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Mechanisms ruling the partition of solutes in ionic-liquid-based aqueous biphasic systems – the multiple effects of ionic liquids

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Abstract

In the past decade it has been demonstrated the remarkable potential of ionic-liquid-based aqueous biphasic systems (IL-based ABS) to extract and purify a large range of valued-added biocompounds. However, the transposal of lab-experiments to an industrial scale has been precluded by a poor understanding of the molecular-level mechanisms ruling the separation or partition of target compounds between the coexisting phases. To overcome this limitation we carried out a systematic evaluation of specific interactions, induced by ILs and several salts used as phase-forming components, and their impact on the partition of several solutes in IL-based ABS. To this end, the physicochemical characterization of ABS composed of imidazolium-based ILs, three salts (Na_2SO_4 , K_2CO_3 and $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$) and water was performed. The ability of the coexisting phases to participate in different solute-solvent interactions (where “solvent” corresponds to each ABS phase), were estimated based on the Gibbs free energy of transfer of a methylene group between the phases in equilibrium, $G(\text{CH}_2)$, and on the Kamlet-Taft parameters – dipolarity/polarizability (π^*), hydrogen-bonding donor acidity (α) and hydrogen-bonding acceptor basicity (β) – of the coexisting phases. Relationships between the partition coefficients, the phases properties expressed as Kamlet-Taft parameters and COSMO-RS descriptors were established, highlighting the ability of ILs to establish specific interactions with given solutes. The assembled results clearly support the idea that the partition of solutes in IL-based ABS is due to multiple effects resulting from both global solute-solvent and specific solute-IL interactions. Solute-IL specific interactions are often dominant in IL-based ABS, explaining the higher partition coefficients, extraction efficiencies and selectivities observed with these systems when compared to more traditional ones majorly composed of polymers.

Introduction

Aqueous biphasic systems (ABS) have been investigated in liquid-liquid extraction processes, demonstrating to be particularly suitable for the separation of biomolecules since they are formed by two immiscible aqueous phases. They can be formed by mixing two polymers, a polymer and a salt, or two salts in aqueous media, and above a given

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Conflicts of interest

There are no conflicts to declare.

concentration the phase separation takes place.^{1–3} Besides the conventional polymer-based ABS, in 2003, Rogers and co-workers⁴ demonstrated that ABS could be created by mixing ionic liquids (ILs) and inorganic salts. After this proof of principle, in the following years it was shown that IL-based ABS present additional advantages over the polymer-based systems, namely a lower viscosity, faster phase separation, and higher extraction efficiencies and tailored selectivity for a wide range of biomolecules. After more than a decade of studies, IL-based ABS are nowadays considered one of the most promising media for the extraction and separation of a broad range of biocompounds.⁵

While very promising at lab-scale, ABS have faced a limited industrial interest, and examples for their commercial application are few.^{1–3,6,7} The use of ABS at an industrial scale has been plagued by a poor understanding of the molecular-level mechanisms which rule the partition of the target solutes/biomolecules/products between the coexisting phases, limiting the ability to design ABS for a specific application, and converting the development of novel ABS-based separation processes on a lengthy trial and error approach. With the purpose of improving the understanding of the partition of target products in ABS, several works reporting insights on the physicochemical characterization of ABS composed of polymer/polymer and polymer/salt combinations have been reported.^{8–16} It seems consensual that the distribution of compounds in these more traditional ABS is ruled by differences in solute-solvent interactions.⁸

Among the approaches available for the ABS physicochemical characterization, the Gibbs free energy of transfer of a methylene group between the phases and the Kamlet-Taft Linear Solvation Energy Relationship (LSER) have been successfully used for the description of the partition of solutes in ABS composed of polymers and salts.^{3,4,8–15,17–19} The relative affinity of the coexisting phases for methylene groups in ABS has shown to be an important factor controlling the molecules partition.^{3,4,17–19} This property can be assessed through the analysis of the partition coefficients of a homologous series of compounds with aliphatic moieties of increasing length. The logarithm of partition coefficients is linearly dependent on the equivalent number of methylene groups in the alkyl side-chain, $n(CH_2)$, as described by Eqn (1).

$$\ln(K) = C + E \cdot n(CH_2) \quad (1)$$

where the parameters C and E are characteristic constants for a given ABS. C corresponds to the partition of the non-alkyl moiety of the molecule, and has been suggested to represent the difference in the polar/electrostatic properties of the phases, if sodium salts of dinitrophenyl-amino-acids (DNP-amino-acids) are partitioned. E is related with the Gibbs free energy of transfer of a methylene group between the phases in equilibrium, $\Delta G(CH_2)$, and has been proposed as a measure of the relative hydrophobicity of the phases, given by the equation:³

$$\Delta G(CH_2) = - RTE \quad (2)$$

where R is the universal gas constant, and T the absolute temperature. $G(CH_2)$ has also been used as a measure of the free energy for cavity formation.¹³

Polarity is widely used to classify solvents and their solvation ability, and is defined as the sum of all specific and non-specific interactions occurring between a solvent and a solute, without considering the interactions associated to solute chemical transformations.^{20,21} Since it is impossible to describe the multiple solute-solvent interactions using a single parameter, Kamlet and Taft proposed a multiparametric approach based on a set of solvatochromic probes, allowing the assessment of different interactions for the same solvent,^{22–24} such as its dipolarity/polarizability (π^*), hydrogen bond donor (α) and hydrogen bond acceptor (β) abilities. These parameters allowed the development of correlations for the description of a variety of properties using the LSER model^{22–24}, given by:

$$(XYZ) = (XYZ)_0 + s\pi^* + a\alpha + b\beta \quad (3)$$

where (XYZ) is the value for a particular solvent-dependent property, $(XYZ)_0$ is the value for the reference system, and s , a , and b are the solute-dependent coefficients characterizing the respective influence of π^* , α and β terms on the (XYZ) property under study.^{22–24} There are several examples of the successful application of this approach to correlate properties of solvents and solvent mixtures.^{8–14,25}

In addition to previous efforts concerning the physicochemical characterization of polymer/polymer and polymer/salt ABS by the determination of the solvatochromic parameters and relative hydrophobicity of the coexisting phases,^{8–10,14,15} their application in IL-based ABS has been limited and hampered by experimental difficulties, as discussed below.^{19,26–28} Although nowadays a large database is available for, π^* , α , and β for pure ILs,^{21,29–32} no solvatochromic parameters for the IL-based ABS phases are available and only relative hydrophobicities for a small number of systems have been reported.^{19,26–28}

Due to the increased interest on IL-based ABS for the extraction, separation and purification of value-added biomolecules and products,⁵ the understanding of the mechanisms which rule the ABS formation and the solute partition in these systems is of extreme relevance aiming the design of effective separation systems, that could ultimately be applied at an industrial scale. To overcome this limitation, we performed a comprehensive study on the physicochemical properties of ABS composed of imidazolium-based ILs, three salts – K_2CO_3 , Na_2SO_4 , and $K_3C_6H_5O_7$ – and water. Different probes were used to assess the coexisting phases' Kamlet-Taft parameters, namely their dipolarity/polarizability (π^*), hydrogen-bond acidity (α), and hydrogen-bond basicity (β). Additionally, the partition coefficients of a series of DNP-amino-acids were determined to infer on the relative hydrophobicity and electrostatic properties of the coexisting phases. Finally, it was assessed the relationship between the DNP-amino-acids partition coefficients and the phases properties as characterized by the Kamlet-Taft parameters measured. COSMO-RS (CONductor-like Screening MOdel for Real Solvents) parameters were used to complement these correlations and to evaluate the relevance of solute-IL specific interactions.

Results and discussion

Phase diagrams

The binodal curves of the ABS formed by imidazolium-based ILs and the salts K_2CO_3 , Na_2SO_4 , and $K_3C_6H_5O_7$ were determined at room temperature ($\approx 25^\circ C$). The definition of the ILs acronyms is provided as a footnote[‡], and their chemical structures are presented in the ESI[†]. The detailed experimental procedure, data and graphical representations of the phase diagrams are given in the ESI[†]. Several experimental tie-lines (TLs), which give the composition of each phase for a given biphasic mixture, and their respective length (TLL), were additionally determined for each ABS. In Table 1 are given the TLs that will be used in further discussions. The remaining TLs are reported in the ESI[†].

Gibbs free energy of a methylene group transfer between the ABS phases

Using a series of homologues DNP-amino-acids (DNP-glycine, DNP-alanine, DNP-valine, and DNP-leucine), the Gibbs free energy of transfer of a methylene group, $G(CH_2)$, from the aqueous salt-rich to the aqueous IL-rich phase was determined for the ABS listed in Table 1. The detailed data corresponding to the DNP-amino-acids partition coefficients are reported in the ESI[†]. The difference of the electrostatic properties and relative hydrophobicity of the coexisting phases (parameters C and E , respectively) were estimated by Eqn 1, while $G(CH_2)$ was obtained from Eqn 2. The respective results are reported in Table 2.

Independently of the salt used and pH, $G(CH_2)$ is always negative, meaning that the transfer of a non-polar $-CH_2$ group from the salt- to the IL-rich phase is favorable, and that methylene groups have a higher affinity to IL-rich than to salt-rich phases. The $G(CH_2)$ values of IL + K_2CO_3 and IL + 111 $K_3C_6H_5O_7$ ABS range from -0.59 to $-1.2 \text{ kJ}\cdot\text{mol}^{-1}$ and -0.37 to $- \text{kJ}\cdot\text{mol}^{-1}$, respectively, while a more restricted range is observed for ABS composed of Na_2SO_4 salt, namely from -0.54 to $-0.7 \text{ kJ}\cdot\text{mol}^{-1}$.

The Gibbs free energies of transfer of a methylene group between the coexisting phases in IL-based ABS were previously reported by Wu et al.^{27,28} The authors evaluated the effect of the IL anion²⁷ and cation²⁸, observing a strong dependency of $G(CH_2)$ according to the ILs hydrophobicity, and thus on the ILs ability to form ABS. Although the $G(CH_2)$ values reported in Table 2 are close to those reported by Wu et al.^{27,28}, the reported behavior was not observed in the systems studied in this work. It should be noted that Wu et al.^{27,28} determined the $G(CH_2)$ values at a fixed mixture composition, while in this work a constant TLL was used (*cf.* ESI[†]). As the length of the TL increases, the two phases become more distinct in compositions and properties, resulting in a change on the driving forces acting upon the molecules partition. Thus, when a fixed mixture composition is used in a set

[‡]ILs acronyms: 1-butyl-3-methylimidazolium triflate ($[C_4C_1im][CF_3SO_3]$); 1-butyl-3-methylimidazolium thiocyanate ($[C_4C_1im][SCN]$); 1-butyl-3-methylimidazolium tosylate ($[C_4C_1im][TOS]$); 1-butyl-3-methylimidazolium dicyanamide ($[C_4C_1im][N(CN)_2]$); 1-butyl-3-methylimidazolium ethylsulfate ($[C_4C_1im][C_2H_5SO_4]$); 1-butyl-3-methylimidazolium methylsulfate ($[C_4C_1im][CH_3SO_4]$); 1-butyl-3-methylimidazolium bromide ($[C_4C_1im]Br$); 1-butyl-3-methylimidazolium dimethylphosphate ($[C_4C_1im][DMP]$); 1-butyl-3-methylimidazolium methylacetate ($[C_4C_1im][CH_3CO_2]$); 1-butyl-3-methylimidazolium methanesulfonate, ($[C_4C_1im][CH_3SO_3]$); 1-butyl-3-methylimidazolium chloride ($[C_4C_1im]Cl$); 1-butyl-3-methylimidazolium octylsulfate ($[C_4C_1im][C_8H_{17}SO_4]$).

of systems which differ in the IL nature, not only the IL nature will influence the molecules extraction or the properties determination, but also the TLL. In the case of, $G(CH_2)$ as the TLL increases (larger differences between the two phases compositions), the Gibbs free energy value also increases,^{4,14} and similar results to those reported by Wu et al.^{27,28} could be obtained. Yet, these would lead to a wrong interpretation of the phases relative hydrophobicities as the results are dominated by the TLL effect rather than by the nature of the phases.

Through the analysis of the $G(CH_2)$ results obtained for the imidazolium-based ILs studied here, it is not possible to identify any type of relation between this parameter and the IL concentration or its nature. Since the Gibbs free energy of transfer of a methyl group is only related with the relative hydrophobicity of the phases (*cf.* Eqn 3), it seems clear that at a fixed TLL, the hydrophobicity of IL-based ABS phases results from a delicate balance between the nature and concentration of all phase forming components – IL, salt and water – not being dominated by just one of them.

The effect of a methylene group in $\ln(K)$ is independent of the nature of the partitioned solutes, meaning that E is a characteristic of the system under study. On the other hand, the contribution of the polar group of the solutes, represented by the constant C , depends on both the composition of the ABS and the solute being partitioned. Since a homologous series of solutes was used, the effect of solute nature will be constant and the differences observed in the parameter C result only from the composition of the studied systems. For the studied IL-based ABS, the parameter C is considerably high, ranging between 1.9 and 6.07 for K_2CO_3 -based ABS, between 1.57 and 4.0 for Na_2SO_4 -based ABS, and between 1.90 and 2.71 for $K_3C_6H_5O_7$ -based ABS (*cf.* Table 2). These results suggest that the polar group of DNP-amino-acids has a significant effect on their partition in IL-based ABS.

In a previous work³³ it was shown that the COSMO-RS^{34–37} hydrogen bond energies, E_{HB} , correlate well with the hydrogen bond donor basicity of ILs. In this work, a linear relationship between the parameter C and E_{HB} of the ILs that compose the K_2CO_3 -, Na_2SO_4 - and $K_3C_6H_5O_7$ -based ABS has additionally been found, as depicted in Fig. 1. Thus, this correlation indicates that for IL-based systems, C is mainly dependent on the chemical nature of the IL and is a result of polar/hydrogen bonding interactions differences of the coexisting phases. This also suggests that specific solute-IL interactions are present and have a significant effect on the partition of biomolecules in IL-based ABS. However, while for K_2CO_3 - and Na_2SO_4 -based ABS the parameter has C a significant dependence on the ILs E_{HB} , in $K_3C_6H_5O_7$ -based ABS this effect is considerably weakened due to the strong salting-out nature of this salt. This suggests that the nature of the IL may be masked by the effect of the salt and that IL specific interactions play a more significant role in the solutes partitioning in ABS based on weaker salting-out salts, as demonstrated before with partition coefficients experimental data.³⁸

The methylene group also plays a relevant role since the partition coefficients of DNP-amino-acids considerably increase with $n(CH_2)$ – *cf.* the ESI†. Nevertheless, considering the partition of DNP-leucine in the ABS composed of $[C_4C_1im][CF_3SO_3] + K_2CO_3 + H_2O$, the contribution of the DNP-amino-acid polar group is represented by the value of C , *i.e.* 1.9,

while the contribution of the methylene groups is equal to 1.7 (product of the parameter E , 0.29, by the number of equivalent methylene groups, in this case 3.75).³ Therefore, the contributions of both the polar and non-polar moieties are positive and similar. On the other hand, considering the partition of the same DNP-amino-acid in $[\text{C}_4\text{C}_1\text{im}]\text{Cl} + \text{K}_2\text{CO}_3 + \text{H}_2\text{O}$ system, the contributions of both polar and non-polar groups are 5.4 and 1.2, respectively, meaning that the influence of the polar groups is more than 4 times higher than that from the non-polar groups, suggesting that polar/hydrogen bonding interactions are the main driving force for this partition. Furthermore, this effect is more evident the smaller is the number of CH_2 equivalent groups present in DNP-amino-acids.

In Fig. 2 is represented the ratio between the contribution of the polar group (*i.e.* polar/hydrogen bonding interactions) over the contribution of non-polar group (*i.e.* hydrophobic interactions) – $R_{\text{polar/non-polar}}$ – for all DNP-amino-acids partition in K_2CO_3 -based ABS. The results obtained for Na_2SO_4 - and $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ -based ABS are reported in the ESI†. The number of equivalent methylene groups for DNP-glycine, DNP-valine, DNP-alanine and DNP-leucine are 0.06, 1.31, 2.65 and 3.75,³ respectively. Since DNP-glycine has a $n(\text{CH}_2)$ close to 0, the contribution of the hydrophobic interactions in its partition is almost null, and therefore the $R_{\text{polar/non-polar}}$ is high. Nevertheless, even when DNP-amino-acids with higher $n(\text{CH}_2)$ are considered, the contribution of the polar group is always higher than that of methylene groups (the ratio is always higher than unity) – Fig. 2. Furthermore, similarly to what was previously observed for the parameter C (Fig. 1), this ratio tends to increase with the E_{HB} of the IL, independently of the salt used, and even for ABS based on the strong salting-out salt $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ – *cf.* ESI†. ILs with higher COSMO-RS hydrogen-bonding energies have a higher ability to hydrogen bond with the solutes and, consequently, the effect of the polar group in the partition of the DNP-amino-acids increases. However, if the system is constituted by an IL with lower E_{HB} values and a higher hydrophobic character, such as $[\text{C}_4\text{C}_1\text{im}][\text{CF}_3\text{SO}_3]$, the influence of the methylene groups in the partition becomes more important and the relative contributions of polar *vs.* non-polar interactions more similar (ratio close to 1).

Figure 3 depicts the comparison of $G(\text{CH}_2)$ values for different types of liquid-liquid systems previously reported in the literature. Since several factors could affect the $G(\text{CH}_2)$ values, whenever possible, $G(\text{CH}_2)$ determined at similar TLLs were chosen. IL-based ABS^{27,28} present similar $G(\text{CH}_2)$ values to polymer-salt ABS^{15,39}, and considerably higher $G(\text{CH}_2)$ values than polymer-polymer systems^{3,13}. Furthermore, binary systems composed of organic solvents and water¹⁷ and micellar systems⁴⁰ are those which present the largest differences, due to their highly distinct phases chemical composition. Whenever possible the contribution of the polar group over the non-polar group ($R_{\text{polar/non-polar}}$) was also calculated, being represented in Fig. 3. The ratio between the polar and non-polar contributions is lower in the systems with higher $G(\text{CH}_2)$; this parameter shows that the polar group contribution is always slightly higher than that of the non-polar group in IL-based ABS, while for polymer-salt ABS this ratio tends to be closer to 1. For organic solvents-water systems, the polar group displays a non-favorable impact on the solutes partition for the organic phase ($C < 0$, and consequently $R_{\text{polar/non-polar}} < 0$). These results clearly reveal the different nature of IL-based ABS when compared with other types of liquid-liquid systems.

Kamlet-Taft parameters

The Kamlet-Taft parameters – solvent dipolarity/polarizability, π^* , solvent hydrogen-bond donor acidity, α , and solvent hydrogen bond acceptor basicity, β – of IL-rich and salt-rich phases of IL- K_2CO_3 , IL- Na_2SO_4 and IL- $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ ABS, and their differences (calculated as the difference of a given parameter between the IL- and salt-rich phases), were determined at a constant TLL. Here, and hereafter, “solvent” refers to the global phase mixture, and not to an individual component, such as water, salt or IL. Each of the Kamlet-Taft parameters was obtained from a set of single probes. The obtained results are given in Table 2. Independently of the salt used, the salt-rich phases always present higher dipolarity/polarizability values than the IL-rich phases. Furthermore, the π^* values range is larger for the IL-rich than for the salt-rich phases, that present dipolarity/polarizability values close to those of pure water ($\pi^*_{\text{H}_2\text{O}}=1.26$). Pure ILs usually display dipolarity/polarizability values considerably lower than water (*cf.* the ESI[†]), and therefore binary solutions of water and ILs are expected to present lower dipolarity/polarizability, as observed for the ABS IL-rich phases. However, these results are highly distinct from those observed for ABS composed of polymers and/or salts, where in general both phases present a higher dipolarity/polarizability than water.^{10,14} Although some discussions could be drawn based on these results, from the solutes partition point of view, the difference in the Kamlet-Taft parameters between the coexisting phases seems to be more relevant. The differences between the π^* values of the coexisting phases in IL-based ABS are slightly higher than those observed in polymer-based systems.^{10,14} Furthermore, considering the π^* values for K_2CO_3 -, Na_2SO_4 - and $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ -based ABS, there is a decrease of this difference with the increase of the IL hydrophilic character (ranked in terms of E_{HB}). These results suggest that non-specific dispersion interactions (dipole-dipole, van der Waals, among others) could be more significant in the partition of solutes in IL-based than in polymer-based systems, and their impact could be tuned by the hydrophilic character of the IL.

The Kamlet-Taft parameter that seems to be less affected by the composition of the IL-based ABS is the hydrogen bond donor acidity. For K_2CO_3 - and Na_2SO_4 -based ABS this parameter is almost constant in salt-rich phases with values close to that of pure water ($\alpha_{\text{H}_2\text{O}}=1.36$). However, for $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ -based ABS, a significant decrease on the salt-rich phase hydrogen bond donor acidity is observed in the systems composed of $[\text{C}_4\text{C}_1\text{im}]\text{Cl}$ and $[\text{C}_4\text{C}_1\text{im}]\text{Br}$, which could be a consequence of the high concentration of IL and salt present in both phases – *cf.* Table 1. In IL-rich phases slight oscillations are observed for this parameter. Although the IL anion plays a dominant role, the hydrogen bond donor acidity is mainly determined by the IL cation, which justifies the small variations observed for this parameter in the studied systems. However, the differences between the coexisting phases (α) are considerably higher than those observed for the dipolarity/polarizability and the hydrogen bond acceptor basicity. This behavior is similar to that observed in polymer-salt-based ABS.^{10,14}

Similarly to polymer-polymer- and polymer-salt-based ABS,^{9,10,14} the hydrogen bond acceptor basicity values of IL-based ABS coexisting phases are considerably higher than those of pure water (*cf.* the ESI[†]), with IL-rich phases presenting higher values than salt-rich phases. ILs present considerably higher hydrogen-bond acceptor ability than water, meaning

that their presence in an aqueous solution will increase the β value of the mixture. The hydrogen-bond acceptor ability is mainly controlled by the IL anion, justifying the large variations observed in β for the various systems studied. As observed in dipolarity/polarizability, also β for K_2CO_3 -based ABS seems to decrease with the IL hydrophobicity. However, a clear tendency was not identified for Na_2SO_4 - and $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ -based ABS.

To better understand the Kamlet-Taft parameters variation on the phase diagram of an IL-based ABS, a more comprehensive analysis was carried out for the water- $[\text{C}_4\text{C}_1\text{im}][\text{TOS}]$ - K_2CO_3 ternary system. The Kamlet-Taft parameters were determined in binary solutions of $[\text{C}_4\text{C}_1\text{im}][\text{TOS}]$ + water and K_2CO_3 + water, and in monophasic and biphasic ternary mixtures of $[\text{C}_4\text{C}_1\text{im}][\text{TOS}]$ - K_2CO_3 ABS. The compositions of each mixture are reported in the ESI†. The π^* , α and β parameters are represented as a contour plot on the ternary phase diagram of $[\text{C}_4\text{C}_1\text{im}][\text{TOS}]$ - K_2CO_3 ABS in Fig. 4.

According to Fig. 4 A, the dipolarity/polarizability is mostly affected by water and K_2CO_3 concentrations. The addition of salt to highly diluted aqueous solutions slightly increases the value of π^* , but when the water concentration is below 80 wt %, the dipolarity/polarizability significantly decreases. On the other hand, the hydrogen-bond donor ability of the ternary mixture seems to be ruled by the water content (Fig. 4 B). Although weaker, a similar tendency is observed for the hydrogen-bond acceptor ability (Fig. 4 C). Concerning the TLL effect on the Kamlet-Taft parameters, the coexisting phases present symmetrical behaviors. For instance, the π^* of salt-rich phases increases and in IL-rich phases decreases with the TLL increase (Fig. 4 A). A similar behavior was observed for the hydrogen-bond acceptor ability (Fig. 4 C). In fact, as the IL concentration in salt-rich phase decreases with the increase of the TLL, π^* and β values for this phase became closer to pure water. The increase of IL concentration in the IL-rich phase induces the opposite behavior. Thus, π^* and β significantly increase with the TLL, contrarily to what is observed for ABS composed of polymers and salts, where these two parameters are essentially independent of the TLL.^{10,14} It was previously shown that in polymer-polymer or polymer-salt ABS the solvatochromic probes do not specifically interact with the phase-forming components (salts and/or polymers).⁴¹ However, the results here reported indicate that in specific IL-solvatochromic probes interactions occur in IL-based ABS, playing a significant role in the solvatochromic behavior of the probes.

In what concerns the α of the coexisting phases, and α , they present a similar behavior to that previously observed for polymer-salt ABS.^{10,14} The salt rich-phase displays a hydrogen bond donor acidity close to pure water and independent of the TLL, while the IL-rich phase value decreases considerably with the TLL. Since the water content of the salt-rich phases is almost constant, while it changes considerably in IL-rich phases with the increase of TLL, these trends are probably related with the water concentration in the phases.

As previously mentioned, the Kamlet-Taft parameters differences between the coexisting phases in ABS are relevant factors to understand the partition of a solute. ABS constituted by ILs and salts present a considerably broader variation of both non-specific and specific interactions, suggesting that this type of systems could be seen as a good option for the

separation of molecules and compounds in which their partition could be ruled by specific interactions. Furthermore, contrarily to the observed in polymer-based systems,^{10,14} these differences are strongly dependent on the type and concentration of IL and salt, as well as on the concentration of water that compose the system, suggesting that IL-based ABS can be tuned for the enhanced and selective extraction of a specific solute.

Relations between the various parameters studied

Previous studies on the characterization of polymer-polymer^{12,42} and polymer-salt ABS^{43,44} have shown that (i) both E and C parameters could be correlated by a linear combination of solvent properties of the coexisting phases of the ABS, and (ii) solute partition could be adequately described through a modified solvatochromic equation, based on Eqn 3. These results led to the conclusion that solute partition in polymer-polymer and polymer-salt ABS is mainly driven by solute-solvent interactions.^{12,42–44}

Analysis of the results obtained in the present work using IL-based ABS show that no such correlations could be derived. For example, the description of the E parameter with the solvent properties π^* , α , and β for the 16 ABS reported (Table 2), gives a correlation coefficient of 0.14, while for the C parameter, using the same procedure within the same ABS, gives a correlation coefficient of 0.58 – *cf.* the ESI†. These results suggest that the driving forces for solutes partition in IL-based are different from conventional polymer-based ABS.

The concept of solute-solvent interactions as main driving force for solute partition in ABS is widely recognized not to be applicable to systems containing charged polymers or polymers with specific ligands.^{45,46} In these systems, the partition is driven by solute-ligand specific interactions, similar to what is observed in IL-based ABS^{47,48}, and in polymer-salt ABS for which ILs were used as additives.⁴⁹ In these works it was shown that the presence of ILs as phase-forming components or simply as additives results in significant improvement in the ABS extraction performance. Specific solute-IL interactions in aqueous media have been recently reported by us,⁵⁰ demonstrating that ILs act as hydrotropes. By dynamic light scattering (DLS), molecular dynamics (MD) simulation and nuclear magnetic resonance (NMR) it was shown that the enhanced solubility of sparingly soluble solutes in water is driven by the formation of solute-IL aggregates, and that this IL-mediated hydrotropic effect is also solute-dependent.

Considering these previous observations⁵⁰ and the high partition coefficients or extraction efficiencies obtained with IL-based ABS, we analyzed the partition data obtained in the present work (i) in the absence of IL-mediated hydrotropic effect, and (ii) in the presence of moderate IL-mediated hydrotropic effect, also by the application of a modified solvatochromic equation, based on Eqn 3. In the absence of IL-mediated hydrotropic effect, similarly to what is observed in polymer-polymer ABS, solute partition in IL-based ABS is mainly driven by solute-solvent interactions. On the other hand, when the partition occurs in presence of moderate IL-mediated hydrotropic effect, the solute partition is governed by a synergistic effect of both solute-solvent and solute-IL specific interactions. The results obtained are illustrated by Eqn 4 for the $[\text{C}_4\text{C}_1\text{im}][\text{SCN}]$ - and $[\text{C}_4\text{C}_1\text{im}][\text{N}(\text{CN})_2]$ -based

ABS, and by Eqn 5 for the [C₄C₁im][SCN]⁻; [C₄C₁im][N(CN)₂]⁻[C₄C₁im][TOS]⁻ and [C₄C₁im][CF₃SO₃]⁻-based ABS:

$$\ln(K_{DNP-Gly}) = 0.1_{\pm 0.1} - 25_{\pm 1} \Delta \pi^* - 7.7_{\pm 0.5} \Delta \alpha \quad (4)$$

$n = 5$, $r^2 = 0.9971$, $F = 348.7$, $SD = 0.06$, $p(0) = 0.5$; $p(\pi^*) = 0.002$; $p(\alpha) = 0.005$; *Outl.*: [C₄C₁im][SCN]⁻-Na₂SO₄ ABS;

$$\ln(K_{DNP-Gly}) = 16_{\pm 3} - 20_{\pm 3} \Delta \pi^* - 8_{\pm 2} \Delta \alpha - 1.5_{\pm 0.2} E_{HB} - 2.1_{\pm 0.4} E_{MF} - 1.2_{\pm 0.2} V_{dw}$$

(5)

$n = 11$, $r^2 = 0.9562$, $F = 21.8$, $SD = 0.22$, $p(0) = 0.003$; $p(\pi^*) = 0.0005$; $p(\alpha) = 0.01$; $p(E_{HB}) = 0.0008$; $p(E_{MF}) = 0.003$; $p(E_{vdw}) = 0.003$; *Outl.*: [C₄C₁im][CF₃SO₃]⁻-K₂CO₃ ABS, where n is the number of experimental $\ln(K)$ values; r is the correlation coefficient; F is the ratio of variance; SD is the standard deviation; p is the common statistic p-value based on the null hypothesis; “*Outl.*” is an outlier in the correlation; all the other parameters are as defined above.

The results illustrated by Eqn 4 indicate that in presence of the [SCN]⁻ and [N(CN)₂]⁻ anions, regardless of the salt used in the formation of ABS, no solute-IL specific interactions seem to be present, and consequently partition data can be adequately described by the solvent parameters π^* and α . These results are similar to those previously described for polymer-polymer and polymer-salt ABS,^{12,42–44} and seem to indicate that DNP-amino-acids partition in [C₄C₁im][SCN]⁻ and [C₄C₁im][N(CN)₂]⁻-based ABS is governed by solute-solvent interactions. However, in the presence of [TOS]⁻ and [CF₃SO₃]⁻ anions, the experimental data can only be adequately described by introducing as additional parameters the COSMO descriptors E_{HB} , E_{MF} and E_{vdw} , as illustrated by Eqn 5. These parameters are estimated through COSMO-RS, which reflect the IL hydrogen-bonding interaction energies, E_{HB} , electrostatic-misfit interactions, E_{MF} , and van der Waals forces, E_{vdw} , accounting therefore for the ability of these ILs to establish specific interactions and thus for solute-IL specific interactions. Similar results were obtained for the remaining DNP-amino-acids, and for the E and C parameters – cf. the ESI†.

In summary, from the overall results obtained in the present work it is shown that the solute partitions in IL-based ABS is a result of multiple effects arising from the solute-IL and solute-solvent interactions, which further depend on the IL chemical nature. The solute-IL specific interactions, both derived from hydrotropic effects or from the enhanced polarity resulting from the $R_{polar/non-polar}$ previously discussed, may thus explain some experimental observations of enhanced partitions and selectivities provided by IL-based ABS.

Conclusions

With the aim to better understand the molecular-level mechanisms which rule the partition of molecules/solutes in ABS composed of ILs and salts, the Gibbs free energy of a methylene group transfer and the Kamlet-Taft parameters of the phases in equilibrium were here studied. The relative hydrophobicity of the coexisting phases of a wide range of ABS composed of [C₄C₁im]-based ILs and K₂CO₃, Na₂SO₄ or K₃C₆H₅O₇, was determined by the partition of a series of DNP-amino-acids. It was found that the partition of DNP-amino-acids in IL-salt ABS is mainly ruled by hydrogen-bond interactions with ILs, and that the nature of ILs is more significant when weaker salting-out salts are used. It was additionally evaluated how the Kamlet-Taft parameters of the ABS coexisting phases – dipolarity/polarizability (π^*), hydrogen-bonding donor acidity (α) and hydrogen-bonding acceptor basicity (β) – change with their composition. ABS constituted by ILs and salts display a considerably high variation of the Kamlet-Taft parameters differences between the coexisting phases, with a significant dependency on the type and concentration of IL and salt. The results obtained suggest that, when compared with polymer-based systems, IL-based ABS are be more favorable for the extraction of biomolecules in which their partition could be ruled by both specific and non-specific interactions.

The correlations found for the DNP-amino-acids partition coefficients with both the solvent properties and ILs ability to stablish specific interactions (here evaluated by the IL interaction energies estimated by COSMO-RS as descriptors), suggests that the driving forces governing the solutes partition in IL-based ABS result from a multiple solute-solvent non-specific interactions and solute-IL specific interactions, both derived from hydrotropic effects or from their enhanced polarity. Contrarily to the dominant solute-solvent non-specific interactions present in conventional polymer-based ABS, the presence of solute-IL specific interactions explains the high partition coefficients, extraction efficiencies, and selectivity usually observed in IL-based ABS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Relationship between C and COSMO-RS hydrogen-bonding energies (E_{HB}) for K_2CO_3 -based ABS (■), Na_2SO_4 -based ABS (◆) and $K_3C_6H_5O_7$ -based ABS (▲).

Fig. 2.

Ratio of the contribution of polar (*i.e.* polar/hydrogen bonding interactions) and non-polar groups (*i.e.* hydrophobic interactions), $R_{\text{polar/non-polar}}$ of DNP-amino-acids (DNP-glycine – blue bars; DNP-valine – red bars; DNP-alanine – green bars and DNP-leucine – purple bars) on their partition in K_2CO_3 -based ABS.

Fig. 3.

Gibbs free energy $-\Delta G(CH_2)$ of transfer of a methylene group between the coexisting phases of different types of liquid-liquid system and the ratio of solutes polar and non-polar groups contributions ($R_{polar/non-polar}$) in the partition of a molecule with $n(CH_2) \approx 4$: ABS composed of IL/salt, 27, 28 polymer/salt (light red – smallest TL; darker red – largest TL), 15, 39 polymer/polymer, 3, 13 and binary systems composed of organic solvents/water¹⁷ and micellar-systems.⁴⁰ ILs and compounds abbreviations: 1-butyl-3-methylimidazolium L-serine ([C₄mim][Ser]), 1-butyl-3-methylimidazolium glycine ([C₄mim][Gly]), 1-butyl-3-methylimidazolium L-alanine ([C₄mim][Ala]) and 1-butyl-3-methylimidazolium L-leucine ([C₄mim][Leu]), tetramethylammonium glycine ([N₁₁₁₁][Gly]), tetraethylammonium glycine ([N₂₂₂₂][Gly]), tetrabutylammonium glycine ([N₄₄₄₄][Gly]), tetrabutylphosphonium glycine ([P₄₄₄₄][Gly]), tetrapentylammonium glycine ([N₅₅₅₅][Gly]), polyethylene glycol (PEG), sodium dodecyl sulphate (SDS), and sodium octyl sulphate (SOS).

Fig. 4.

The effect of ABS composition in the Kamlet-Taft parameters: (A) π^* , (B) α , and (C) β . [C₄C₁im][TOS]-K₂CO₃-based ABS: binary mixtures of [C₄C₁im][TOS] and water (red circles), binary mixtures of K₂CO₃ and water (purple circles); IL-rich phases (blue circles); salt-rich phases (green circles).

Table 1Experimental data for TLs and TLLs of [C₄C₁im]-based ILs + salt + H₂O ABS at 25°C.^a

IL	Weight fraction composition / wt %						TLL
	[IL] _{IL}	[salt] _{IL}	[IL] _M	[salt] _M	[IL] _{salt}	[salt] _{salt}	
IL + K ₂ CO ₃ + H ₂ O ABS							
[C ₄ C ₁ im][CF ₃ SO ₃]	58.32	1.04	37.05	4.40	8.85	9.13	50.13
[C ₄ C ₁ im][SCN]	54.83	1.86	27.78	8.77	3.96	14.97	52.9
[C ₄ C ₁ im][TOS]	45.96	4.19	26.85	10.27	6.29	16.81	41.62
	46.91	3.99	28.34	10.16	4.28	18.16	44.92
	50.46	3.31	26.85	12.01	1.37	21.40	52.32
	53.31	2.82	28.01	12.21	0.96	22.25	55.85
	55.81	2.43	29.55	12.25	0.67	23.05	58.86
[C ₄ C ₁ im][N(CN) ₂]	60.81	3.04	29.08	10.80	3.01	17.18	59.51
[C ₄ C ₁ im]Br	49.87	3.11	26.80	15.50	3.33	28.23	52.89
[C ₄ C ₁ im][DMP]	45.32	5.78	23.50	21.59	2.09	37.10	53.38
[C ₄ C ₁ im]Cl	43.10	4.46	21.94	19.92	3.11	33.67	49.53
IL + Na ₂ SO ₄ + H ₂ O ABS							
[C ₄ C ₁ im][CF ₃ SO ₃]	52.55	1.30	35.13	3.93	11.19	7.54	41.82
[C ₄ C ₁ im][SCN]	46.37	1.56	34.06	5.00	5.91	12.85	42.01
[C ₄ C ₁ im][TOS]	44.35	3.33	32.76	8.00	4.35	19.50	43.14
[C ₄ C ₁ im][N(CN) ₂]	41.68	2.42	31.69	6.40	1.95	18.40	42.82
[C ₄ C ₁ im]Br	40.32	4.14	29.73	11.50	6.79	28.01	41.15
IL + K ₃ C ₆ H ₅ O ₇ + H ₂ O ABS							
[C ₄ C ₁ im][CF ₃ SO ₃]	50.78	2.91	35.00	10.00	3.02	24.36	52.36
[C ₄ C ₁ im][SCN]	56.77	2.57	30.00	9.60	9.61	16.64	49.21
[C ₄ C ₁ im][TOS]	51.24	6.21	32.06	15.39	6.34	27.71	49.79
[C ₄ C ₁ im][N(CN) ₂]	52.21	3.62	24.70	15.13	6.98	22.55	49.03
[C ₄ C ₁ im]Br	53.25	5.78	30.00	22.00	9.65	36.2	53.16
[C ₄ C ₁ im]Cl	45.94	11.64	27.50	30.00	10.45	46.97	50.08

^a [IL]_M, [IL]_{IL} and [IL]_{salt} correspond to IL weight fraction percentages in the initial mixture, and IL- and salt-rich phases, respectively; [salt]_M, [salt]_{IL} and [salt]_{salt} correspond to salt weight fraction percentages in the initial mixture, and IL- and salt-rich phases, respectively.

Table 2

Parameters C and E (Eqn 1), Gibbs free energy of transfer of a methylene group, $G(CH_2)$ (Eqn 2), and Kamlet-Taft parameters^a and their differences between the coexisting phases of IL-based ABS at 25°C.

IL	TLL	C	E	$G(CH_2)$ (kJ·mol ⁻¹)	π_{IL}^*	π_{salt}^*	π^*	α_{IL}	α_{salt}	α	β_{IL}	β_{salt}	β
IL + K₂CO₃ + H₂O ABS													
[C ₄ C ₁ im][CF ₃ SO ₃]	50.13	1.9 ± 0.1	0.29 ± 0.04	-0.7 ± 0.1	1.09	1.24	-0.15	1.13	1.34	-0.21	0.44	0.34	0.10
[C ₄ C ₁ im][SCN]	52.91	3.3 ± 0.1	0.31 ± 0.04	-0.8 ± 0.1	1.21	1.29	-0.08	1.17	1.34	-0.17	0.41	0.34	0.07
[C ₄ C ₁ im][TOS]	52.32	3.92 ± 0.02	0.237 ± 0.009	-0.59 ± 0.02	1.12	1.24	-0.12	1.21	1.33	-0.12	0.56	0.44	0.12
[C ₄ C ₁ im][N(CN) ₂]	59.51	3.9 ± 0.2	0.50 ± 0.07	-1.2 ± 0.2	1.18	1.28	-0.10	1.15	1.33	-0.18	0.41	0.35	0.06
[C ₄ C ₁ im]Br	52.89	4.1 ± 0.2	0.45 ± 0.08	-1.12 ± 0.08	1.27	1.29	-0.02	1.21	1.31	-0.10	0.49	0.44	0.05
[C ₄ C ₁ im][DMP]	53.38	6.07 ± 0.03	0.46 ± 0.03	-1.13 ± 0.07	1.20	n.s. ^b	---	1.18	1.30	-0.12	0.59	n.s. ^b	---
[C ₄ C ₁ im]Cl	49.53	5.4 ± 0.1	0.33 ± 0.04	-0.8 ± 0.1	1.26	n.s. ^b	---	1.21	1.31	-0.10	0.53	n.s. ^b	---
IL + Na₂SO₄ + H₂O ABS													
[C ₄ C ₁ im][CF ₃ SO ₃]	41.82	1.57 ± 0.04	0.24 ± 0.01	-0.60 ± 0.04	1.12	1.21	-0.09	1.17	1.35	-0.18	0.41	0.37	0.04
[C ₄ C ₁ im][SCN]	42.01	2.29 ± 0.02	0.218 ± 0.007	-0.54 ± 0.02	1.23	1.28	-0.05	1.23	1.35	-0.12	0.40	0.36	0.04
[C ₄ C ₁ im][TOS]	43.14	2.9 ± 0.1	0.30 ± 0.06	-0.7 ± 0.1	1.17	1.24	-0.07	1.26	1.35	-0.09	0.53	0.46	0.07
[C ₄ C ₁ im][N(CN) ₂]	42.82	2.54 ± 0.01	0.218 ± 0.005	-0.54 ± 0.01	1.20	1.27	-0.07	1.21	1.34	-0.13	0.39	0.34	0.05
[C ₄ C ₁ im]Br	41.15	4.0 ± 0.1	0.25 ± 0.04	-0.6 ± 0.1	1.31	1.32	-0.01	1.26	1.36	-0.10	0.46	0.41	0.05
IL + K₃C₆H₅O₇ + H₂O ABS													
[C ₄ C ₁ im][CF ₃ SO ₃]	52.36	2.21 ± 0.08	0.45 ± 0.03	-1.11 ± 0.07	1.13	1.23	-0.10	1.12	1.33	-0.21	0.50	0.46	0.04
[C ₄ C ₁ im][SCN]	49.21	1.90 ± 0.06	0.26 ± 0.02	-0.66 ± 0.06	1.26	1.30	-0.04	1.20	1.31	-0.11	0.41	0.39	0.02
[C ₄ C ₁ im][TOS]	49.79	2.36 ± 0.06	0.15 ± 0.02	-0.37 ± 0.06	1.15	1.18	-0.03	1.19	1.30	-0.11	0.54	0.50	0.04
[C ₄ C ₁ im][N(CN) ₂]	49.03	2.56 ± 0.06	0.20 ± 0.02	-0.49 ± 0.06	1.19	1.25	-0.02	1.03	1.29	-0.26	0.38	0.38	0
[C ₄ C ₁ im]Br	53.16	2.71 ± 0.03	0.18 ± 0.01	-0.44 ± 0.03	1.31	1.33	-0.02	1.19	1.27	-0.08	0.53	0.50	0.03
[C ₄ C ₁ im]Cl	50.08	2.56 ± 0.01	0.202 ± 0.005	-0.50 ± 0.01	1.28	1.29	-0.01	1.13	1.22	-0.09	0.61	0.60	0.01

^a the indexes M, IL and salt correspond to the initial mixture, and IL- and salt-rich phases, respectively

^b not soluble (n.s.).