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

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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 K₃PO₄ 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 diagrams⁴,⁵,⁹,¹⁴,¹⁵ (where the ion’s influence follows 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 diagrams⁴,⁵,⁹,¹⁴,¹⁵ (where the ion’s influence follows

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14 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.¹⁰–¹² 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 (K₃PO₄).
Ternary phase diagrams (binodal curves and tie-lines) for systems of hydrophilic ILs + K₃PO₄ + 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. 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). 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. 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 K₃PO₄, ≥98 % w/w pure from Sigma, and different aqueous solutions of hydrophilic ILs. The chloride-based ILs studied were imidazolium chloride, [C₄mim]Cl; methylimidazolium chloride, [C₃mim]Cl; ethylimidazolium chloride, [C₂mim]Cl; 1,3-dimethylimidazolium chloride, [C₃mim]Cl; 1-ethyl-3-methylimidazolium chloride, [C₅mim]Cl; 1-butyl-3-methylimidazolium chloride, [C₆mim]Cl; 1-hexyl-3-methylimidazolium chloride, [C₇mim]Cl; 1-allyl-3-methylimidazolium chloride, [C₈mim]Cl; 1-hydroxyethyl-3-methylimidazolium chloride, [OHC₆mim]Cl; and 1-benzyl-3-methylimidazolium chloride, [C₇H₇mim]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 [C₃mim]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 ¹H and ¹³C 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 at 298 ± 1 K. The experimental procedure adopted was validated with the phase diagram obtained for the [C₄mim]Cl + water + K₃PO₄ ternary system against literature data. Aqueous solutions of K₃PO₄ 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 ±10⁻⁴ g.

Figure 1. Chemical structure of the ILs studied: (i) [C₁mim]Cl; (ii) [C₂mim]Cl; (iii) [C₃mim]Cl; (iv) [C₄mim]Cl; (v) [C₅mim]Cl; (vi) [C₆mim]Cl; (vii) [OHC₆mim]Cl; (viii) [im]Cl; (ix) [C₁im]Cl; (x) [C₂im]Cl.
The tie-lines (TLs) were determined by a gravimetric method described by Merchuck et al.\textsuperscript{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.\textsuperscript{23} For that purpose the experimental binodal curves were correlated using eq 1:\textsuperscript{23}

\[ Y = A \exp[(BX^{0.5}) - (CX^3)] \] (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, \( K_{\text{Trp}} \), are defined as the ratio of the concentration of L-tryptophan in the IL and in the \( \text{K}_3\text{PO}_4 \) aqueous-rich phases, described by eq 2:

\[ K_{\text{Trp}} = \frac{[\text{Trp}]_{\text{IL}}}{[\text{Trp}]_{\text{K}_3\text{PO}_4}} \] (2)

where \([\text{Trp}]_{\text{IL}}\) and \([\text{Trp}]_{\text{K}_3\text{PO}_4}\) are the concentration of L-tryptophan in the IL and in the \( \text{K}_3\text{PO}_4 \) 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 spectrosopy 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.\textsuperscript{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.\textsuperscript{14}

The experimental phase diagrams at 298 K and at atmospheric pressure for each IL + \( \text{K}_3\text{PO}_4 + \text{H}_2\text{O} \) 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 \([\text{amim}]\text{Cl}; [\text{OHC}_2\text{mim}]\text{Cl}; [\text{C}_7\text{H}_7\text{mim}]\text{Cl}; [\text{C}_6\text{mim}]\text{Cl}; [\text{C}_4\text{mim}]\text{Cl}; [\text{C}_2\text{mim}]\text{Cl}; \) and Figure 3 shows the binodal curve for \([\text{C}_2\text{im}]\text{Cl}; [\text{C}_1\text{im}]\text{Cl}; \) and \([\text{im}]\text{Cl}\).
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. 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 formation of 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 than their disubstituted analogues. In PEG-based ABS the salt anion has the major impact on polymer solubility and hence in the ABS phase diagrams, while the cation has a minor (though sizable) effect. For ILs, it is here shown that the IL cation has a huge influence in the ABS-promoting ability as a result of the higher hydrophilicity of the IL when compared with \([\text{C}_2\text{mim}]\text{Cl}\). Also the presence of a double bond at the allyl group of the imidazolium cation (\([\text{amim}]\text{Cl}\)) or the presence of a benzyl group (\([\text{C}_7\text{H}_7\text{mim}]\text{Cl}\)) as a substituent group decreases the ability to form ABS, although in a less pronounced way.

The experimental binodal curves were fit to the empirical relationship described by eq 1,\(^\text{23}\) 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 \([\text{C}_4\text{mim}]\text{Cl} + \text{K}_3\text{PO}_4 + \text{water} \) at 298 K: (\(\square\)) binodal curve data; (\(\bigcirc\)) TL data.

Partitioning of \(\text{L-Tryptophan}\) in ABS. Hydrophobic interactions play a major role in the tertiary structure of proteins and 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.\(^\text{30}\) 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.

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

<table>
<thead>
<tr>
<th>IL</th>
<th>wt fraction composition (%)</th>
<th>TL eq: IL (wt %) = a (wt %) + bK3PO4 (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL</td>
<td>K3PO4</td>
</tr>
<tr>
<td>[amim]Cl</td>
<td>26.91</td>
<td>15.91</td>
</tr>
<tr>
<td>[OHC2mim]Cl</td>
<td>40.57</td>
<td>16.05</td>
</tr>
<tr>
<td>[C3mim]Cl</td>
<td>25.90</td>
<td>14.90</td>
</tr>
<tr>
<td>[C4mim]Cl</td>
<td>25.35</td>
<td>15.97</td>
</tr>
<tr>
<td>[C5H7mim]Cl</td>
<td>25.11</td>
<td>18.30</td>
</tr>
<tr>
<td>[C7mim]Cl</td>
<td>15.36</td>
<td>30.53</td>
</tr>
<tr>
<td>[im]Cl</td>
<td>15.24</td>
<td>32.80</td>
</tr>
</tbody>
</table>

The results obtained in this work show that the magnitudes of the 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 hydrophobic ILs to be used in liquid–liquid extractions, and new phase equilibrium data for systems involving hydrophilic imidazolium-based ILs + K3PO4 + 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 [C4mim]Cl > [C5H7mim]Cl > [C7mim]Cl ≥ [C1im]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 substituted 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.

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Supporting Information Available: Synthesis of [C1mim]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

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