ± 0.02	3.44 ± 0.29	1.32 ± 0.76	48.10 ± 0.02	69.52
24.88 ± 0.04			25.12 ± 0.04	69.38 ± 0.02	3.08 ± 0.32	1.23 ± 0.82	36.83 ± 0.03	76.05	
[N _{4,4,4,4}]Br		20.00 ± 0.05	21.26 ± 0.05	61.74 ± 0.02	3.90 ± 0.26	5.38 ± 0.19	27.34 ± 0.04	61.04	
		40.08 ± 0.03	17.46 ± 0.06	50.83 ± 0.02	7.36 ± 0.14	8.19 ± 0.12	47.40 ± 0.02	58.50	
		43.01 ± 0.02	19.15 ± 0.05	57.42 ± 0.02	4.71 ± 0.21	2.80 ± 0.36	59.44 ± 0.02	77.32	
[C ₄ mim]Cl	39.43 ± 0.03	20.51 ± 0.05	52.25 ± 0.02	7.94 ± 0.13	7.67 ± 0.13	51.67 ± 0.02	62.45		
KH ₂ PO ₄ /K ₂ HPO ₄ at pH 7	[N _{3,3,3,3}]Br	27.98 ± 0.04	12.11 ± 0.08	35.59 ± 0.03	4.59 ± 0.22	1.79 ± 0.56	38.02 ± 0.03	47.54	
		25.02 ± 0.04	12.85 ± 0.08	39.51 ± 0.03	3.60 ± 0.28	2.76 ± 0.36	27.06 ± 0.04	43.60	
	[N _{4,4,4,4}]Br	29.99 ± 0.03	15.39 ± 0.07	53.09 ± 0.02	1.45 ± 0.69	0.78 ± 0.01	33.01 ± 0.03	61.10	
		15.00 ± 0.07	30.00 ± 0.03	31.88 ± 0.03	7.21 ± 0.14	2.65 ± 0.38	46.68 ± 0.02	49.12	
	[N _{2,2,2,2}]Cl	15.00 ± 0.07	26.96 ± 0.04	27.02 ± 0.04	10.43 ± 0.10	4.30 ± 0.23	41.68 ± 0.02	38.63	
		28.09 ± 0.04	16.85 ± 0.06	40.47 ± 0.03	2.02 ± 0.49	0.04 ± 0.01	50.43 ± 0.02	63.07	
	[N _{3,3,3,3}]Cl	40.44 ± 0.03	17.85 ± 0.06	58.53 ± 0.02	0.32 ± 0.01	0.00 ± 0.01	57.06 ± 0.02	81.52	
		29.88 ± 0.03	26.05 ± 0.04	58.24 ± 0.02	0.33 ± 0.01	0.00 ± 0.01	53.14 ± 0.02	78.61	
	[P _{4,4,4,1}][CH ₃ SO ₄]	22.30 ± 0.05	15.01 ± 0.07	37.52 ± 0.03	5.21 ± 0.19	3.30 ± 0.30	27.25 ± 0.04	40.70	
	[P _{4,4,4,1}][Tos]	23.22 ± 0.04	12.07 ± 0.08	37.49 ± 0.03	7.03 ± 0.14	6.10 ± 0.16	18.13 ± 0.06	33.3	
	[P _{4,4,4,4}]Cl	18.09 ± 0.06	18.08 ± 0.06	32.52 ± 0.03	6.12 ± 0.16	3.50 ± 0.29	30.17 ± 0.03	37.68	
	[P _{4,4,4,4}]Br	20.00 ± 0.05	11.03 ± 0.09	33.52 ± 0.03	5.36 ± 0.19	10.79 ± 0.09	14.88 ± 0.07	24.64	
	[Ch][Sal]	39.56 ± 0.03	18.16 ± 0.06	61.29 ± 0.02	3.33 ± 0.30	0.46 ± 0.01	44.84 ± 0.02	73.65	
	[BCh]Cl	34.86 ± 0.03	18.06 ± 0.06	55.18 ± 0.02	4.68 ± 0.21	1.34 ± 0.75	40.13 ± 0.03	64.46	
40.40 ± 0.03		17.83 ± 0.03	63.77 ± 0.02	1.41 ± 0.71	0.19 ± 0.01	46.07 ± 0.02	77.70		
		35.03 ± 0.03	18.02 ± 0.06	57.07 ± 0.02	2.55 ± 0.39	0.56 ± 0.01	42.21 ± 0.02	69.04	
K ₂ CO ₃ at pH ~13	[N _{3,3,3,3}]Br	29.86 ± 0.03	9.49 ± 0.11	39.69 ± 0.03	3.86 ± 0.26	2.26 ± 0.44	25.29 ± 0.04	43.13	
		19.78 ± 0.05	12.43 ± 0.08	46.81 ± 0.02	2.90 ± 0.34	3.23 ± 0.31	18.26 ± 0.06	46.21	
	[N _{4,4,4,4}]Br	25.11 ± 0.04	13.94 ± 0.07	61.62 ± 0.02	1.51 ± 0.66	0.97 ± 0.01	22.17 ± 0.05	64.07	
		39.93 ± 0.03	17.98 ± 0.06	57.62 ± 0.02	1.53 ± 0.65	0.03 ± 0.01	55.08 ± 0.02	78.64	
	[N _{2,2,2,2}]Cl	30.13 ± 0.03	25.91 ± 0.04	57.03 ± 0.02	1.61 ± 0.62	0.06 ± 0.01	53.07 ± 0.02	76.78	
		27.96 ± 0.04	16.97 ± 0.06	46.95 ± 0.02	2.46 ± 0.41	0.17 ± 0.01	38.21 ± 0.03	58.87	
	[N _{3,3,3,3}]Cl	20.12 ± 0.05	16.98 ± 0.06	35.94 ± 0.03	5.18 ± 0.19	1.32 ± 0.75	30.99 ± 0.03	43.18	
		23.00 ± 0.04	16.84 ± 0.06	55.46 ± 0.02	1.55 ± 0.65	0.85 ± 0.01	27.28 ± 0.04	60.37	
	[N _{4,4,4,4}]Cl	20.13 ± 0.05	15.08 ± 0.07	45.68 ± 0.02	2.81 ± 0.36	2.32 ± 0.43	23.63 ± 0.04	48.10	
		18.06 ± 0.06	14.05 ± 0.07	52.00 ± 0.02	2.40 ± 0.42	3.06 ± 0.33	19.20 ± 0.05	51.75	
	[P _{4,4,4,1}][CH ₃ SO ₄]	17.22 ± 0.06	13.02 ± 0.08	47.04 ± 0.02	3.14 ± 0.32	5.95 ± 0.17	16.75 ± 0.06	43.29	
	[P _{4,4,4,1}][Tos]	19.88 ± 0.05	11.03 ± 0.09	55.27 ± 0.02	3.35 ± 0.30	1.62 ± 0.62	15.00 ± 0.07	54.90	
	[P _{4,4,4,4}]Cl	22.20 ± 0.05	11.14 ± 0.09	34.72 ± 0.03	5.46 ± 0.18	4.63 ± 0.22	19.11 ± 0.05	33.04	
	[P _{4,4,4,4}]Br	17.05 ± 0.06	9.56 ± 0.10	40.62 ± 0.03	4.09 ± 0.24	11.52 ± 0.09	10.84 ± 0.09	29.87	
	[Ch][Sal]	39.92 ± 0.03	18.09 ± 0.06	70.74 ± 0.02	4.44 ± 0.23	3.46 ± 0.29	34.25 ± 0.03	73.59	
	[BCh]Cl	34.54 ± 0.03	17.92 ± 0.06	56.42 ± 0.02	7.35 ± 0.14	4.86 ± 0.21	32.26 ± 0.03	57.26	
		39.65 ± 0.03	18.14 ± 0.06	67.80 ± 0.02	3.31 ± 0.30	1.56 ± 0.64	38.20 ± 0.03	74.87	
		34.92 ± 0.03	19.03 ± 0.05	62.65 ± 0.02	4.13 ± 0.24	2.12 ± 0.47	36.64 ± 0.03	68.71	

[IL + (K₂HPO₄/KH₂PO₄ or C₆H₅K₃O₇/C₆H₈O₇ or K₂CO₃) + water] chosen at the biphasic region were gravimetrically prepared with an uncertainty of $\pm 10^{-4}$ g and vigorously agitated. After stirring, the system was allowed to equilibrate for at least 12 h at $T = (298 \pm 1)$ K, using small ampoules (10 cm³) especially designed for this purpose, aiming at the complete separation of the coexisting

phases. At this point, both phases were carefully separated and individually weighed. At the conditions used in this work, the system presents the IL-rich phase as the top layer while the salt-rich phase is the bottom layer.

Each TL was determined by the lever-arm rule [60] through the relationship between the top phase and the total system

composition. Moreover, for each TL calculation, the following system of four equations (2)–(5), (being equations (2) and (3) the mass balance for each one of the main components, specifically IL and salt) and four unknown parameters (Y_T , Y_B , X_T , X_B) was applied:

$$Y_T = A \exp[(BX_T^{0.5}) - (CX_T^3)], \quad (2)$$

$$Y_B = A \exp[(BX_B^{0.5}) - (CX_B^3)], \quad (3)$$

$$Y_T = \frac{Y_M}{\alpha} - \frac{1 - \alpha}{\alpha} \times Y_B, \quad (4)$$

$$X_T = \frac{X_M}{\alpha} - \frac{1 - \alpha}{\alpha} \times X_B, \quad (5)$$

where the subscripts M , T and B designate, respectively, the initial mixture, the top and bottom phases. The value α is the ratio between the mass of the top phase and the total mass of the mixture experimentally determined. Finally, the tie-line length (TLL) was determined through the application of the following equation:

$$TLL = \left((X_T - X_B)^2 - (Y_T - Y_B)^2 \right)^{1/2}. \quad (6)$$

The pH of the buffer solutions was measured at $T = (298 \pm 1)$ K using a Mettler Toledo S47 SevenMulti™ dual meter pH equipment with an uncertainty of ± 0.02 .

3. Results and discussion

The present study addresses the capacity of different ammonium-, phosphonium-, cholinium-, imidazolium- and pyridinium-based ILs for ABS formation with distinct salts, namely K_2CO_3 , K_2HPO_4/KH_2PO_4 and $C_6H_5K_3O_7/C_6H_8O_7$. All phase diagrams were determined at $T = (298 \pm 1)$ K and atmospheric pressure. The experimental weight fraction data for all the studied systems are provided in the Supporting information, tables S2 to S41. The solubility curve for the system $[C_4mim]Cl + C_6H_5K_3O_7/C_6H_8O_7$ at pH 7 + H_2O previously reported by Passos *et al.* [54] is in close agreement with our results as shown in the Supporting Information, figure S2. The experimental solubility curves for all systems were correlated using equation (1). The regression parameters are presented in table 2 and were used in the determination of the TLs. All TLs obtained (at least one for each system), and the corresponding TLLs were calculated by the gravimetric method described above (table 3). As an example, the binodal curve (in mass fraction units) and the respective graphical representation of the TLs is depicted in figure 2, for the system $[N_{3,3,3,3}]Br + C_6H_5K_3O_7/C_6H_8O_7$ at pH 8. Considering the example represented in figure 2, it is possible to conclude that, because the TLL represents the difference between the IL and salt concentrations in both phases, the longer the TLL, the higher is the IL concentration in the top phase and the salt concentration at the bottom phase. This information is important when these ABS were applied in the extraction or purification of any biomolecule, since it was shown that the top and bottom phase compositions may have an important influence in the migration of the target compounds [11,29,49].

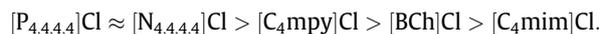
3.1. pH effect

Although the impact of a wide variety of salts on the formation of IL-ABS has been widely studied and discussed [21], little attention has been devoted to the pH influence, which can be of crucial importance when the extraction of diverse biomolecules is the main objective. In fact, the use of distinct salts generates different pH values at the coexisting phases. In general, the literature reports that the formation of ABS is more easily achieved under alkaline

conditions than under acidic or neutral conditions [21,28]. Zafarani-Moattar *et al.* [48] studied the pH effect on the ABS formation of systems composed of imidazolium-based ILs and $C_6H_5K_3O_7/C_6H_8O_7$ solutions (pH 5, 6, 7 and 8) [48] showing that a decrease in the pH value leads to a decrease in the phase-separation ability. In this work, to evaluate the pH effect on the ABS formation, different $C_6H_5K_3O_7/C_6H_8O_7$ buffers (pH 5, 6, 7 and 8) were prepared. The capacity of two quaternary ammonium ($[N_{3,3,3,3}]Br$ and $[N_{4,4,4,4}]Br$), one imidazolium- ($[C_4mim]Cl$) and one pyridinium-IL ($[C_4mpy]Cl$) to induce ABS formation was studied, being the system $[N_{4,4,4,4}]Br + C_6H_5K_3O_7/C_6H_8O_7$ at various pH values + water depicted in figure 3, as a representative example of the pH effect. The phase diagrams composed of $[N_{3,3,3,3}]Br$, $[C_4mim]Cl$ and $[C_4mpy]Cl$ are plotted in figures S1 to S3 at Supporting information. Note that the ternary systems composed of $[C_4mim]Cl$ or $[C_4mpy]Cl + C_6H_5K_3O_7/C_6H_8O_7$ at pH 5 + water were not measured due to salt precipitation. In general, the phase-separation ability increases with the pH, which is in close agreement with recent literature [48]. It is important to note that the pH effect is more pronounced for the quaternary ammonium compounds than for the imidazolium- and pyridinium- based ILs studied. The different behavior in the ABS formation is in this case correlated with the different surface charges or degrees of protonation [48] considered in the different buffer structures, as previously described [53]. In accordance with the speciation curve of the salt species (figure S16 in the Supporting Information file), it is clear that in the pH range studied, the charge of the salt species changes and with it, the type and strength of the interactions.

3.2. Cation core effect

Five families of ILs were investigated, two with aliphatic cations ($[P_{4,4,4,4}]$ and $[N_{4,4,4,4}]$), two with aromatic cations ($[C_4mim]$ and $[C_4mpy]$) and one cholinium based-IL with a benzene ring ($[BCh]Cl$). According to figure 4, the ability of phase formation for the aforementioned ionic compounds using the $C_6H_5K_3O_7/C_6H_8O_7$ at pH 7 is the following:



This trend is in good agreement with those previously reported in the literature with K_3PO_4 [25], K_2HPO_4 [25], K_2CO_3 [25], KOH [25], K_2HPO_4/KH_2PO_4 [34] and $C_6H_5K_3O_7/C_6H_8O_7$ [47], as reviewed by Freire *et al.* [21]. The higher ABS formation ability of the two quaternary salts ($[P_{4,4,4,4}]Cl$ and $[N_{4,4,4,4}]Cl$) may be explained by the fact that they have highly shielded charges, located on the heteroatom surrounded by four butyl alkyl chains, which is facilitating the two phase formation [25]. The smaller and hydroxyl containing chains of the cholinium enhance its water solvation reducing its ability to induce ABS formation. Finally, larger cations such as pyridinium (containing a 6-atom ring) are more able to induce the ABS formation than the imidazolium (containing a 5-atom ring). The comparison of the ABS here reported using K_2HPO_4/KH_2PO_4 at pH 7 with literature results [34] is reported in Supporting information, figure S4. The ABS formation trend for this salt ($[N_{4,4,4,4}]Cl \approx [P_{4,4,4,4}]Cl > [C_4mpy]Cl > [C_4mpip]Cl > [C_4mim]Cl \approx [BCh]Cl \approx [C_4mpyr]Cl$) is in close agreement with the results obtained with $C_6H_5K_3O_7/C_6H_8O_7$ at the same pH value.

3.3. Alkyl side chain length and anion effect

Although several authors have addressed the influence of the IL alkyl chain length on the ABS formation, most of these experimental works deal mainly with the imidazolium family [10,32–35,38,61,62]. Meanwhile, this work reports the evaluation of the capacity of different ammonium compounds (with distinct alkyl

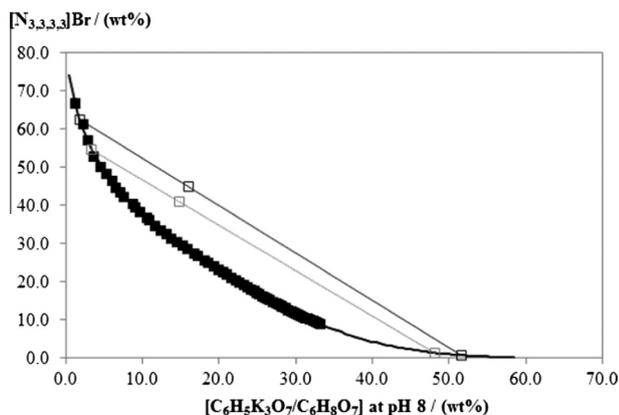


FIGURE 2. Phase diagram for $[N_{3,3,3,3}]\text{Br}$ and $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 8 and $T = (298 \pm 1) \text{ K}$: binodal curve data (■), TL data (□).

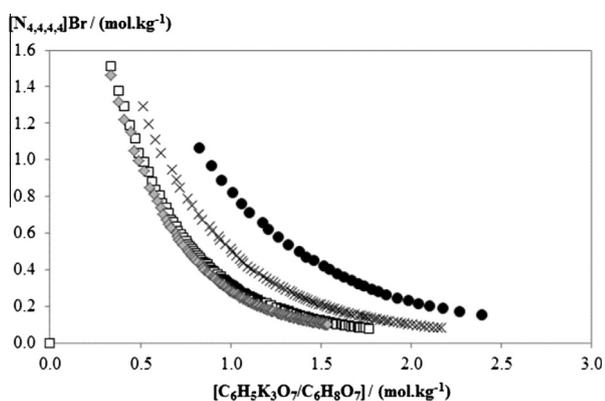


FIGURE 3. Phase diagrams for the system $[N_{4,4,4,4}]\text{Br} + \text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ (pH 5, 6, 7 and 8) + H_2O at $T = (298 \pm 1) \text{ K}$, atmospheric pressure and different pH values: pH 5 (●), pH 6 (×), pH 7 (□) [53], pH 8 (◆).

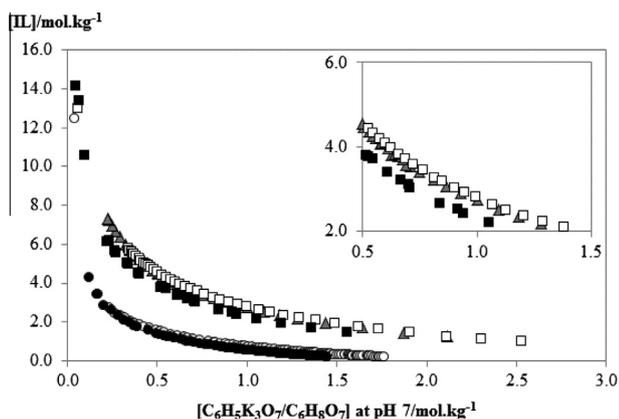


FIGURE 4. Phase diagrams for the chloride-based ILs + $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH 7 + H_2O systems at $T = (298 \pm 1) \text{ K}$ and atmospheric pressure: $[\text{C}_4\text{mim}]\text{Cl}$ (□), $[\text{Bch}]\text{Cl}$ (▲), $[\text{C}_4\text{mpy}]\text{Cl}$ (■), $[\text{N}_{4,4,4,4}]\text{Cl}$ (○) [53], $[\text{P}_{4,4,4,4}]\text{Cl}$ (●).

chain lengths) on the formation of ABS, conjugated with the K_2CO_3 salt (pH ~ 13), the results being plotted in figure 5, with the $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ (pH 5, 6, 7 [53] and 8) and $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH 7) systems depicted in figures S5 to S8 at Supporting information. All systems studied show that the longer the alkyl side chain, the greater is its hydrophobicity, and thus the ability of the quaternary ammonium to promote the phase separation. This phenomenon

and the correlation between the solubility in water [63,64] of different ILs and their hydrophobic character was already largely addressed [10,34,38,53,61,62] and reviewed by Freire *et al.* [21].

Few authors have studied the influence of the IL anion nature and type on the formation of ABS [24,36,38,65]. In this work, the effect of two halogenate anions was studied and is reported in figure 5(A)–(C). The results suggest that these two halogenate anions, Cl^- and Br^- , have different capacities to promote ABS formation in presence of K_2CO_3 (pH ~ 13) and $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ at pH 7 (shown in figure S8 at Supporting Information). The binodal data shows that, in good agreement with literature [24,47,53,66], the bromide salts present a higher capability to form ABS. Although the anions effect is more pronounced with $[\text{N}_{2,2,2,2}]$ cation, neither the cation alkyl chain length nor the salt used affects the sequence on the anion ability to promote ABS formation.

3.4. Salt effect

The effect of different salts (K_2CO_3 , $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ and $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$) on the ABS formation was evaluated using different IL families, namely four phosphonium, five ammonium and two cholinium compounds. The results are reported in figure 6 and figures S9 to S15 from Supporting Information.

Figure 6 shows the ability of the different salts here studied to induce the formation of a second aqueous phase in presence of four different ionic compounds, namely $[\text{P}_{4,4,4,4}]\text{Br}$ (figure 6(A)), $[\text{N}_{4,4,4,4}]\text{Br}$ (figure 6(B)), $[\text{N}_{3,3,3,3}]\text{Cl}$ (figure 6(C)) and $[\text{Ch}][\text{Sal}]$ (figure 6(D)). An analysis of figures 6(A) and (B) shows that the capacity of the different salts, in general follows the tendency described by: $\text{C}_6\text{H}_5\text{K}_3\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at pH7 > K_2CO_3 at pH ~ 13 > $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ at pH7. This tendency is similar when the phosphonium-based ILs are considered (figures S9 to S11 from Supporting information file) and is in close agreement with the behavior of $[\text{N}_{2,2,2,2}]\text{Br}$ discussed by Silva *et al.* [53] and others [39]. Trying to understand this behavior, the speciation of each buffer used (figures S16 and S17) and the capacity of the salt anion to interact with water [39], were considered. In fact, anions with higher valence have a higher capacity to interact with water, more easily forming hydration complexes and, consequently, inducing an easier ABS formation [39]. Surprisingly this tendency is not observed for all the ILs studied in this work, as shown in figure 6(C) and (D), representing the ILs $[\text{N}_{3,3,3,3}]\text{Cl}$ and $[\text{Ch}][\text{Sal}]$, respectively. The differences in the ability of the salts to promote the ABS formation correlate primarily with their aptitudes for being hydrated by the water molecules, and thus with the molar entropy of hydration of the ions [21]. However, in some particular situations for weakly hydrated salts or strongly hydrated ILs the dominating interactions may become more complex and the simple reasoning based on the salting-out ability of the salt ions, resulting from their entropy of hydration, is no longer adequate. These systems here identified, with a more complex behavior suggesting limitations to the prevalent interpretation on ABS formation, are quite interesting for the questions they rise and that may lead towards the development of a more comprehensive model for explaining the ABS formation. But this is outside the scope of the present work.

4. Conclusions

In this work novel ABS were determined as a platform for distinct extraction processes. Taking into account the phase diagram behavior, the effect of pH, IL cation core, alkyl side chain length, IL anion nature, and distinct salts in what concerns their capacity induce ABS formation was presented and discussed. In general, the ability to form ABS increases with the pH and alkyl chain length for all systems studied. Moreover, the influence of the cation core and anion nature of the ILs on their capacity to ABS formation

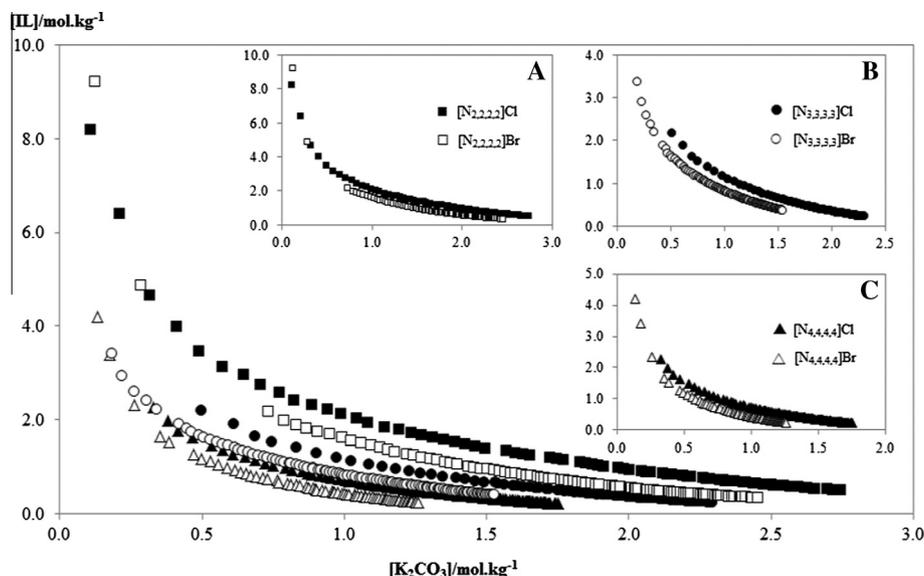


FIGURE 5. Phase diagrams for the systems $[N_{n,n,n,n}]X$ ($n = 2, 3$ and 4) + K_2CO_3 + H_2O at $T = (298 \pm 1)$ K and atmospheric pressure: $[N_{2,2,2,2}]Cl$ (■), $[N_{3,3,3,3}]Cl$ (●), $[N_{4,4,4,4}]Cl$ (▲), $[N_{2,2,2,2}]Br$ (□) [53], $[N_{3,3,3,3}]Br$ (○), $[N_{4,4,4,4}]Br$ (△). To study the anion effect, the comparison between the binodal curves obtained for the $[N_{2,2,2,2}]X$ (A), $[N_{3,3,3,3}]X$ (B) and $[N_{4,4,4,4}]X$ (C) were highlighted in figure to help the analysis.

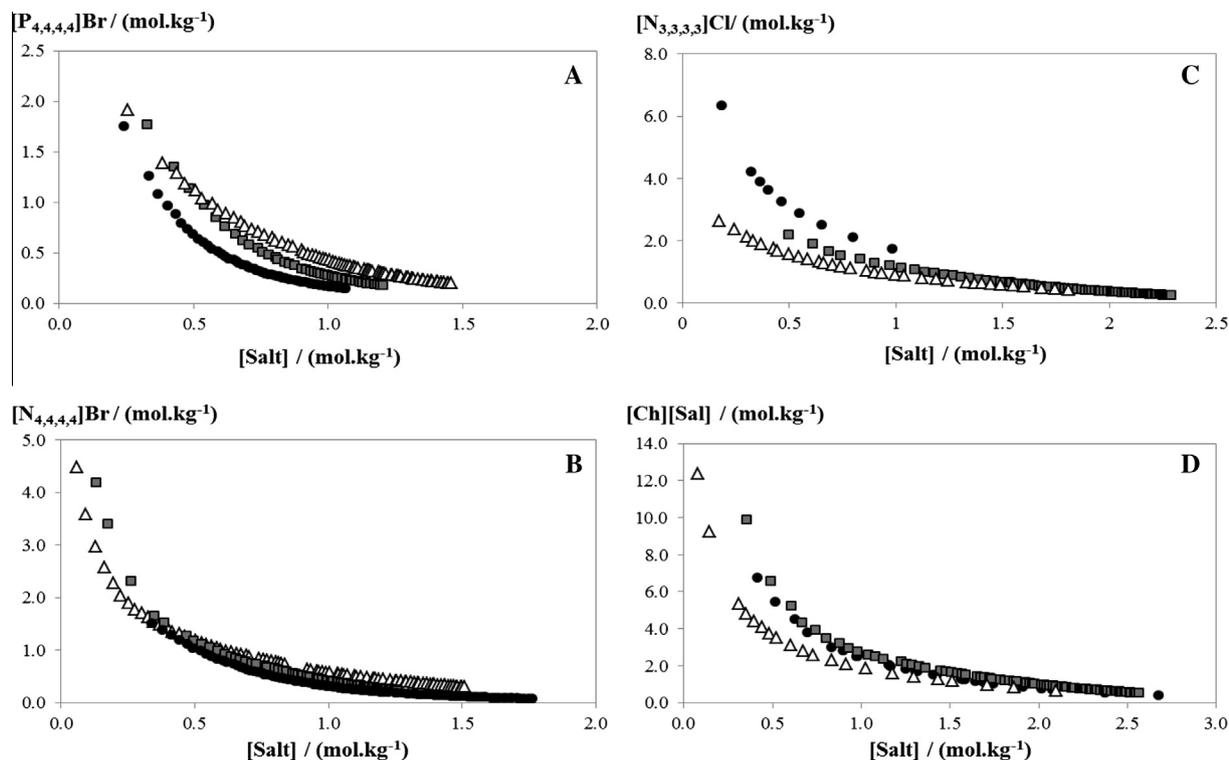


FIGURE 6. Phase diagrams for the systems (A) $[P_{4,4,4,4}]Br$ + salt + H_2O , (B) $[N_{4,4,4,4}]Br$ + salt + H_2O , (C) $[N_{3,3,3,3}]Cl$ + salt + H_2O and (D) $[Ch][Sal]$ + salt + H_2O for the salts KH_2PO_4/K_2HPO_4 at pH 7 (△), K_2CO_3 (■) and $C_6H_5K_3O_7/C_6H_8O_7$ at pH 7 (●), at $T = (298 \pm 1)$ K and atmospheric pressure.

is dominated by the IL (hydrophobic/hydrophilic) nature. Finally, and concerning the effect of different salts the results suggest that, in agreement with previous works, their ability to form ABS closely correlates with the ILs capacity to form hydration complexes with water, meaning their salting-out ability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jct.2013.10.024>.

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