# Evaluation of Cation Influence on the Formation and Extraction Capability of Ionic-Liquid-Based Aqueous Biphasic Systems

**ARTICLE** *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · MAY 2009

Impact Factor: 3.3 · DOI: 10.1021/jp900293v · Source: PubMed



**5 AUTHORS**, INCLUDING:

Sónia P M Ventura
University of Aveiro
**58** PUBLICATIONS **1,119** CITATIONS
SEE PROFILE

Mara G Freire
University of Aveiro
**174** PUBLICATIONS **5,515** CITATIONS
SEE PROFILE

Isabel Marrucho
New University of Lisbon
**250** PUBLICATIONS **6,964** CITATIONS
SEE PROFILE

Joao A. P. Coutinho
University of Aveiro
**482** PUBLICATIONS **12,290** CITATIONS
SEE PROFILE

# Evaluation of Cation Influence on the Formation and Extraction Capability of Ionic-Liquid-Based Aqueous Biphasic Systems

**Catarina M. S. S. Neves,[†] Sónia P. M. Ventura,[†] Mara G. Freire,[†,‡] Isabel M. Marrucho,[†,‡] and João A. P. Coutinho*[,†]**

*CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal, and Instituto de Tecnologia Química e Biológica, ITQB2, Universidade Nova de Lisboa, Av. República, Apartado 127, 2780-901 Oeiras, Portugal*

*Received: January 12, 2009; Revised Manuscript Received: February 10, 2009*

In addition to the large range of applications proposed in literature, ionic liquids (ILs) have been recently reported to be able to form aqueous biphasic systems (ABS). They could thus be interesting media in biotechnological applications for the separation and purification of vital biomolecules. Therefore, in this work, a systematic study involving a large number of imidazolium-based ILs was conducted to provide new information related to ILs' ABS-promoting capability and extraction ability. For that purpose, the influence of the number of alkyl groups present at the cation, the cation side alkyl chain length, and the presence of double bonds, aromatic rings, and hydroxyl groups on this alkyl chain were evaluated. Ternary phase diagrams of the ABS formed by these ILs and $K_3PO_4$ and the respective tie-lines were measured and presented. The ABS here investigated were further characterized for the first time accordingly to their extractive potential for amino acids, where L-tryptophan was selected as a model biomolecule. The partition coefficients here obtained were shown to be substantially larger than those observed in conventional ABS, demonstrating therefore the fine potential of IL-based ABS for biomolecules separation and purification.

## Introduction

The efficiency and viability of any biotechnological process depends largely on downstream processing that ensures the purity and quality of biomolecules and represents about 60−90% of the cost of the final product.[1] Many metabolites and/or bioproducts present narrow tolerance limits of pH, ionic strength, temperature, osmotic pressure, and surface charges; thus, the extraction and isolation techniques must be specific and compatible with the product.[2] Conventional techniques used for product recovery from biotechnological processes are usually expensive and present low yields.[3] There have been, therefore, considerable efforts from the industrial and academic communities for the development of cost-effective separation techniques,[4] such as liquid−liquid extraction in aqueous biphasic systems (ABS). These systems are formed when two mutually incompatible, though both miscible with water, polymer/polymer, polymer/salt, or salt/salt systems are employed. Above a critical concentration of those components, spontaneous phase separation takes place, and the extraction of biomolecules can be achieved by the manipulation of their affinity for each of the aqueous-rich phases.

Water-immiscible organic solvents have long been commonly used in industrial applications. Nevertheless, environmental concerns about the use of volatile organic compounds (VOCs) has increased in the past few years, and there is an emergent interest for the development of "green" solvents for separation processes.[5] In this context, ionic liquids (ILs) have appeared as possible nonhazardous candidates. Their particular characteristics, including high solvation abilities and coordination properties, general inflammability, high thermal and chemical stabilities, and negligible vapor pressures,[6,7] make them suitable candidates for a large range of industrial and biotechnological applications. Moreover, the possibility of controlling their inherent physicochemical properties by a wise combination of the cation and/or anion makes possible the manipulation of the extraction phase properties for enhanced yield of product recovery. In addition, IL-based ABS offer the opportunity to combine the purification process of active biocatalysts with the improved performance of some enzymes in the presence of ionic media.[8]

The first suggestion that ILs could be used to prepare ABS was reported by Gutowski et al.[9] The potential advantages of factual IL-based ABS have motivated previous studies on the interactions between water and ILs and between ILs and salts in aqueous solutions, with the goal of achieving a deeper understanding of the molecular phenomena governing the IL-based ABS scenario.[10−13] Despite the scattered results that have been reported concerning IL-based ABS, there are still many gaps in the characterization of ILs, and a general picture of the situation that would allow the finest selection of an IL is still lacking. Research regarding the use of IL-based ABS has been so far mostly centered on the influence of several inorganic salts on the phase diagrams[4,5,9,14,15] (where the ion's influence follows the well-known Hofmeister series)[13] or in the use of carbohydrates for IL-based ABS formation.[16−19] Surprisingly, one of the most interesting practical issues, evaluation of the extraction ability of those IL-based ABS, was seldom studied; only testosterone, epitestosterone, penicillin G, and opium alkaloids were previously used as partitioning solutes.[20−22] No reports concerning the extraction of amino acids or even proteins using IL-based ABS were found in literature.

In this work we evaluate the influence of cations on promoting IL-based ABS, maintaining the same inorganic salt ($K_3PO_4$).

* To whom correspondence should be addressed. Tel.: +351 234 401 507. Fax: +351 234 370 084. E-mail: jcoutinho@ua.pt.
† Universidade de Aveiro.
‡ Universidade Nova de Lisboa.

10.1021/jp900293v CCC: $40.75 
Published on Web 03/23/2009

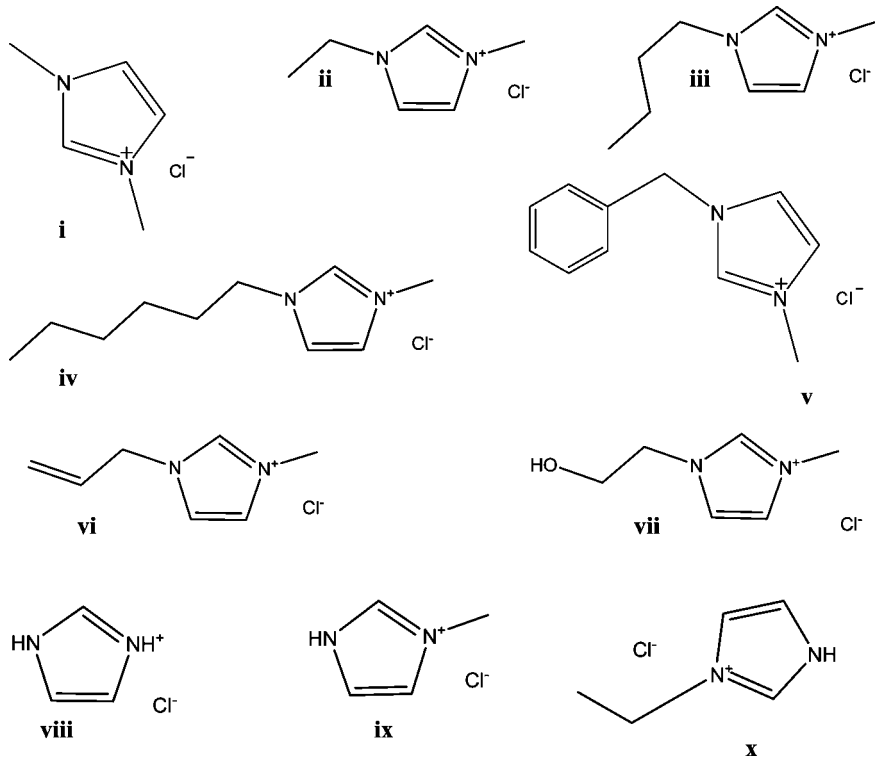


**Figure 1.** Chemical structure of the ILs studied: (i) [$C_1$mim]Cl; (ii) [$C_2$mim]Cl; (iii) [$C_4$mim]Cl; (iv) [$C_6$mim]Cl; (v) [$C_7H_7$mim]Cl; (vi) [amim]Cl; (vii) [$OHC_2$mim]Cl; (viii) [im]Cl; (ix) [$C_1$im]Cl; (x) [$C_2$im]Cl.

Ternary phase diagrams (binodal curves and tie-lines) for systems of hydrophilic ILs + $K_3PO_4$ + water, at 298 K and atmospheric pressure, were determined. The binodal curves were fit to a three-parameter equation, and the tie-lines were estimated using the Merchuck et al. mathematical approach.[23] The main objective of this study was to establish the impact of different characteristics of the cation's structure on promoting ABS, such as the alkyl side chain length, the number of substituents at the cation, and the presence of double bonds, benzyl groups, or hydroxyl groups. The ILs selected were hydrophilic imidazolium- and chloride-based ILs. It is known that the anion has a small effect on IL toxicity (although chloride-based ILs are nonfluoride candidates and therefore less toxic).[24] The IL toxicity is mainly determined by the imidazolium cation and directly correlates with the length of the side alkyl chain and/or with the hydrophobicity of the IL.[24] Thus, the studied ILs are the less toxic of the imidazolium-based family since this study focused essentially in hydrophilic and short side alkyl chain length ILs.

The revised ILs were further analyzed accordingly to their potential biomolecule extractive ability, for which L-tryptophan was selected as a model compound of biotechnological interest. Amino acids are important compounds in several biotechnological processes, and the development of methods for their separation and purification still is a focal dilemma.

## Experimental Section

**Materials.** The ABS studied in this work were established by using an aqueous solution of $K_3PO_4$, ≥98 % w/w pure from Sigma, and different aqueous solutions of hydrophilic ILs. The chloride-based ILs studied were imidazolium chloride, [im]Cl; methylimidazolium chloride, [$C_1$im]Cl; ethylimidazolium chloride, [$C_2$im]Cl; 1,3-dimethylimidazolium chloride, [$C_1$mim]Cl; 1-ethyl-3-methylimidazolium chloride, [$C_2$mim]Cl; 1-butyl-3-methylimidazolium chloride, [$C_4$mim]Cl; 1-hexyl-3-methylimidazolium chloride, [$C_6$mim]Cl; 1-allyl-3-methylimidazolium chloride, [amim]Cl; 1-hydroxyethyl-3-methylimidazolium chloride, [$OHC_2$mim]Cl; and 1-benzyl-3-methylimidazolium chloride, [$C_7H_7$mim]Cl. The molecular structures of the ILs are described in Figure 1. All ILs used in this work were acquired at Iolitec with the exception of [$C_1$mim]Cl, which was synthesized in our laboratory (see Supporting Information). To reduce the water and volatile compound content to negligible values, individual samples of the ILs were dried under constant stirring at moderate vacuum and temperature (~353 K) for a minimum of 48 h. After this procedure, the purity of each ionic liquid was further checked by $^1$H and $^{13}$C NMR spectra and found to be >99.0 % w/w for all samples. The water used was ultrapure, double distilled water, passed through a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus. The L-tryptophan with a purity >99.0 % w/w was from Fluka.

## Experimental Procedure

**Phase Diagrams and Tie-Lines.** The phase diagram binodals were determined through the cloud point titration method[25,26] at 298 ± 1 K. The experimental procedure adopted was validated with the phase diagram obtained for the [$C_4$mim]Cl + water + $K_3PO_4$ ternary system against literature data.[27] Aqueous solutions of $K_3PO_4$ at 40 % w/w and aqueous solutions of the different hydrophilic ILs at variable concentrations were prepared and used for the phase diagram binodal determinations. Repetitive dropwise addition of the aqueous inorganic salt solution to the aqueous solution of IL was carried out until detection of a cloudy solution, followed by the dropwise addition of ultrapure water until detection of a monophasic region (clear and limpid solution). Dropwise additions were carried out under constant stirring. The ternary system compositions were determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g.

The tie-lines (TLs) were determined by a gravimetric method described by Merchuck et al.[23] For the TL determinations a mixture at the biphasic region was prepared, vigorously stirred and allowed to reach equilibrium by the separation of both phases for 12 h at 298 K using small ampoules (ca. 10 mL) especially designed for the purpose. After the separation step, both top and bottom phases were weighed. Each individual TL was determined by application of the lever rule to the relationship between the top mass phase composition and the overall system composition.[23] For that purpose the experimental binodal curves were correlated using eq 1:[23]

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

where $Y$ and $X$ are, respectively, the IL and salt weight percentages, and $A$, $B$, and $C$ are constants obtained by the regression.

**Partitioning of L-Tryptophan.** The partition coefficients of L-tryptophan, $K_{Trp}$, are defined as the ratio of the concentration of L-tryptophan in the IL and in the $K_3PO_4$ aqueous-rich phases, described by eq 2:

$$K_{Trp} = \frac{[Trp]_{IL}}{[Trp]_{K_3PO_4}} \quad (2)$$

where $[Trp]_{IL}$ and $[Trp]_{K_3PO_4}$ are the concentration of L-tryptophan in the IL and in the $K_3PO_4$ aqueous-rich phases, respectively.

A mixture in the biphasic region was selected and used to evaluate the L-tryptophan partitioning at 298 K (the mixture composition is described in Table 3 for each system). For this purpose aqueous solutions of L-tryptophan with a concentration of approximately 0.78 g $dm^{-3}$ were used. The biphasic solution was left to equilibrate for 12 h (a time period established in previous optimizing experiments) to achieve a complete L-tryptophan partitioning between the two phases. The amino acid quantification, in both phases, was carried out by UV spectroscopy using a SHIMADZU UV-1700, Pharma-Spec spectrometer, at a wavelength of 279 nm and using a calibration curve previously established. At least three samples of each individual aqueous-rich phase were quantified. Moreover, both phases were weighed, and the corresponding TLs were obtained as previously described.

## Results and Discussion

**Phase Diagrams and Tie-Lines.** Because ILs are ionic by nature, IL-based ABS are more complex than typical poly(ethylene glycol) (PEG)-based ABS as a result of the possibility of ion exchange and/or ion-pairing between both salt phases. Bridges et al.[14] have shown that there is ion partition between both phases, yet electroneutrality is maintained. However, the overall deviations observed are small enough to be considered as a source of error in the cloud point titration (as determined by radioanalytical results), thus yielding a satisfactory representation of the ion concentration present at any TL.[14]

The experimental phase diagrams at 298 K and at atmospheric pressure for each IL + $K_3PO_4$ + $H_2O$ system are presented in Figures 2 and 3 in molality units for a detailed understanding of the impact of the ILs on ABS formation (cf. Supporting Information for experimental weight fraction data). Figure 2 presents the binodal curve for the ILs [amim]Cl, [$OHC_2$mim]Cl, [$C_7H_7$mim]Cl, [$C_6$mim]Cl, [$C_4$mim]Cl, [$C_2$mim]Cl, and [$C_1$mim]Cl, and Figure 3 shows the binodal curve data for [$C_2$im]Cl, [$C_1$im]Cl, and [im]Cl.

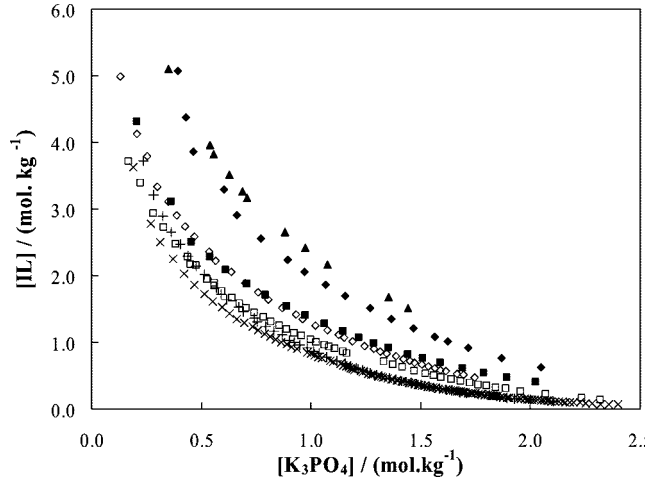


**Figure 2.** Phase diagram for the disubstituted imidazolium-based ternary systems composed by IL + $K_3PO_4$ + $H_2O$ at 298 K: (◆) [$C_1$mim]Cl; (■) [$C_2$mim]Cl; (□) [$C_4$mim]Cl; (×) [$C_6$mim]Cl; (+) [$C_7H_7$mim]Cl; (◇) [amim]Cl; (▲) [$OHC_2$mim]Cl.

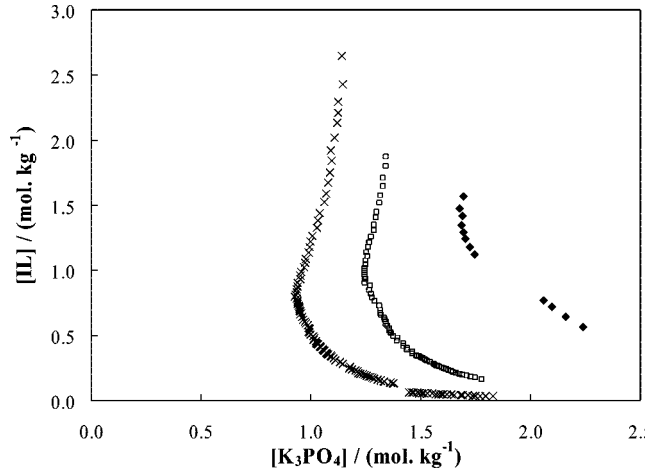


**Figure 3.** Phase diagram for the un- and monosubstituted imidazolium-based ternary systems composed by IL + $K_3PO_4$ + $H_2O$ at 298 K: (◆) [im]Cl; (□) [$C_1$im]Cl; (×) [$C_2$im]Cl.

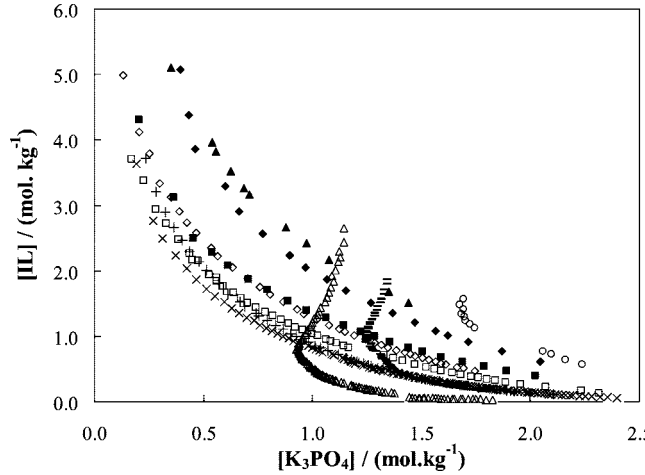


**Figure 4.** Phase diagram for all the studied imidazolium-based ternary systems composed by IL + $K_3PO_4$ + $H_2O$ at 298 K: (○) [im]Cl; (—) [$C_1$im]Cl; (Δ) [$C_2$im]Cl; (◆) [$C_1$mim]Cl; (■) [$C_2$mim]Cl; (□) [$C_4$mim]Cl; (×) [$C_6$mim]Cl; (+) [$C_7H_7$mim]Cl; (◇) [amim]Cl; (▲) [$OHC_2$mim]Cl.

**TABLE 1: Correlation Parameters Used in Eq 1 To Describe the Binodals**

| IL + $K_3PO_4$ + water system | $A$ | $B$ | $10^5$ $C$ |
|---|---|---|---|
| [$C_1$mim]Cl | 109.9 | −0.3735 | 1.991 |
| [$C_2$mim]Cl | 78.25 | −0.3389 | 2.697 |
| [$C_4$mim]Cl | 72.64 | −0.3185 | 4.070 |
| [$C_6$mim]Cl | 84.02 | −0.3563 | 5.462 |
| [$C_7H_7$mim]Cl | 94.67 | −0.3545 | 6.128 |
| [amim]Cl | 72.00 | −0.2924 | 4.088 |
| [$OHC_2$mim]Cl | 103.7 | −0.3067 | 1.004 |

**TABLE 2: Experimental Data for TLs and Respective TLLs**

| | wt fraction composition (%) | | TL eq: IL (wt %) = $a$ (wt %) + $b$$K_3PO_4$ (wt %) | | |
|---|---|---|---|---|---|
| IL | IL | $K_3PO_4$ | $a$ | $b$ | TLL |
| [$C_1$mim]Cl | 25.20 | 16.55 | 47.48 | −1.347 | 31.75 |
| [$C_2$mim]Cl | 26.30 | 14.93 | 42.79 | −1.104 | 47.03 |
| | 24.88 | 20.85 | 49.83 | −1.196 | 59.46 |
| [$C_4$mim]Cl | 15.75 | 22.67 | 42.27 | −1.170 | 45.97 |
| | 17.69 | 21.55 | 42.91 | −1.171 | 47.67 |
| | 21.36 | 23.04 | 51.40 | −1.304 | 61.20 |
| [$C_6$mim]Cl | 10.78 | 26.04 | 45.65 | −1.340 | 47.79 |
| | 15.16 | 25.33 | 52.62 | −1.479 | 58.32 |
| [$C_7H_7$mim]Cl | 20.17 | 20.05 | 53.86 | −1.680 | 52.78 |
| | 17.56 | 26.09 | 60.57 | −1.649 | 66.29 |
| [amim]Cl | 26.64 | 16.60 | 47.32 | −1.246 | 53.95 |
| | 26.80 | 21.20 | 52.85 | −1.229 | 65.40 |
| [$OHC_2$mim]Cl | 41.17 | 15.36 | 86.86 | −2.976 | 66.66 |

The observation of Figure 2 indicates that the larger cation alkyl chain, the greater is the IL's ability for ABS formation. It is well-known that an increase in cation alkyl chain length leads to an increase of the IL's hydrophobic nature and therefore to a poorer affinity for water.[10] The higher the affinity for water and/or hydrophilic nature of the IL, the less effective is the IL in promoting ABS. Moreover, the presence of a terminal hydroxyl group at the alkyl chain leads to a large decrease in the ABS-promoting ability as a result of the higher hydrophilicity of the IL when compared with [$C_2$mim]Cl. Also the presence of a double bond at the allyl group of the imidazolium cation ([amim]Cl) or the presence of a benzyl group ([$C_7H_7$mim]Cl) as a substituent group decreases the ability to form ABS, although in a less pronounced way.

When a second electrolyte is added to a non-electrolyte or electrolyte aqueous solution, the solubility of the solute usually decreases as a consequence of the salting-out effect. In general, ILs with lower water affinity require less salt to promote separation of the two phases, resulting in a binodal curve closer to the axis and in a larger biphasic region. The results here obtained show that the ability of the ILs to form ABS follows the order [$C_6$mim]Cl > [$C_7H_7$mim]Cl > [$C_4$mim]Cl > [$C_2$mim]Cl ≈ [amim]Cl > [$C_1$mim]Cl > [$OHC_2$mim]Cl.

Figure 3 illustrates the experimental binodal curves obtained for the mono- and unsubstituted imidazolium chlorides. These systems reveal an "atypical" behavior for ABS involving ILs and not previously reported. The asymmetrical behavior of these aqueous biphasic systems has been previously observed for ABS of PEG and low weight polysaccharides,[28,29] and they may constitute an interesting approach for product separations. For the same $K_3PO_4$ molality, two monophasic and one biphasic regions are present, and thus the system can be moved between the various phases by simple variation of the IL concentration. Comparing the three ILs, the ABS-forming ability ranks [$C_2$im]Cl > [$C_1$im]Cl > [im]Cl. The number of alkyl substituents

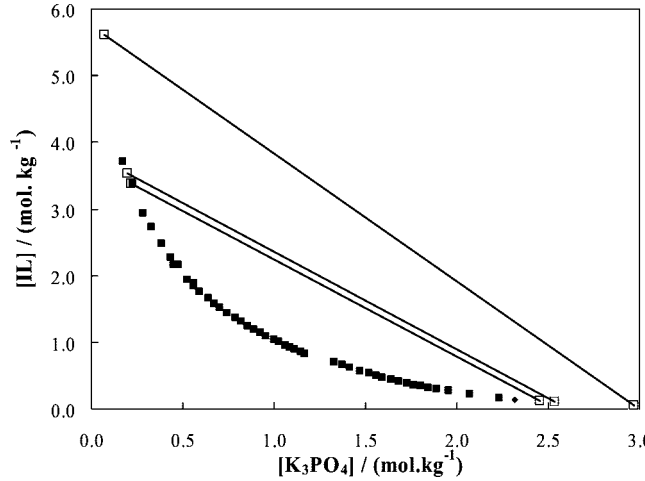


**Figure 5.** Phase diagram for the [$C_4$mim]Cl + $K_3PO_4$ + water ternary system at 298 K: (■) binodal curve data; (□) TL data.

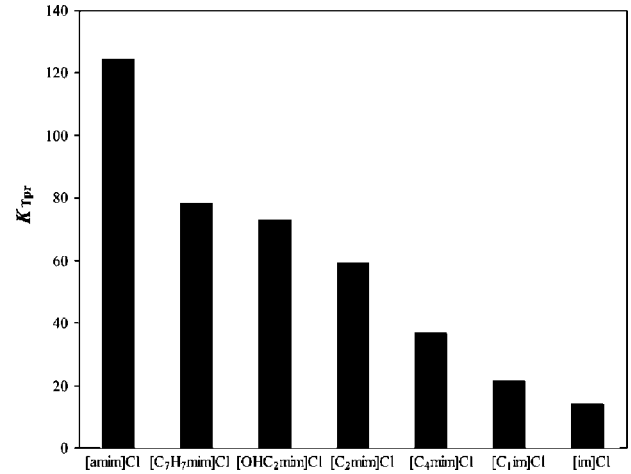


**Figure 6.** Partition coefficients of L-tryptophan between the IL- and the $K_3PO_4$-rich aqueous phases at 298 K.

and again the alkyl chain length increase lead to a higher ABS-inducing capacity. Curiously, comparing the results reported in Figures 2 and 3 and depicted in Figure 4, there are specific ranges of IL concentration where the monosubstituted imidazolium-based ILs are more effective in promoting phase separation than their disubstituted analogues.

In PEG-based ABS the salt anion has the major impact on the polymer solubility and hence in the ABS phase diagrams, while the cation has a minor (though sizable) effect.[30] For ILs, it is here shown that the IL cation has a huge influence in the phase diagrams behavior, allowing the IL-based ABS to be tailored to meet the specific requirements of a particular separation.

The experimental binodal curves were fit to the empirical relationship described by eq 1.[23] The regression parameters and the tie-line equations obtained for each ternary system, as well as the tie-line lengths (TLLs), are reported in Tables 1 and 2, respectively. Representation of TLs is shown in Figure 5 for the ternary system [$C_4$mim]Cl + $K_3PO_4$ + water as an example measured in this work. For shorter TLLs the TLs are approximately parallel, whereas for longer TLLs the tie-line slopes start to deviate. These deviations in the TLs slopes are in agreement with literature[31] and are related with the fact that the $K_3PO_4$-rich phase is increasingly free of IL at longer TLLs.

**Partitioning of L-Tryptophan in ABS.** Hydrophobic interactions play a major role in the tertiary structure of proteins and

**TABLE 3: Weight Fraction Composition and Partition Coefficients of L-Tryptophan in ILs-ABS Systems at 298 K**

| | wt fraction composition (%) | | TL eq: IL (wt %) = $a$ (wt %) + $b$$K_3PO_4$ (wt %) | | | |
|---|---|---|---|---|---|---|
| IL | IL | $K_3PO_4$ | $a$ | $b$ | TLL | $K_{Trp}$ |
| [amim]Cl | 26.91 | 15.91 | 47.20 | −1.28 | 52.53 | 124 ± 5 |
| [$OHC_2$mim]Cl | 40.57 | 16.05 | 87.17 | −2.90 | 68.38 | 73.1 ± 0.8 |
| [$C_2$mim]Cl | 25.90 | 14.90 | 42.36 | −1.10 | 45.73 | 59.2 ± 0.4 |
| [$C_4$mim]Cl | 25.35 | 15.97 | 43.46 | −1.15 | 50.03 | 36.6 ± 0.6 |
| [$C_7H_7$mim]Cl | 25.11 | 18.30 | 56.62 | −1.72 | 57.62 | 78.4 ± 0.5 |
| [$C_1$im]Cl | 15.36 | 30.53 | | | | 21.3 ± 0.3 |
| [im]Cl | 15.24 | 32.80 | | | | 14.2 ± 0.4 |

biological membranes and control the partition coefficients in ABS. The partition coefficients of biomolecules also depend on electrostatic forces, molecular size, solubility, and affinity for both phases, and their magnitudes further depend on the two-phase compositions and on the nature of the biomolecules.[32]

As shown in Figure 6, the partition coefficients of L-tryptophan ($K_{Trp}$) at 298 K in IL-based ABS are substantially higher than in the usual PEG-polysaccharide systems (with $K_{Trp} \approx 1$)[33] or PEG-inorganic salts systems (with $K_{Trp} \approx 1-7$).[34] The IL-based ABS are thus far more efficient for L-tryptophan separation than conventional ABS. Table 3 presents the composition of the ternary system employed for the L-tryptophan partition studies, as well as the corresponding TLs and TLLs. During the partitioning of L-tryptophan between the two phases there are several competing interactions between the IL, the inorganic salt, the L-tryptophan, and water: hydrogen-bonding interactions, $\pi\cdots\pi$ interactions, hydrophobic interactions, and electrostatic interactions. In general, the results indicate that $K_{Trp}$ increases with the IL cation's hydrophilic nature and thus the main interactions between the IL and the amino acid determining L-tryptophan partitioning are hydrophobic and hydrogen-bonding type interactions. For the studied ILs the L-tryptophan partition coefficient follows the rank [amim]Cl > [$C_7H_7$mim]Cl > [$OHC_2$mim]Cl > [$C_2$mim]Cl > [$C_4$mim]Cl > [$C_1$im]Cl > [im]Cl. The highest partition coefficient occurs for [amim]Cl followed by [$C_7H_7$mim]Cl and [$OHC_2$mim]Cl as a result of the presence of a double bond, an aromatic ring and a hydroxyl group of the alkyl side chains, respectively. Increasing the IL cation alkyl chain decreases the L-tryptophan partitioning in the disubstituted imidazolium-based ILs. The mono- and unsubstituted imidazolium-based ILs present lower partition coefficients for L-tryptophan, which could result from the decrease of the hydrophobic interactions between them.

Wang and co-workers[35] reported the extraction of amino acids from an aqueous phase using hydrophobic water-immiscible ILs. The results obtained in this work show that the magnitudes of L-tryptophan partition coefficients using IL-based ABS can be as much as 1000 times larger compared to the water-immiscible ILs results. Indeed our results show that nearly quantitative extraction of L-tryptophan can be achieved in a single-step extraction procedure. In addition to the higher affinity of hydrophilic ILs for L-tryptophan, another factor should be taken into account. In IL ABS the presence of an inorganic salt also leads to the amino acid salting-out from the aqueous phase, further enhancing the distribution ratio of L-tryptophan. This was previously shown by Fan et al.[36] with the extraction of endocrine-disrupting phenols by the use of hydrophobic ILs in the absence and presence of inorganic salts. However, the effect of the IL on the extraction ability of these systems can be gauged from the results of Salabat et al.,[34] where a conventional PEG-based ABS was used for L-tryptophan extraction with much lower partition coefficients ($K_{Trp} \approx 1-7$). The high partition coefficients obtained with IL-based ABS for the extraction of L-tryptophan show that these systems may be a successful and clean approach for biomolecules separation and purification in biotechnological processes.

## Conclusions

The problems of employing traditional solvents for the separation of biomolecules in biotechnological processes are driving the exploration of ILs as new promising extraction media. The ability of ILs to form salt-salt ABS allows hydrophilic ILs to be used in liquid−liquid extractions, and new phase equilibrium data for systems involving hydrophilic imidazolium-based ILs + $K_3PO_4$ + water have been presented.

The tunability of IL-based ABS was demonstrated by the evaluation of the IL cation influence in promoting ABS. The results showed that IL-based ABS can be obtained over a large range of concentrations for both ILs and the inorganic salt. The ability of imidazolium-based ILs for aqueous phase separation was shown to follow the order [$C_6$mim]Cl > [$C_7H_7$mim]Cl > [$C_4$mim]Cl > [$C_2$mim]Cl ≈ [amim]Cl > [$C_1$mim]Cl > [$OHC_2$mim]Cl and [$C_2$im]Cl > [$C_1$im]Cl > [im]Cl. The results obtained indicated that the IL cation has a significant influence on the behavior of the binodal curves and in the promotion of ABS. Increasing the alkyl chain length (for mono- or disubstituted ILs) increases the phase separation ability, whereas the insertion of a double bond, a benzyl group, or a hydroxyl group leads to a decrease of ABS promotion capability.

The capacity of the IL-based ABS as extraction media was demonstrated with the high L-tryptophan partitioning coefficients obtained. It was shown that depending on the nature of the IL used the partition coefficients can vary between 10 and 120. The ability of ILs to extract L-tryptophan increases with the cation hydrophilicity. These values are substantially higher than those obtained with conventional PEG-based ABS or with water-immiscible IL two-phase extractions.

**Acknowledgment.** The authors are grateful for financial support from Fundação para a Ciência e a Tecnologia for the project PTDC/EQU-FTT/65252/2006, Ph.D. grant SFRH/BD/37830/2007 to S.P.M.V., and post-doctoral grant SFRH/BPD/41781/2007 to M.G.F. The authors also thank Bernd Schröder for the synthesis of [$C_1$mim]Cl.

**Supporting Information Available:** Synthesis of [$C_1$mim]Cl and experimental binodal curve mass fraction data. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Kula, M.-R.; Kroner, K. H.; Hustedt, H. *Adv. Biochem. Eng./Biotechnol.* **1982**, *24*, 73.

(2) Banik, R. M.; Santhiagu, A.; Kanari, B.; Sabarinath, C.; Upahyay, S. N. *World J. Microbiol. Biotechnol.* **2003**, *19*, 337.

(3) Silva, M. E.; Franco, T. T. *Braz. J. Chem. Eng.* **2000**, *17*, 1.

(4) Pei, Y.; Wang, J.; Liu, L.; Wu, K.; Zhao, Y. *J. Chem. Eng. Data* **2007**, *52*, 2026.
(5) Zafarani-Moattar, M. T.; Hamzehzadeh, S. *J. Chem. Eng. Data* **2007**, *52*, 1686.
(6) Freemantle, M. *Chem. Eng. News* **1998**, *76*, 32.
(7) Anderson, J. L.; Jie, D.; Welton, T.; Armstrong, D. W. *J. Am. Chem. Soc.* **2002**, *124*, 14247.
(8) Zhao, H. *J. Mol. Catal. B: Enzym.* **2005**, *37*, 16.
(9) Gutowski, K. E.; Broker, G. A.; Willauer, H. D.; Huddleston, G. J.; Swatloski, R. P.; Holbrey, J. D.; Rogers, R. D. *J. Am. Chem. Soc.* **2003**, *125*, 6632.
(10) Freire, M. G.; Neves, C. M. S. S.; Carvalho, P. J.; Gardas, R. L.; Fernandes, A. M.; Marrucho, I. M.; Santos, L. M. N B. F.; Coutinho, J. A. P. *J. Phys. Chem. B* **2007**, *111*, 13082.
(11) Freire, M. G.; Santos, L. M. N. B. F.; Fernandes, A. M.; Coutinho, J. A. P.; Marrucho, I. M. *Fluid Phase Equilib.* **2007**, *261*, 449.
(12) Freire, M. G.; Carvalho, P. J.; Gardas, R. L.; Marrucho, I. M.; Santos, L. M. N. B. F.; Coutinho, J. A. P. *J. Phys. Chem. B* **2008**, *112*, 1604.
(13) Freire, M. G.; Carvalho, P. J.; Silva, A. M. S.; Santos, L. M. N. B. F.; Rebelo, L. P. N.; Marrucho, I. M.; Coutinho, J. A. P. *J. Phys. Chem. B* **2009**, *113*, 202.
(14) Bridges, N. J.; Gutowski, K. E.; Rogers, R. D. *Green Chem.* **2007**, *9*, 177.
(15) Visak, Z. P.; Canongia Lopes, J. N.; Rebelo, L. P. N. *Monatsh. Chem.* **2007**, *138*, 1153.
(16) Wu, B.; Zhang, Y.; Wang, H. *J. Phys. Chem. B* **2008**, *112*, 6426.
(17) Wu, B.; Zhang, Y.; Wang, H.; Yang, L. *J. Phys. Chem. B* **2008**, *112*, 13163.
(18) Wu, B.; Zhang, Y. M.; Wang, H. P. *J. Chem. Eng. Data* **2008**, *53*, 983.
(19) Zhang, Y.; Zhang, S.; Chen, Y.; Zhang, J. *Fluid Phase Equilib.* **2007**, *257*, 173.
(20) He, C.; Li, S.; Liu, H.; Li, K.; Liu, F. *J. Chromatogr. A* **2005**, *1082*, 143.
(21) Li, S.; He, C.; Liu, H.; Li, K.; Liu, F. *J. Chromatogr. B* **2005**, *826*, 58.
(22) Liu, Q.; Yu, J.; Li, W.; Hu, X.; Xia, H.; Liu, H.; Yang, P. *Sep. Sci. Technol.* **2006**, *41*, 2849.
(23) Merchuk, J. C.; Andrews, B. A.; Asenjo, J. A. *J. Chromatogr. B* **1998**, *711*, 285.
(24) Romero, A.; Santos, A.; Tojo, J.; Rodríguez, A. *J. Hazard. Mater.* **2008**, *151*, 268.
(25) Willaure, H. D.; Huddleston, J. G.; Rogers, R. D. *Ind. Eng. Chem. Res.* **2002**, *41*, 1892.
(26) Galaev, I. Y.; Mattiasson, B. *Enzyme Microbiol. Technol.* **1993**, *15*, 354.
(27) Deng, Y.; Chen, J.; Zhang, D. *J. Chem. Eng. Data* **2007**, *52*, 1332.
(28) Chetana, S.; Rastogi, N. K.; Raghavarao, K. S. M. S. *Biotechnol. Lett.* **2006**, *28*, 25.
(29) Closs, C. B.; Conde-Petit, B.; Roberts, I. D.; Tolstoguzov, V. B.; Escher, F. *Carbohydr. Polym.* **1999**, *39*, 67.
(30) Rogers, R. D.; Bauer, C. B. *J. Chromatogr. B* **1996**, *680*, 237.
(31) Huddleston, J. G.; Willauer, H. D.; Rogers, R. D. *J. Chem. Eng. Data* **2003**, *48*, 1230.
(32) Albertsson, P. A. *Partition of Cell Particles and Macromolecules*, 3rd ed.; Wiley: New York, NY, 1986.
(33) Lu, M.; Tjerneld, F. *J. Chromatogr. A* **1997**, *766*, 99.
(34) Salabat, A.; Abnosi, M. H.; Motahari, A. *J. Chem. Eng. Data* **2008**, *53*, 2018.
(35) Wang, J.; Pei, Y.; Zhao, Y.; Hu, Z. *Green Chem.* **2005**, *7*, 196.
(36) Fana, J.; Fana, Y.; Peia, Y.; Wua, K.; Wanga, J.; Fan, M. *Sep. Purif. Technol.* **2008**, *61*, 324.

JP900293V