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(Extraction of biomolecules using) aqueous biphasic systems formed by ionic liquids and aminoacids

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ABSTRACT

The increasing emphasis on cleaner and environmentally benign extraction procedures has led to the systematic investigation of systems containing ionic liquids (ILs)—a new class of non-volatile alternative solvents. In this work, aqueous biphasic systems (ABS) composed by hydrophilic ILs and aminoacids were studied aiming at obtaining new evidences regarding their ABS formation ability and their capacity for the extraction of specific biomolecules. On the basis of the IL cation 1-butyl-3-methylimidazolium, the IL anion influence on ABS formation was assessed through its combination with tetrafluoroborate, triflate, and dicyanamide anions, with three different aminoacids: L-lysine, D,L-lysine HCl and L-proline. Ternary phase diagrams (and respective tie-lines) formed by these aqueous solutions of the ILs and the selected aminoacids, were measured at 298 K and atmospheric pressure. The results indicate that the ability of an IL to produce ABS closely follows the decrease in the hydrogen-bond accepting strength of the IL anion. In addition, the ability of aminoacids to form ABS follows the order: L-lysine ≈ D,L-lysine HCl > L-proline. Finally, the extraction capability of the studied ABS was evaluated through their application to the extraction of three biomolecules (caffeine, ciprofloxacin and ciprofloxacin HCl).

1. Introduction

Separation and purification stages of biotechnological processes usually require numerous steps with high energy and chemical consumption and have often a large impact on the cost of the final product [1,2]. One of the major industrial challenges in biotechnology is the development of fast, efficient and cost-effective downstream processes for the recovery of biomolecules from fermentation media, while minimizing the environmental impact of the processes adopted. Among others [3–6], liquid–liquid extraction techniques seem to have the greatest potential, offering advantages such as higher capacity, better selectivity and integration between recovery and purification, and providing higher yields, purities and lower costs [2,7]. In spite of its effectiveness, liquid–liquid extraction processes may have some negative environmental impact and often rise concerns related with the purity and quality of the extracted biomolecules due to the toxic and denaturating characters of most volatile organic solvents commonly employed [2]. For this reason, in the past few years, there has been an active search for alternative approaches [8–10] to overcome those environmental and operational problems. In this context, the replacement of ordinary solvents by ionic liquids (ILs) as extraction media has emerged as one of the most promising techniques. In fact, besides their ambient-friendly thermophysical properties such as negligible vapour pressure, non-flammability and high chemical stability [11], ILs present excellent solvation qualities [12] and usually provide a non-denaturing environment for biomolecules, maintaining protein structure and enzymatic activity [13,14]. Since these properties are strongly dependent on the solvent nature, the possibility of customizing and manipulating the properties of the IL through the selection of the anions and cations that compose them represents an additional advantage. While the low vapour pressures of these compounds and consequent reduced air pollution risks is one of their most claimed advantages, the release of these liquids into aquatic environments may lead to water contamination because they have at least some miscibility with water. Recent reports [15–17] show that the ecotoxic character of ILs seems to increase with their hydrophobicity; hydrophilic ILs such as those most convenient for biochemical separations present very low toxicities.

Most biotechnological applications of liquid–liquid extraction using ILs are based on aqueous biphasic systems (ABS). Since

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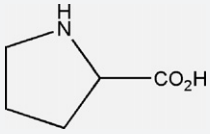
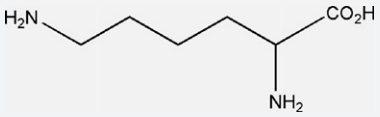
Table 1
Structure of the aminoacids studied and their dissociation constants [43,44] and solubilities in water (*s*) [44].

Aminoacid	Molecular structure	pK_a (–COOH)	pK_a (–NH_3)	pK_a (–R)	*s*/g (100 g^{-1})
Pro		2.00	10.60	–	162.3
Lys		2.18	8.95	10.53	Very soluble

Gutowski et al. [18] reported that the addition of potassium phosphate to an aqueous solution of a hydrophilic IL produces ABS, the advantages of ABS technology – clarification, concentration and purification integrated in one step and high selectivity and scaling-up facility – have been investigated the tailoring properties of ILs to develop specific extraction and isolation procedures. A large amount of studies on the phase behaviour of ternary systems composed of ILs + water + salting-out inducing inorganic ions and on the ability of the last to form IL-based ABS can now actually be found in literature [19–26], along with some reports on the extraction potential of those systems for some biomolecules, such as testosterone and epitestosterone [23], opium alkaloids [24], antibiotics [25,26] and aminoacids [20,21]. Although often used, these kinds of IL/inorganic salt separation systems are highly ionic media and therefore might not be compatible with the extracted product, since many biomolecules present narrow tolerance limits of ionic strength. Moreover, the ion exchange between salt and IL can complicate the separation procedure and the recyclability of the IL from aqueous solutions while the salt disposal might cause a significant impact on wastewater treatment [27]. The use of sugars [28,29] and, very recently, of aminoacids [30] to form more environmentally benign and less aggressive IL-based ABS has been suggested to overcome these issues. Nevertheless, both liquid–liquid equilibrium results and extraction ability data are still very scarce and systematic work is still required to develop the potentialities of those new ABS. Actually, to the best of our knowledge there is a single investigation study [30] concerning aminoacid-ILs ABS-promoting capability and no reports on the extraction ability of those systems for biomolecules were found.

In this work, we evaluate the capability of ILs and aminoacids to promote ABS and study the influence of both the IL anion nature and the aminoacid structure on ABS formation. For that purpose, three imidazolium-based ILs—1-butyl-3-methylimidazolium triflate ([C_4mim][CF_3SO_3]), 1-butyl-3-methylimidazolium dicyanamide ([C_4mim][$N(CN)_2$]) and 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim][BF_4])—and two structurally different aminoacids—proline (Pro) and lysine (Lys)—were selected. Additionally, the monohydrochloride form of lysine (Lys·HCl) was also considered in order to assess the effect of the addition of the HCl group on the promotion of the ABS. The ILs were chosen on the basis of a previous systematic study [20] which indicates that imidazolium-based ILs comprising triflate and dicyanamide anions present higher ABS formation capability in the presence of K_3PO_4. This fact is very important in the present work since aminoacids have a less pronounced salting-out inducing behaviour than the inorganic salts commonly used (e.g. K_3PO_4). In addition, imidazolium-based ILs with short cation alkyl chains are known to have low toxicities [15–17,31,32].

Ternary phase diagrams for different systems composed by hydrophilic IL + aminoacid + water were determined, at 298 K and atmospheric pressure, maintaining either the aminoacid or the IL to establish, respectively, the effect of the IL anion nature and of aminoacid structure on promoting ABS. The binodal curves were fitted to a three-parameter equation, and the tie-lines were estimated using the Merchuck et al. [33] approach. The ABS studies were further analysed according to their potential for the extraction of biomolecules, for which one alkaloid (caffeine) and two forms of an antibiotic with different aqueous solubilities (ciprofloxacin and ciprofloxacin·HCl) were selected as model compounds of biotechnological interest. The development of methods for the recovery and determination of alkaloids [24] and for the production of antibiotics at lower manufacturing costs [25,34,35] is still a significant challenge. Besides this specific practical interest, this work opens up new possibilities in the separation of other drugs from biological samples and gives another contribution towards the understanding of the molecular interactions which control the separation and purification processes by ILs [36–39].

2. Experimental

2.1. Materials

The ABS studied in this work were established using an aqueous solution of each aminoacid and different aqueous solutions of hydrophilic ILs. The aminoacids—L-proline (Sigma, >99%), L-lysine (Fluka, >98%) and D,L-lysine monohydrochloride (BHD Chemicals, >98.5%) were used as received without further purification. Their molecular structures are represented in Table 1. The ILs studied – [C_4mim][CF_3SO_3], [C_4mim][$N(CN)_2$] and [C_4mim][BF_4] – were supplied by Iolitec and are depicted in Fig. 1. To reduce the water and volatile compound contents to negligible values, IL individual samples were dried under constant agitation at vacuum and moderate temperature (353 K) for a minimum of 48 h. After this procedure, the purity of each IL was further checked by ^{1}H, ^{13}C

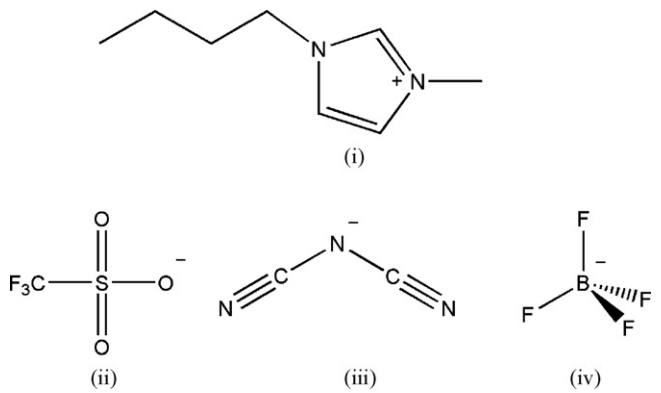


Fig. 1. Chemical structure of the cation and anions composing the ILs studied: (i) $[C_4mim]^+$; (ii) $[CF_3SO_3]^-$; (iii) $[N(CN)_2]^-$; (iv) $[BF_4]^-$.

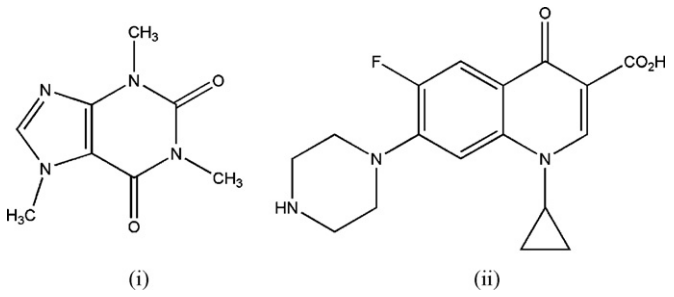


Fig. 2. Chemical structure of the partitioning solutes considered in this work: (i) caffeine; (ii) ciprofloxacin.

and ^{19}F NMR spectra and found to be >99% (w/w) for all samples. The water used was ultrapure, double distilled, passed through a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus. The compounds used as partitioning solutes were caffeine >99.0% (w/w) pure acquired from José M. Vaz Pereira S.A., ciprofloxacin >99.8% (w/w) generously provided by Bayer HealthCare AG and ciprofloxacin·HCl >98% (w/w) from Fluka. Their structures are presented in Fig. 2.

3. Experimental procedure

3.1. Phase diagrams and tie-lines

The phase diagram binodals were determined through the cloud point titration method [20,21] at 298 K (±1 K) and atmospheric pressure. Aqueous solutions of aminoacids at ≈25–35 (w/w%) and aqueous solutions of the different hydrophilic ILs at ≈85 (w/w%) were prepared and used for the determination of the binodal curves. The ternary system compositions were determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g. Details of the experimental procedure adopted and respective validation are described elsewhere [20,21]. The pH of the aminoacid solutions was measured with a pH meter (Hanna Instruments, Model 9321), with an associated uncertainty of 0.01. At the biphasic region the upper phase is composed by the aminoacid-rich aqueous phase while the lower phase is the IL-rich phase for all ILs and aminoacids investigated (the relative position of the phases is inverted comparing to IL/K_3PO_4 ABS [20,21]). The tie-lines (TLs) were determined using the Merchuck et al. [33] gravimetric method. For the TL determinations, a ternary mixture within 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. 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. For that purpose, the binodal curves were fitted to the empirical correlation [33]:

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

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

3.2. Partitioning of caffeine, ciprofloxacin and ciprofloxacin·HCl

A ternary mixture in the biphasic region was selected and used to evaluate the partitioning of the biomolecules at 298 K. Aqueous solutions of caffeine ($\approx 7.8 \times 10^{-2}$ mol dm^{-3}), ciprofloxacin ($\approx 1.7 \times 10^{-4}$ mol dm^{-3}) and ciprofloxacin·HCl ($\approx 6.9 \times 10^{-4}$ mol dm^{-3}) were used in the aqueous ternary composition. The biphasic solution was hand stirred and left to equilibrate for 12 h to achieve a complete partitioning of the biomolecules between the two phases. This time period was established in previous optimizing experiments. The partitioning solute quantification, in both phases, was performed by UV spectroscopy using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, and calibration curves previously established. Three samples of each aqueous-rich phase were quantified to determine the biomolecules partition coefficients and the respective standard deviations were determined. The partition coefficients of the biomolecules, K_{Bio}, were determined as the ratio of the concentration of each partitioning compound in the IL and in the aminoacid (aa) aqueous-rich phases, according to Eq. (2):

$$K_{Bio} = \frac{[Bio]_{IL}}{[Bio]_{aa}} \quad (2)$$

where $[Bio]_{IL}$ and $[Bio]_{aa}$ are the concentrations of the each individual biomolecule in the IL and in the aminoacid aqueous-rich phases, respectively.

4. Results and discussion

4.1. Phase diagrams and tie-lines

Contrarily to common inorganic salts, whose addition to ionic aqueous systems could lead to ion exchange and/or ions pairing between the salts present in both aqueous-rich phases, aminoacids are a known type of inner salts which may preclude ion exchange. Therefore, among other advantages, IL-based ABS formed by the addition of these biomolecules are less complex and more gentle systems for biomolecule extraction expanding the range of potential salts for ABS formation. Nevertheless, since aminoacids are weak salting-out inducing species, the ILs employed have to be carefully selected so that the phase separation can actually occur. Previous results [20,21] indicate that the ILs used in this work are adequate for this purpose. Indeed, the [C_4mim][halogenate] ILs are not sufficiently salted out by the addition of aminoacids to allow ABS formation.

Although [C_4mim][BF_4] is able to form ABS, the hydrolysis of such anion in aqueous solutions, precludes its use in separation processes due to the formation of hydrofluoridric acid in solution [40]. However, the phase diagrams were determined in short time periods (around 2–4 h) and at a moderate temperature (298.15 K). Under these conditions, hydrolysis of $[BF_4]^-$ is not significantly extensive.

Although the effect of salts on ABS formation has been largely studied [18,22–24,41] and seems to be follow the Hofmeister series [42], the ability of aminoacids to promote phase separation of hydrophilic IL + water systems has seldom been approached [30] and the underlying molecular-level mechanisms have hardly ever been studied. Being the aminoacids charged particles it is possible to use some of the insights previously gained on the formation of IL-based ABS systems with inorganic salts [20,21] and on the aminoacid solubility effects in water + IL systems [36] in order to understand their ability to promote ABS. As can be seen from the pK_a data [43,44] in Table 1, at the pH considered in this work (≈7) the aminoacids under study are in their zwitterionic form. Proline possessing a neutral side chain has no net charge. Lysine has a basic character, thus acquiring a positively charged side chain.

The solubility of a given solute in water is affected by the presence of other species. As far as aminoacid solubility effects are concerned, the information available indicates that proline, L-serine and glycine can salt out [C_4mim][BF_4] + water mixtures [30] while proline has a salting-in effect in aqueous solutions of a less hydrophilic imidazolium-based IL, such as [C_4mim][$C(CN)_3$] [36]. Lysine induces a slight increase on the mutual solubilities of [C_4mim][$C(CN)_3$] and water [36]. We are not aware of any report on Lys·HCl solubility inducing behaviour in ILs + water systems.

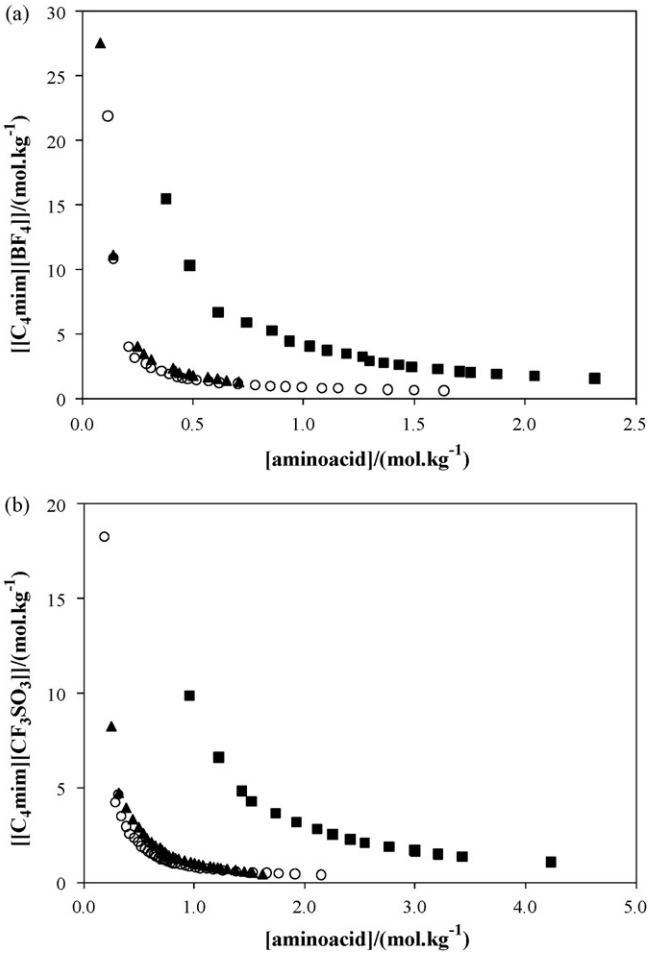


Fig. 3. Phase diagrams for the [C_4mim]-based ternary systems, at 298 K, composed by (a) [C_4mim][BF_4] + aminoacid + H_2O; (b) [C_4mim][CF_3SO_3] + aminoacid + H_2O: ○, Lys; ▲, Lys·HCl; ■, Pro.

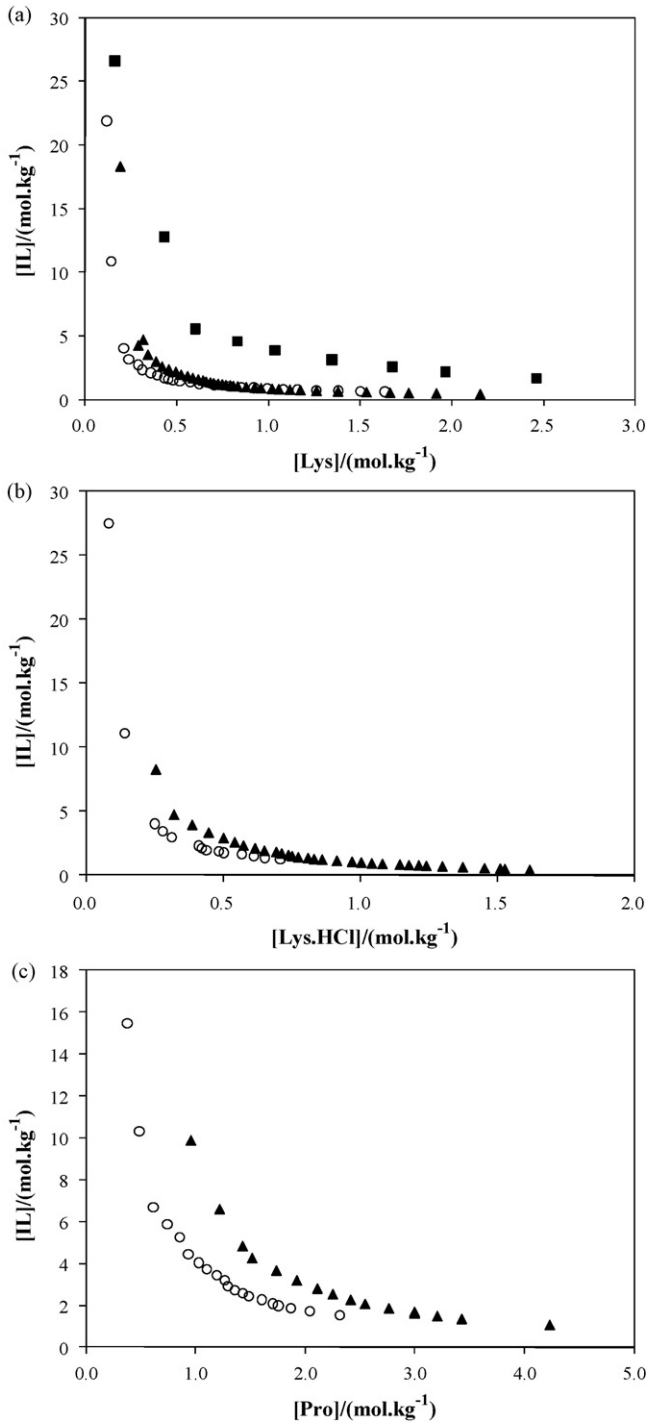


Fig. 4. Phase diagrams for the [C_4mim]-based ternary systems, at 298 K, composed by (a) IL + Lys + H_2O; (b) IL + Lys·HCl + H_2O; (c) IL + Pro + H_2O: ○, [C_4mim][BF_4]; ▲, [C_4mim][CF_3SO_3]; ■, [C_4mim][$N(CN)_2$].

As can be seen from the experimental phase diagrams obtained with [C_4mim][BF_4] and [C_4mim][CF_3SO_3] reported in Fig. 3, all the aminoacids investigated can induce salting-out of these ILs from aqueous solutions leading to the formation of ABS (*cf.* Supporting Information with the experimental weight fraction data). The trend observed for the aminoacids ability to form an ABS is similar for both ILs and the distance between the origin and the binodal curves follows the order Lys < Lys·HCl < Pro, meaning that a lower molality of Lys is needed to form an ABS for both ILs + water systems studied. These results indicate that the difference in the aminoacid hydrophobicities and, consequently, their solubilities in water, are responsible for the observed effects. Actually, as can be seen from Table 1, Lys (and naturally Lys·HCl) is more soluble than Pro [44] due to the presence of an additional $-NH_2$ group in its side chain. This enhanced aminoacid hydrophilicity leads to a more pronounced salting-out inducing effect. The aminoacids seem to behave just like ordinary salting-out inducing salts, acting mainly through the formation of water–aminoacid hydration complexes, which, due to the nature of the aminoacids and the ILs, is a more favourable process than the direct binding to the IL moieties [38,39]. It is worth to note, however, that the addition of the hydrochloride group to Lys does not change significantly the aminoacid's ABS promotion ability, as expected.

It is possible to obtain further insight into the salting-out inducing mechanism of aminoacids in the ILs + water systems if the current results are compared with data on the impact of aminoacids on the LLE behaviour of [C_4mim][$C(CN)_3$] [36] aqueous solutions. Accordingly to the model proposed to rationalize the effects of a series of aminoacids on the mutual solubilities of water and [C_4mim][$C(CN)_3$] [36], the salting-in and salting-out phenomena are the result of a delicate balance among water-aminoacid side chain, IL-aminoacid side chain and water-IL interactions, determined by the relative affinities of the biomolecule side chains to water and to IL. Proline promotes a slight increase on water + [C_4mim][$C(CN)_3$] mutual solubilities because its small, neutral and less hydrophobic allowing the establishment of interactions with water, which compensate its direct binding to the

Table 2
Hydrogen-bond basicity (β) of [C_4mim]-based ILs [45].

IL anion	β
$[N(CN)_2]^-$	0.64
$[CF_3SO_3]^-$	0.57
$[BF_4]^-$	0.55

hydrophobic moieties of the IL cation, typical of the mechanism of salting-in [38,39]. Lysine contains a more hydrophilic side chain and has, therefore, a preferential hydration characteristic of salting-out. When the IL anion is changed to $[BF_4]^-$ and $[CF_3SO_3]^-$, as in the current work, the solubility effects observed are curiously inverted. Since the systems under comparison only differ in the IL anion, it is reasonable to attribute those different behaviours to the interactions established between the aminoacids and the IL anion. In this work the interactions of Pro and Lys with the two fluorinated anions are rather unfavourable and therefore both aminoacids promote salting-out due to their preferential hydration. Consequently, it is just the hydrophilic character of aminoacids that determines their ability to decrease the mutual solubilities of water and ILs. Hence, the more hydrophobic the side chain, the less the ability of the aminoacid to promote phase separation, as confirmed by the results here obtained.

From the results presented in Fig. 4 it is possible to evaluate the effect of the nature of the anion of the imidazolium-based ILs on ABS formation. Independently of the aminoacid used, the ability of the ILs to form ABS follows the order [C_4mim][BF_4] > [C_4mim][CF_3SO_3] > [C_4mim][$N(CN)_2$]. The closer to the *y*-axis the binodal curve is located, the larger is the IL anion salting-in inducing behaviour. This sequence was also observed for IL-based ABS formed by the addition of K_3PO_4 [20]. According to the molecular interpretation given [20], ABS formation reflects the competition between the inorganic salt and the IL ions for the creation of water-ion hydration complexes and is related to the anion's hydrogen-bond basicity of the ILs. Since, as discussed above, the aminoacids under study behave like salting-out inducing salts, it is reasonable to apply the same type of interpretation to the current case. The hydrogen-bond basicity (β) data of [C_4mim]-based ILs using the solvatochromic probe $[Fe(phen)_2(CN)_2]ClO_4$ [45] are reported in Table 2. When the value of β increases, the hydrogen-bond accepting strength of the IL anion increases, enhancing its ability to be preferentially hydrated and resulting in a lower ABS formation capacity of the correspondent IL.

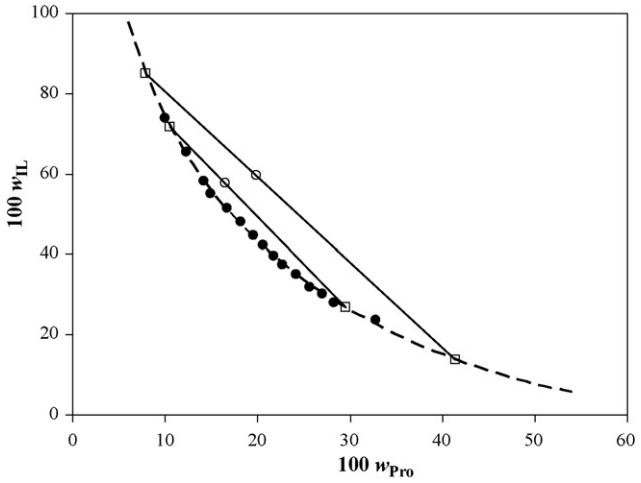


Fig. 5. Phase diagram for the [C_4mim][CF_3SO_3] + Pro + water ternary system at 298 K: ●, binodal experimental data; ○, experimental data used for the TL determination; □, TL data; — — —, fitting by Eq. (1).

The binodal curves were fitted to Eq. (1) where the regression parameters were estimated by least-squares regression (*cf.* Supporting Information). The tie-line (TL) equations obtained for each ternary system and correspondent lengths (TLLs) are given in Table 3. A graphical representation of the TLs and their fitting by Eq. (1) for the [C_4mim][CF_3SO_3]/Pro ABS is depicted in Fig. 5.

4.2. Partitioning of caffeine, ciprofloxacin and ciprofloxacin·HCl

The partition coefficients of caffeine, ciprofloxacin and ciprofloxacin·HCl in the studied ABS and the correspondent mixture compositions are presented in Table 4. The partition coefficients of caffeine were determined for all the IL-based ABS formed with lysine and for one of the IL-based ABS formed with proline. The partition coefficients of the other solutes were only determined using ABS formed by the strongest salting-out inducing aminoacid (Lys) and [C_4mim][CF_3SO_3].

Table 3
Experimental data for TLs and respective TLLs.

IL	Weight fraction composition/wt%		TL equation IL (wt%) = $a + b \times$ aa (wt%)		TLL
	IL	Pro	a	b	
[C_4mim][BF_4]	63.51	18.43	106.1	−2.309	108.2
	40.16	19.98	94.73	−2.732	77.69
[C_4mim][CF_3SO_3]	59.62	19.87	101.8	−2.124	78.57
	57.74	16.48	96.75	−2.367	48.85
IL	Weight fraction composition/wt%		TL equation IL (wt%) = $a + b \times$ aa (wt%)		TLL
	IL	Lys	a	b	
[C_4mim][BF_4]	24.98	14.90	82.62	−3.868	64.44
[C_4mim][CF_3SO_3]	30.37	15.04	82.01	−3.433	61.47
[C_4mim][$N(CN)_2$]	49.69	19.89	90.20	−2.037	73.42
IL	Weight fraction composition/wt%		TL equation IL (wt%) = $a + b \times$ aa (wt%)		TLL
	IL	Lys·HCl	a	b	
[C_4mim][BF_4]	56.81	8.500	74.25	−2.052	48.42
	37.96	14.17	68.69	−2.169	44.59
[C_4mim][CF_3SO_3]	43.17	12.89	110.4	−5.219	59.74
	42.03	10.50	81.55	−3.765	40.67

Table 4
Weight fraction composition and partition coefficients of caffeine, ciprofloxacin and ciprofloxacin·HCl (K_{Bio}), and respective standard deviations (σ), in IL-based ABS at 298 K.

Biomolecule	ABS	Weight fraction composition/wt%		$K_{Bio} \pm \sigma$
		IL	aa	
Caffeine	[C_4mim][CF_3SO_3] + Pro	49.34	19.95	2.5 ± 0.2
	[C_4mim][BF_4] + Lys	39.94	17.97	0.23 ± 0.01
	[C_4mim][CF_3SO_3] + Lys	39.98	20.15	0.145 ± 0.009
	[C_4mim][$N(CN)_2$] + Lys	39.97	18.01	5.8 ± 0.6
Ciprofloxacin	[C_4mim][CF_3SO_3] + Lys	39.96	19.88	2.7 ± 0.4
Ciprofloxacin·HCl	[C_4mim][CF_3SO_3] + Lys	40.02	20.01	2.37 ± 0.03

For the correct design of extraction processes, it is essential to understand the physicochemical issues leading the partition of biomolecules between the two equilibrium aqueous-rich phases. The higher the biomolecules partition coefficients, the higher the tendency for the solute to migrate to the aqueous IL-rich phase. The partition coefficients of caffeine with [C_4mim]-based ILs and Lys systems follow the IL order: [C_4mim][$N(CN)_2$] > [C_4mim][BF_4] > [C_4mim][CF_3SO_3]. Moreover, maintaining the same IL and changing the aminoacid ([C_4mim][CF_3SO_3] + Pro and [C_4mim][CF_3SO_3] + Lys systems) the highest partition coefficient for caffeine was obtained when Pro was used. On the other hand, retaining the [C_4mim][CF_3SO_3] + Lys ABS the biomolecule partition coefficients follow the rank: ciprofloxacin ≈ ciprofloxacin·HCl > caffeine. Actually, the values obtained for the two forms of the antibiotic are not significantly different. The effect of the addition of the hydrochloride group is only an enhancement in the aqueous solubility [34,35] and in this case it is present in such small quantity in the solution that it does not have any role in the extraction process. More complex and hydrophobic molecules are better extracted by an IL with a smaller affinity for water. It is expected that the solute–IL interactions are complex and will involve van der Waals forces, electrostatic interactions, hydrogen-bonding, and $\pi\cdots\pi$ stacking between the imidazolium ring and the aromatic ring of each biomolecule. In fact, $\pi\cdots\pi$ stacking between imidazolium and benzene molecules has been reported previously [46]. Therefore, the partition coefficient of a specific biomolecule is the result of several competing interactions in aqueous phases. The values obtained in this work for the partition coefficients are reasonably good, indicating that relatively high amounts of the model biomolecules under study can be extracted from aqueous phases using the extraction systems considered. Furthermore, the results show that the extractive potential of ABS depends more on the IL nature than on the aminoacid structure and make it clear that it is possible to manipulate phase properties producing higher selectivities for the biomolecules of interest. Nevertheless, further studies regarding ILs aiming at obtaining a complete perspective of the molecular interactions controlling the biomolecules partition behaviour is of utmost importance.

5. Conclusions

[C_4mim]-based hydrophilic ILs combined with [BF_4], [CF_3SO_3] and [$N(CN)_2$] anions have been shown to be able to induce aqueous phase separation in the presence of aminoacids, and thus to form ABS. Novel experimental equilibrium data for the compositions of coexisting phases of ABS involving hydrophilic IL + aminoacid + H_2O, at the same conditions of temperature and pressure (298 K and atmospheric pressure), were studied and reported.

The ability of [C_4mim]-based ILs for aqueous phase separation was shown to follow the order [BF_4] > [CF_3SO_3] > [$N(CN)_2$]—inversely proportional to the hydrogen-bond basicity of the anion composing the IL. Moreover, the ability of the aminoacids to form ABS follows the rank Lys ≈ Lys·HCl > Pro. The results indicate that IL-based ABS can be obtained by the addition of more benign salting-out agents – aminoacids – and that such systems can be finely tuned by the adjustment of the IL anion and/or aminoacid employed.

The aptitude of the IL-based ABS as prospective extraction media in biotechnological processes was demonstrated by the partition coefficients obtained for three biomolecules of significance (caffeine, ciprofloxacin and ciprofloxacin·HCl) and where higher values are attained using aqueous phases with lower affinities among them.

Neither the ABS formation ability of Lys, nor the partition coefficients of ciprofloxacin are significantly changed if the correspondent monohydrochloride forms are considered.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.seppur.2010.01.008.

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