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Tryptophan extraction using hydrophobic ionic liquids

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ABSTRACT

The application of hydrophobic ionic liquids (ILs) as promising alternatives to volatile organic solvents in liquid–liquid extraction processes of biomolecules was evaluated through the determination of the partition coefficients of the aminoacid L-tryptophan, taken as model biomolecule, between aqueous solutions and distinct ILs. Factors affecting the effectiveness of the recovery such as the pH of the aqueous medium, the nature of the IL anion and the nature and the chemical structure of the IL cation were assessed. The results show that the pH of the aqueous phase strongly influences the success of the separation and that the anion/cation hydrophobic characters are main structural factors ruling the extraction efficiency. The evidences gathered in this work suggest that L-tryptophan partitioning between the aqueous and IL phases is ruled by a complex interplay of intermolecular forces between the solute and the IL solvent, such as electrostatic interactions between the cationic form of the aminoacid and the anion of the IL and interactions established at the level of the IL cation. These findings support earlier molecular interpretations of the mechanisms that govern the partitioning of biomolecules between ILs and aqueous phases.

1. Introduction

The recovery of proteins and other biomolecules from natural sources and fermentation media is an essential task in biotechnology. The demand for fast, cost-effective and eco-friendly separation and purification processes has led to a significant effort to develop clean manufacturing methods and easily scaled-up industrially relevant techniques to increase selectivity and capacity and reduce overall costs [1]. Among others [2–5], liquid–liquid extraction processes seem to have the greatest potential due to their effectiveness, technological simplicity and economic nature, and have been often a favored choice in process engineering [1,6–12]. Despite economical advantages, the application of conventional solvents in extraction techniques brings up some difficulties associated to their toxic and denaturing nature. The complex balance between the hydrophilic and lipophilic character of many biomolecules can also constitute a further disadvantage. Alternative approaches such as the use of water [13] or supercritical fluids [14] as solvents, and the addition of complexing agents or hydrophobic counter ions [8] have been identified to overcome those operational and environmental problems, but the most recent and promising alternative has been the use of ionic liquids (ILs), as substitutes for ordinary solvents [8,15–17]. Actually, besides their remarkable and advantageous thermophysical properties such as excellent solvation ability, negligible vapour pressure, non-flammability and high chemical stability and tunability [18], ILs are often biocompatible, by not inactivating enzymes, and thus assuring the structural integrity of these biomolecules [1,19]. Therefore, the unique features of their chemistry are currently being combined with the advantages of solvent extraction itself to improve the efficiency of the separation of important biomolecules such as aminoacids and proteins, carbohydrates, lactic acid, butanol, antibiotics and alkaloids from different media [7–9,20–26]. In this area, research has been focusing on two different approaches, each of which with their own advantages and disadvantages: the direct use of hydrophobic ILs as organic solvent replacements in liquid–liquid separations and the use of IL-based aqueous biphasic systems (ABS) formed by the addition of salting-out inducing agents to aqueous solutions of hydrophilic ILs. Aqueous two-phase extraction systems using ILs have actually become a powerful technique for the purification and extraction of a wide range of (bio)compounds [7,21,22,24–28] partly due to the fact that they represent considerable operational cost savings and also because hydrophilic ILs are more numerous than their hydrophobic counterparts. However, resorting to IL-based ABS inevitably introduces inorganic ions which might complicate the separation procedure, not to mention the impact on waste water treatment that the salt disposal may cause [29].

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These problems might be overcome by the direct application of hydrophobic ILs. ILs with fluorinated anions have been extensively and successfully employed in the extraction of various biocompounds including aminoacids [8,9] and antibiotics [7], but their use has been discouraged by their high costs of production [30].

To successfully apply ILs as extraction solvents or to correctly design new ILs to meet the requirements of any particular task, it is crucial to effectively manipulate the experimental parameters that influence the partitioning of biomolecules between ILs and aqueous media together with a deep understanding of the interactions established in solution. Although some recent studies [31,32] provided helpful correlations between the extraction of biomolecules by ILs and different experimental factors, there is still a lack in the fundamental knowledge required to develop and optimize separation procedures, particularly as far as other hydrophobic ILs are concerned. Moreover, the molecular level interactions behind the extraction, occurring in these systems, are far from being established. In a previous study [33] we evaluated, through liquid–liquid equilibria type studies, how the nature, polarity and chemical properties of different aminoacids affect the behaviour of aqueous solutions of a partially miscible IL, and we interpreted these results in terms of a proposed molecular model. In the current work, we report a detailed study of the extraction of the aminoacid L-tryptophan, taken as model biomolecule, from aqueous solutions into distinct hydrophobic ILs, selected in order to investigate how the nature of IL anions and cations, length of the alkyl side chain and polarity of the IL affect the recovery of the aminoacid between the two phases. The pH dependence of the partition coefficients was additionally evaluated.

Besides constituting a valuable source of information with commercial, industrial and biochemical relevance, the data gathered in this work, once related with other information [33], can give further insights into the mechanisms that govern the solvation of biomolecules in ILs and aqueous phases and in clarifying crucial aspects related to the separation and purification processes not only of aminoacids using ILs, but also of more complex biomolecules such as proteins and enzymes, and their influence on the recovery, activity and stability of enzymes in these solvents.

2. Materials and methods

2.1. Materials

The ILs employed in this work were purchased from IoLitec with mass fraction purities >99%. The abbreviations used for the ILs are listed in Table 1 and the structure of their constituting ions are represented in Fig. 1. To reduce the water and volatile compounds content to negligible values, the ILs were dried under constant agitation at vacuum and moderate temperature (353 K) for a minimum of 48 h. After this procedure, their purity was checked by ^{1}H, ^{13}C and ^{19}F NMR spectra. The water used for the preparation of the aqueous solutions was double-distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus. It has a resistivity of 18.2 MΩ cm, a TOC smaller than 5 μg dm^{-3} and it is free of particles greater than 0.22 μm. The aminoacid studied, L-tryptophan (Trp) (Fluka, >99 wt%), was used as acquired without further purification. Its chemical structure is represented in Fig. 2. Hydrochloric acid (Riedel-de-Haen, 37 v/v % aqueous solution) was used to adjust the pH of aqueous solutions.

2.2. Experimental procedure

The IL/water partition coefficients of Trp were determined at 298.15 K and atmospheric pressure. Aqueous saturated solutions of each IL were prepared (by mutual IL and water equilibration under slow stirring for a minimum of 48 h) and, after adjusting their pH to the desired value by addition of HCl, they were used to prepare solutions of Trp of approximately 3.8×10^{-3} mol dm^{-3}. The saturation of the aqueous phase with each IL eliminates the further variations in pH values by the IL migration to the aqueous-rich phase as well as reduces the volume changes. The pH was measured with a pH meter (Hanna Instruments, Model 9321), with an associated uncertainty of 0.01 and the temperature was controlled with a calibrated Pt 100 temperature sensor with an uncertainty of ±0.01 K. 2.0 mL of the aminoacid aqueous solution was added to 2.0 mL of pure IL in stoppered glass test tubes and vigorously stirred. This mixture was placed under moderate stirring for 3 h. This is the minimum time required for the extraction process to be completed and was established in preliminary tests. After stirring, the two phases were carefully separated using a centrifuge (Eppendorf, Model 5804). The concentration of Trp in the aqueous-rich phase was determined using a UV–vis spectrophotometer (Shimadzu UV-1700) at a wavelength of 279 nm for all ILs except for pyridinium-based ones, where the wavelength of 287 nm was used (due to interferences of pyridinium-based aromatic rings absorption in the UV region). The corresponding aminoacid concentration in the IL-rich phase was calculated by mass balance. Since the aqueous phase was saturated by the IL before the partition experiment, it was possible to better control the desired pH and, considering additionally that the mutual solubilities of the ILs under study and water are negligible [34,35], to assume that only the aminoacid was transferred from one phase to another, being thus correct to calculate its concentration in the IL-rich phase by mass balance. The partition coefficients of Trp between the IL and the aqueous-rich phases were calculated

Table 1
ILs studied and correspondent abbreviations.

IL	Abbreviation
$[C_2mim][Tf_2N]$	1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_3mim][Tf_2N]$	1-Propyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_4mim][Tf_2N]$	1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_5mim][Tf_2N]$	1-Pentyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_6mim][Tf_2N]$	1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_7mim][Tf_2N]$	1-Heptyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_8mim][Tf_2N]$	1-Octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_6mim][BF_4]$	1-Hexyl-3-methylimidazolium tetrafluoroborate
$[C_8mim][BF_4]$	1-Octyl-3-methylimidazolium tetrafluoroborate
$[C_4mim][PF_6]$	1-Butyl-3-methylimidazolium hexafluorophosphate
$[C_8mim][PF_6]$	1-Octyl-3-methylimidazolium hexafluorophosphate
$[C_3mpyrr][TF_2N]$	1-Methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide
$[C_3mpyr][TF_2N]$	1-Propyl-3-methylpyridinium bis(trifluoromethylsulfonyl)imide
$[C_3mpip][TF_2N]$	1-Propyl-3-methylpiperidinium bis(trifluoromethylsulfonyl)imide
$[C_2C_2im][TF_2N]$	1,3-Diethylimidazolium bis(trifluoromethylsulfonyl)imide
$[C_4mpyrr][Tf_2N]$	1-Butyl-3-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide
$[C_4mmim][Tf_2N]$	1-Butyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide

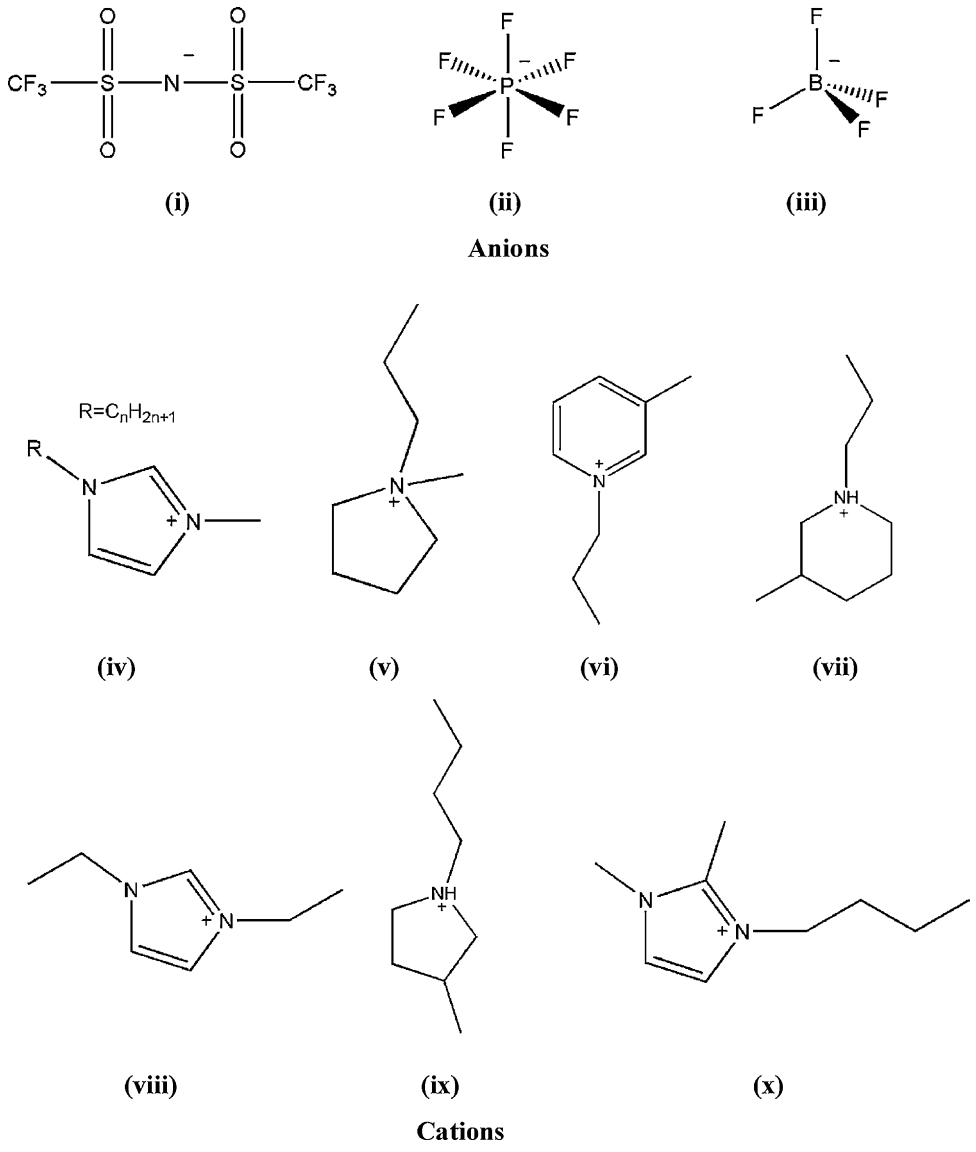

Fig. 1. Chemical structure of the anions and cations composing the ILs studied: (i) $[Tf_2N]^-$; (ii) $[PF_6]^-$; (iii) $[BF_4]^-$; (iv) $[C_n mim]^+$ (n, number of carbon atoms); (v) $[C_3 mpyrr]^+$; (vi) $[C_3 mpyr]^+$; (vii) $[C_3 mpip]^+$; (viii) $[C_2C_2 im]^+$; (ix) $[C_4 mpyrr]^+$; (x) $[C_4 mmim]^+$.

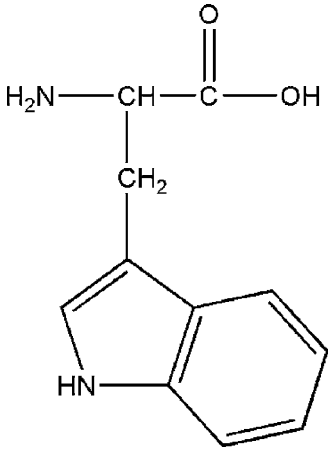

Fig. 2. Chemical structure of tryptophan.

by:

$$P_{IL/W} = \frac{[Trp]_{IL}}{[Trp]_W} \quad (1)$$

where $[Trp]_{IL}$ and $[Trp]_W$ refer to the L-tryptophan concentration in the IL and water-rich phases, respectively. At least two distinct extractions and quantifications were carried out for each IL and pH under study and the partition data presented are an average of the obtained values.

3. Results and discussion

The results obtained for the partition coefficients of Trp between the IL and the water phases, at 298.15 K and at different values of pH are reported in Table 2. In order to better visualize and evaluate the effect of the different experimental conditions (chemical structure of ILs and pH) at which extraction equilibrium experiments were performed, the measured data are represented in Figs. 3–7 and discussed in the subsections below.

In a previous study [33], the effects of a series of aminoacids on the solubility of a partially miscible IL, 1-butyl-3-

Table 2
Partition coefficients, $P_{IL/W}$, of Trp between the IL and aqueous-rich phases as a function of the pH.

IL	pH	$P_{IL/W}$	IL	pH	$P_{IL/W}$
$[C_2mim][Tf_2N]$	1.00	4.5 ± 0.1	$[C_6mim][BF_4]$	1.01	7.8 ± 0.1
$[C_3mim][Tf_2N]$	0.98	1.80 ± 0.02	$[C_8mim][BF_4]$	0.98	2.8 ± 0.1
$[C_4mim][Tf_2N]$	1.05	0.93 ± 0.02	$[C_4mim][PF_6]$	0.95	0.42 ± 0.02
	1.48	0.477 ± 0.009		1.17	0.238 ± 0.003
	1.50	0.451 ± 0.004	$[C_8mim][PF_6]$	0.99	0.020 ± 0.005
	1.68	0.382 ± 0.009	$[C_3mpyrr][Tf_2N]$	0.97	2.2 ± 0.1
	1.89	0.218 ± 0.005	$[C_3mpyr][Tf_2N]$	0.96	1.75 ± 0.02
	2.50	0.074 ± 0.005	$[C_3mpip][Tf_2N]$	0.99	1.30 ± 0.08
	2.85	0.002 ± 0.001	$[C_2C_2im][Tf_2N]$	1.01	1.80 ± 0.01
$[C_5mim][Tf_2N]$	1.00	0.35 ± 0.07	$[C_4mpyrr][Tf_2N]$	1.00	0.79 ± 0.05
$[C_6mim][Tf_2N]$	0.98	0.256 ± 0.001	$[C_4mmim][Tf_2N]$	1.01	0.51 ± 0.02
$[C_7mim][Tf_2N]$	0.98	0.11 ± 0.03			
$[C_8mim][Tf_2N]$	0.98	0.030 ± 0.005			

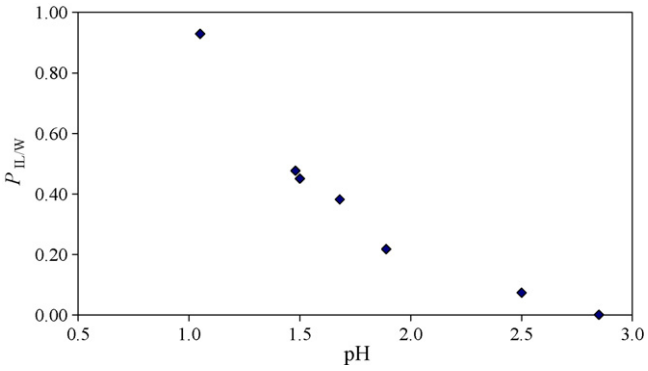

Fig. 3. pH dependence of partition coefficients ($P_{IL/W}$) of Trp between $[C_4mim][Tf_2N]$ and water-rich phases.

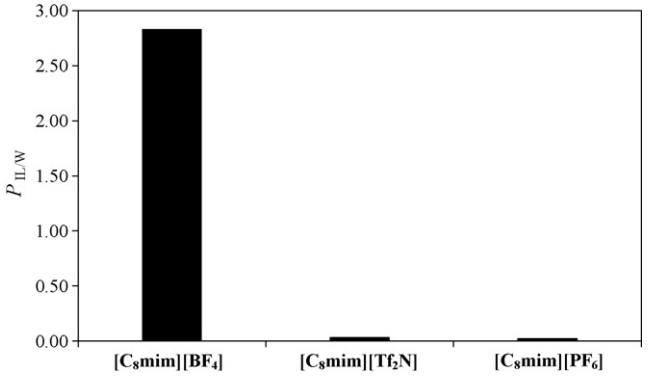

Fig. 4. Partition coefficients ($P_{IL/W}$) of Trp between $[C_8mim]$-based ILs and water-rich phases comprising anions from different families.

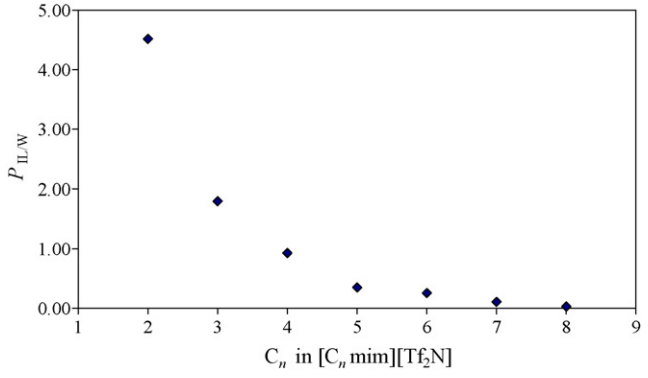

Fig. 5. Partition coefficients ($P_{IL/W}$) of Trp between $[C_nmim][Tf_2N]$ ILs and water-rich phases, at pH 1.0. *n* is the number of carbons constituting the alkyl chain of the cations.

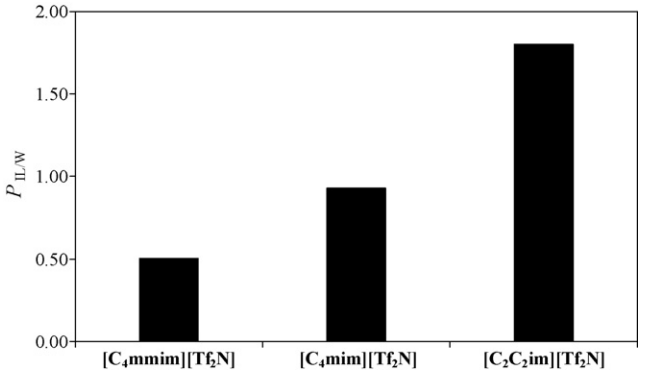

Fig. 6. Partition coefficients ($P_{IL/W}$) of Trp between $[Tf_2N]$-based ILs and water-rich phases comprising imidazolium-based cations with different number of substitutions in the alkylic chain at pH 1.0.

methylimidazolium tricyanomethane, in water were shown to be dependent on the charge, size and polarity of the aminoacids side chains and were explained in terms of a delicate balance between (water–aminoacid side chain), (IL–aminoacid side chain) and (water–IL) interactions, determined by the relative affinities of the biomolecules side chains to water and to IL. The partition coefficients between hydrophobic ILs and water of a model biomolecule, such as Trp, at different experimental conditions, can thus give a more quantitative perspective of the phase preference of the aminoacid and also a more comprehensive view of the factors determining the underlying interactions established in solution. Those are likely to involve van der Waals forces, electrostatic interactions, hydrogen-bonding and $\pi\cdots\pi$ stacking between imidazolium rings and the aromatic ring of L-tryptophan. Indeed,

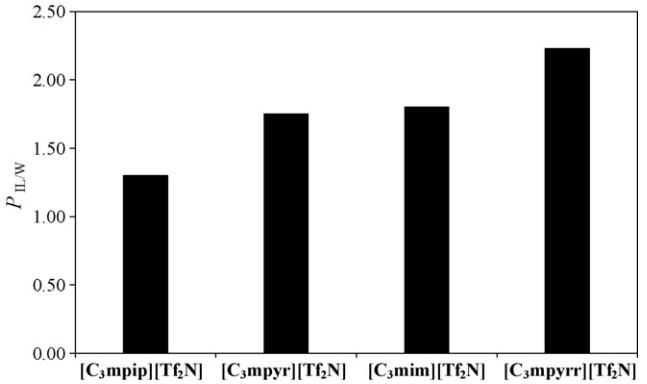

Fig. 7. Partition coefficients ($P_{IL/W}$) of Trp between $[Tf_2N]$-based ILs and water-rich phases comprising cations from different families.

$\pi\cdots\pi$ stacking between imidazolium and benzene molecules has been previously reported [36].

3.1. pH dependence of partition coefficients

It is well known that, depending on the pH of the solution, aminoacids can be present in the form of different species due to the ionization/protonation of their characteristic functional groups, –COOH and $–NH_2$. Since for Trp, protonation constants values are $pK_1 = 2.38$ and $pK_2 = 9.39$ [9], the aminoacid is predominately on its cationic form (and therefore positively charged) in the experiments performed at pH below 2.38 and is a zwitterion (with no net charge) or an anion if the $pK_1 < pH < pK_2$ or $pH > pK_2$, respectively. In any case, the side chain is always neutral. Globally speaking, in face of the results obtained in this work, the pH has a deep influence on the aminoacid distribution between the IL and the aqueous phases. This feature is clearly shown in the example depicted in Fig. 3 for the partition coefficients of Trp into $[C_4mim][Tf_2N]$ and can be further confirmed in the two experiments with $[C_4mim][PF_6]$ at two different pHs. The trend observed is a steep decrease of $P_{IL/W}$ in the range $pH < pK_1$; when the condition is $pH > pK_1$, the magnitude of the partition coefficients drops significantly, although the decrease becomes less accentuated in that pH range. Actually, for pH values of 2.50 and 2.85, the $P_{IL/W}$ of Trp into $[C_4mim][Tf_2N]$ approaches a negligible value. As far as the range $pH < pK_1$ is concerned, the aminoacid is present in the cationic form and the percentage of the latest decreases with increasing the pH of the aqueous phase. It is therefore reasonable to assume that the interactions responsible for the extraction will possibly be established between the Trp cation and the anion of the IL. On the other hand, in the absence of anions in the aqueous phase, for the Trp cation to transfer from water into the IL phase, and not violate the electroneutrality requirement, an ion-exchange process between the cationic form of Trp and the IL cation must be operating. Therefore, the strongest the interaction between Trp and the anion of the IL, the more efficient is the extraction. This mechanism might be understood in the same way that metal ions extraction into an IL in the presence of a neutral extractant can be interpreted [37,38]. As the amount of cationic form decreases, the electrostatic interactions established with the anion of the IL are weakened, and the exchange of the Trp cation with the imidazolium cation is disfavored, resulting in a smaller affinity of the solute for the IL phase. When the zwitterionic form is dominant, as it happens for pH values between 2.50 and 2.85, the interaction of the solute with the IL becomes different in nature and smaller in magnitude. Simultaneously, the polarity of the zwitterion confers to the aminoacid a more advantageous interaction with water than with the IL, which results on a pronounced decrease in $P_{IL/W}$.

3.2. Effect of the chemical structure of the IL anion

As can be seen from Table 2 and Fig. 4, the partition coefficients of Trp into $[C_8mim][BF_4]$ are much higher than those of $[C_8mim][Tf_2N]$ and $[C_8mim][PF_6]$, under the same conditions. The latter two are quite similar, although the value for $[C_8mim][PF_6]$ is slightly lower. This trend is confirmed by the extractions with $[C_4mim][Tf_2N]$ and $[C_4mim][PF_6]$. The data obtained in this work for ILs comprising different anions further supports the importance of electrostatic interactions between the IL anion and the positively charged solute on the extraction of the aminoacid. Actually, the results gathered suggest that the more localized electrostatic charge of $[BF_4]^-$ originates stronger electrostatic interactions with the cationic form of Trp, determining a higher affinity of the aminoacid for the IL. It is worth noticing that the same type of interactions was observed in other studies concerning the extraction of other aminoacids, including Trp, into other ILs [9] and in the $[C_4mim][C(CN)_3]$/water solubility behaviour induced by the positively charged side chains of alanine and lysine [33]. In spite of the good results obtained for the partition coefficients, it is not prudent, however, from a practical point of view, to use $[BF_4]$-based ILs for the extraction of biomolecules from aqueous media since, as shown before, those ILs are not stable in the presence of water under several experimental pH and temperature conditions [39]. Although chemically and thermally stable at moderate experimental conditions, decomposition only becomes significant for $[PF_6]^-$ under acidic media or high temperatures where PF_6-based ILs might also encounter processing problems or have environmental hazardous impacts [39].

3.3. Effect of the chemical structure of the IL cation

Although the anionĭs nature of the IL would be likely to play the most important role in the extraction process of Trp, since the aminoacid is in the cationic form at the studied experimental conditions, the effect of the chemical structure of the IL cation was also taken into account in this study. In fact, the results presented below indicate that factors such as the length of the alkyl chain, the number of substitutions in the hydrophobic moiety and the chemical family of the IL cation also have a significant impact on the phase distribution of the aminoacid.

Thermodynamic data gathered in a previous work [33] suggest that aminoacids when present in (hydrophobic IL + water) mixtures, interact with the IL through a complex interplay of electrostatic and dispersive interactions between the non-polar moieties of the IL cation and the alkyl chains of the aminoacid, (positively charged group of the aminoacid–IL anion), (positively charged group of the aminoacid–hydrophobic moiety of the IL cation) and well-known hydrophobic interactions [40,41]. These interactions are therefore governing the relative affinity of the aminoacid with the two phases. It is the balance between those types of interactions that determines the aminoacid partitioning observed between the phases.

From the data represented in Fig. 5 for the partition of Trp into $[C_nmim][Tf_2N]$, with $2 \geq n \leq 8$, it is clear that the preference of the aminoacid for the IL phase decreases as the length of the longer alkyl substituent in the cation increases. As can be seen from Table 2, these facts are further confirmed, not only for imidazolium-based ILs comprising other fluorinated anions, but also for ILs constituted by cations belonging to a different family ($[C_3mpyrr][Tf_2N]$ and $[C_4mpyrr][Tf_2N]$). It is also worth to note that previous works [9] on the extraction of Trp from $[C_6mim][BF_4]$ and $[C_8mim][BF_4]$ aqueous mixtures have also reported this trend, although the absolute values obtained for the partition coefficients are slightly different from those got in this work, probably due to a non total match of experimental conditions, namely the pH of the aqueous solutions which, as discussed before, has a profound influence on the partition coefficients. Independently of the IL anion type, Trp has thus a larger affinity for ILs with shorter alkylic chains, or, in other words, the magnitude of the interactions (aminoacid–IL cation/anion) is higher for these systems. Two aspects have to be considered: a decrease in the length of the longer alkyl substituent of the IL cation implies a decrease in its hydrophobicity, which will facilitate its interaction with the cationic form of the aminoacid; a long alkyl side chain of the IL cation originates a screening effect and therefore less effective electrostatic interactions between Trp and the anion of the ILs [9]. The same type of arguments can be used to explain the increase of the partition coefficient values with the number of substitutions in the IL cation, shown in Fig. 6. In fact, IL rings with more substitutions (and thus higher number of carbon atoms) are more hydrophobic and do not favor interactions with the cationic form of the aminoacid. Moreover, a steric hindrance effect promoted by large size and volume of these IL cations will

weaken the electrostatic interactions of Trp with the anion of the IL.

Finally, as indicated by the results displayed in Fig. 7, the chemical family of the IL cation has a noticeable influence on the extraction of Trp. The affinity of Trp for the ILs increases in the order $[C_3mpip][Tf_2N] < [C_3mpyr][Tf_2N] \approx [C_3mim][Tf_2N] < [C_3mpyrr][Tf_2N]$. Due to the $\pi\cdots\pi$ stacking between the imidazolium or pyridinium rings and the aromatic ring of Trp and to additional N–H$\cdots\pi$ interactions, where the pyrrole of Trp acts as NH donor and the aromatic imidazolium or pyridinium rings as acceptors, the interactions aminoacid-IL cation are strengthened relatively to those established with the cation of $[C_3mpip][Tf_2N]$. As a consequence, the partition coefficient of Trp into the last IL is smaller than into the former two ILs. Actually, interactions involving an aromatic π system as a donor or acceptor [42] and $\pi\cdots\pi$ stacking between imidazolium and benzene molecules [36] are well established, and N–H$\cdots\pi$(pyrrole) hydrogen-bonding processes were reported to arise in the crystal structure of porphyrin precursors [43]. In spite of the absence of any aromaticity, the value obtained for $[C_3mpyrr][Tf_2N]$ is the highest. Although this result might look at first rather intriguing, it actually leads to suggestive conclusions, once compared with the $[C_4mpyrr][Tf_2N]$ system, whose cation only comprises one additional methylene group, but has two different substitution sites. Despite being, as discussed above, quite evident that an increase in the length of the longer alkyl substituent in the IL cation originates a decrease of the affinity of the aminoacid for the ILs, the difference found in the magnitude of $P_{IL/W}$ of Trp into $[C_3mpyrr]$ and $[C_4mpyrr][Tf_2N]$ cannot be only attributed to that fact. Actually, the dialkylic substitution at the same site in $[C_3mpyrr][Tf_2N]$ turns the cation of this IL more compact and that will have consequences not only as far as (IL cation–Trp) interactions are concerned, but also at the level of the intermolecular forces established between the aminoacid and the IL anion. In fact, if on one hand there is a much less screening effect for the electrostatic attractive interactions of Trp with the anion of $[C_3mpyrr]$ than of $[C_4mpyrr]$, on the other hand there is a much more effective package of the former cation around the aminoacid molecule, resulting in stronger interactions. In fact, if the interaction (IL cation–aminoacid) is thought as occurring simultaneously between (N^+ of the IL cation–COO^- of Trp) and (hydrophobic moiety of IL cation–hydrophobic moiety of Trp), it is definitely easier for $[C_3mpyrr]^+$ to orientate itself around Trp and originate more effective electrostatic and dispersive interactions than for $[C_4mpyrr]^+$. Furthermore, the dispersive interactions of the former cation with the hydrophobic moieties of Trp will be stronger since those are strictly dependent on contact areas and ramification of alkyl chains. This interpretation for the difference of affinity of Trp between these two ILs can be used to explain the trend observed for the $P_{IL/W}$ of Trp between water and ILs comprising cations belonging to different families.

4. Conclusions

The potential use of hydrophobic ILs as substitutes for organic solvents in liquid–liquid extraction processes of biomolecules was evaluated by the determination of the partition coefficients of an aminoacid, Trp, taken as model biomolecule, between different ILs and water.

The partition coefficients determined are strongly dependent on the pH of the aqueous phase, on the nature of the anion of the IL and on the chemical structure of the IL cation. The more efficient extractions are obtained in the range $pH < pK_1$ and when ILs with the $[BF_4]$ anion are employed. The ILs extraction ability for Trp decreases with the increase of the length of the alkyl side chain and with the increasing number of substitutions at the IL cation. Pyrrolidinium-based ILs are much better extraction phases than ILs comprising imidazolium and pyridinium rings, while those provide a higher degree of extraction than the correspondent piperidinium-based ones.

From a molecular point of view, the evidence reported suggests that, besides the obvious determinant attractive electrostatic interactions between the cationic form of Trp and the IL anion, a complex interplay of other important interactions established at the level of the IL cation govern the phase preference of the aminoacid, supporting also earlier molecular interpretations. Furthermore, ion-exchange processes play a significant role in the mechanism of phase transfer and are determinant in interpreting the behaviour of such systems at different pH values.

From a practical point of view, the information gathered highlights the suitability of (TF_2N^--based hydrophobic ILs/water) mixtures as liquid–liquid extraction systems. In spite of the good results obtained, BF_4^--based ILs are not, in practice, recommended for the extraction of biomolecules from aqueous media, particularly in acidic media, due to their low stability. The results reported might thus be used for the optimization of biochemical recovery and purification procedures through the manipulation of the experimental conditions and the development of new ILs that can meet the requirements of any particular task.

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