

Combining ionic liquids and polyethylene glycols to boost the hydrophobic–hydrophilic range of aqueous biphasic systems†

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This work reveals, for the first time, that polymer–ionic-liquid-based aqueous biphasic systems (ABS) exhibit a much wider hydrophilic–hydrophobic range than conventional systems reported to date. Three probe dyes were used to demonstrate that either the polymer-rich or the ionic-liquid-rich layer can serve as the most hydrophobic phase. It was found that the phase polarities can be easily tuned by the choice of an appropriate ionic liquid.

Aqueous biphasic systems (ABS) consist of two immiscible aqueous-rich phases based on polymer–polymer, polymer–salt, or salt–salt combinations. When appropriately selected, these systems may constitute a greener alternative to typical solvent-extraction methods which use volatile and hazardous solvents.¹ ABS have a high water content (*ca.* 60–80% on a weight fraction basis) and are able to maintain the native conformation and biological activity of diverse biomolecules.¹

Polymer-based ABS have largely been employed in the separation and purification of proteins, enzymes, blood cells and antibiotics.^{1,2} However, and despite their advantages, polymer-based ABS exhibit limited polarity differences between the phases, a fact which restricts their application due to narrow extraction yields and purification factors. ABS constituted by two distinct polymers display coexisting phases of similar polarities, whereas polymer–salt ABS consist of a hydrophobic and an ionic, particularly hydrophilic layer. The limited array of polarity differences between the two-phases in these conventional systems has led to the search for alternative strategies, such as polymer

derivatization or the use of additives, aiming at improving the extraction yields of a target molecule.³ Nevertheless, these approaches make the process either more costly or complex.

Ionic liquids (ILs) have been proposed as alternatives to polymers as novel phase-forming components of ABS.⁴ They exhibit several interesting properties and one of the most remarkable and valuable features in the field of separation processes is the prospect to tailor their extraction ability by selection of the IL constituting ions.⁵ Indeed, ABS composed of ILs have already been shown to be enhanced platforms for the extraction of diverse biomolecules from aqueous media.⁶ In addition to ABS constituted by ILs and inorganic–organic salts, it has recently been demonstrated that the IL-based ABS concept can be extended up to polymer–IL combinations.⁷ Most studies in this area address the use of polypropylene glycol (PPG) due to its higher propensity for liquid–liquid demixing in the presence of “salting-out” inducing ILs.⁸ Nonetheless, the ABS formed by ILs and polyethylene glycol (PEG), a more hydrophilic polymer than PPG, are far more interesting and complex.⁹ For example, it has been observed that, despite the hydration capability of the isolated ions in aqueous media, the interactions occurring between the ionic fluid and the polymer play a major role in the formation of the respective ABS.^{9,10}

Here we use the ability to tailor the solvation of ILs by an adequate choice of their constituting ions, as well as the hydrophilic character of low molecular weight PEG, to expand the hydrophobic–hydrophilic range of polymer-based ABS, thus overcoming one of their major limitations. To initiate this work, ternary phase diagrams of ABS, composed of four different water-miscible chloride-based ILs† and PEG 1500, were determined at 323 K and atmospheric pressure by turbidimetric titration.⁹ The experimental binodal curves are shown in Fig. 1, while the detailed experimental weight fraction data and respective correlations^{9,11} are provided in the ESI.†

The data in Fig. 1 are provided in units of molality (mole of solute per kg of solvent) and reveal that the ability of the ILs to induce a PEG-1500-based ABS follows the trend: $[C_4mpip]Cl > [C_4mpyr]Cl > [C_2mim]Cl \approx [C_4mim]Cl$. This order correlates

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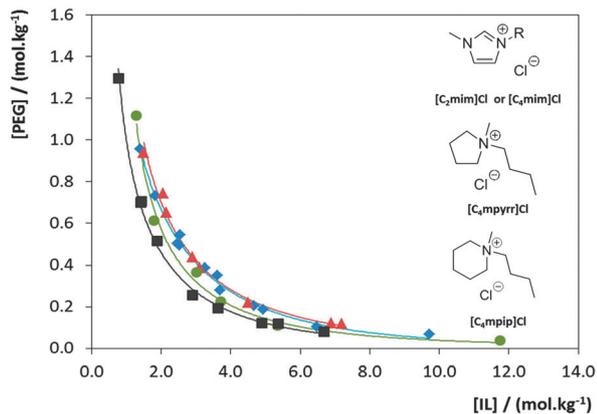


Fig. 1 Experimental solubility data and correlations for IL/PEG 1500 ABS at 323 K and atmospheric pressure: (■) [C₄mpip]Cl; (●) [C₄mpyrr]Cl; (◆) [C₂mim]Cl; (▲) [C₄mim]Cl. The molecular structures of the ILs are provided in the inset.

well with the relative hydrophobic nature of the IL, with the most hydrophobic species showing cloud points at lower concentrations of IL (for a given concentration of PEG). As previously reported in binary water–IL systems, imidazolium-based compounds exhibit stronger interactions with water as a result of their aromatic character.¹² On the other hand, piperidinium and pyrrolidinium are 5- and 6-sided saturated rings, respectively, and are thus less hydrophilic based ILs and with a lower propensity for interaction with water.¹² Furthermore, the increase of the alkyl side chain length also has a significant impact on the affinity of ILs for water. ILs with longer aliphatic tails are less water-soluble.¹²

Researchers reporting results for PPG⁸ suggested that the higher the IL ion's ability to create hydration complexes and/or hydrophilic character, the more easily the IL salts-out the polymer (*i.e.*, it takes less IL to salt-out a given amount of PPG). However, ABS formation with the less hydrophobic PEG is in fact more complex. In the current work, it was observed that the higher the affinity for water and/or the hydrophilic nature of the IL, the less effective such an IL is in promoting the PEG phase separation. According to the salting-out inducing hypothesis which has been presented for PPG,⁸ the less hydrophilic IL investigated, [C₄mpip]Cl,¹² should be the least effective in promoting the liquid–liquid regime. Instead, this IL is the most proficient in the formation of PEG-1500-based ABS. As detailed elsewhere,^{9,10} the interactions between PEGs and ILs, and how they are affected by water, cannot be neglected and have a significant impact on their phase separation. PEG is a polyether and, thus, hydrogen-bonding interactions are expected to occur with the chloride anion as well as with aromatic cations. The presence of π electrons leads to strong interactions with PEG and thus explains the low ability of imidazolium-based ILs to form ABS with PEG 1500. The enhanced affinity between these compounds would require a higher quantity of both solutes for liquid–liquid demixing. Indeed, results on the phase behaviour of binary systems constituted by polyether compounds and ILs proved that the polymers are more soluble in ionic fluids containing aromatic cations.¹³ In summary, when dealing with

ABS composed of ILs and PEGs, the molecular-level phenomena are more intricate than anticipated. The IL–PEG interactions cannot be neglected and are behind their ABS formation aptitude.^{9,10}

One of the major features of ILs is the wide variety of chemical structures that can be rearranged to create a target compound. It is well accepted that ILs display a wide polarity range,¹⁴ and hence, it is conceivable to tune the phase polarities in ABS by the adequate selection of the IL used as the phase-forming component.

In order to demonstrate the relative hydrophobicity–hydrophilicity of the two phases in each ABS, the partitioning behaviours of three dyes of different polarities (used as probe molecules) were investigated: chloranilic acid (CA, octanol–water partition coefficient – $\log K_{ow} = 1.22$), Indigo Carmine (IC, $\log K_{ow} = 1.627$), and Indigo Blue (IB, $\log K_{ow} = 3.188$).¹⁵ ABS combining PEG 1500 and 4 different ILs ([C₂mim]Cl, [C₄mim]Cl, [C₄mpyrr]Cl, and [C₄mpip]Cl) were prepared. A mixture point in the biphasic region, ≈ 38 wt% of PEG and ≈ 52 wt% of IL, was selected and used to evaluate the partitioning of the dyes. For comparison, the partitioning in ABS constituted by PEG 4000/Dextran 40 000 (≈ 10 wt%/ ≈ 10 wt%) and PEG 1500/Na₂SO₄ (≈ 16 wt%/ ≈ 23 wt%) was also studied. After an equilibration period of 3 h at 323 K, the top and bottom phases were separated and the partition coefficient (K) – defined as the concentration of each dye in the IL-rich phase to that in the PEG-rich phase – was measured (Fig. 2 and 3). Additional details on the experimental procedure, phase compositions, and partition coefficients are provided in the ESI.†

CA has pK_a values of 0.58 and 3.18.¹⁵ The respective dissociation curves and the pH values of the coexisting phases of each ABS are presented in the ESI.† In aqueous media CA may

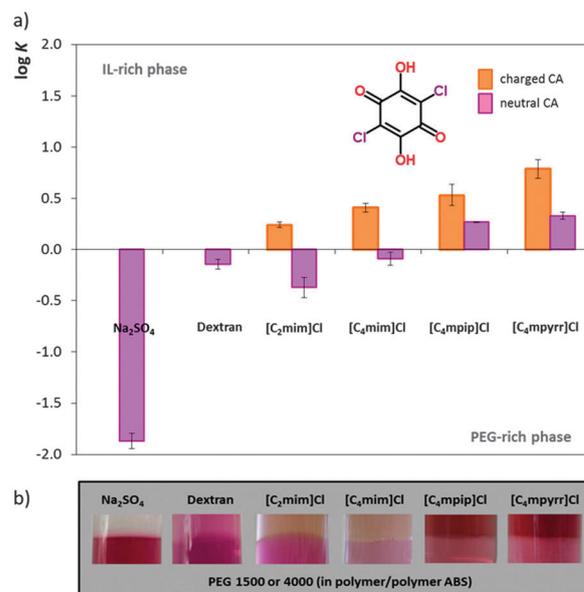


Fig. 2 (a) Log of the partition coefficients ($\log K$) of CA in several IL-based ABS at 323 K. (b) The colour of CA allows visual inspection of the phase preferences. The molecular structure of CA is provided in the inset.

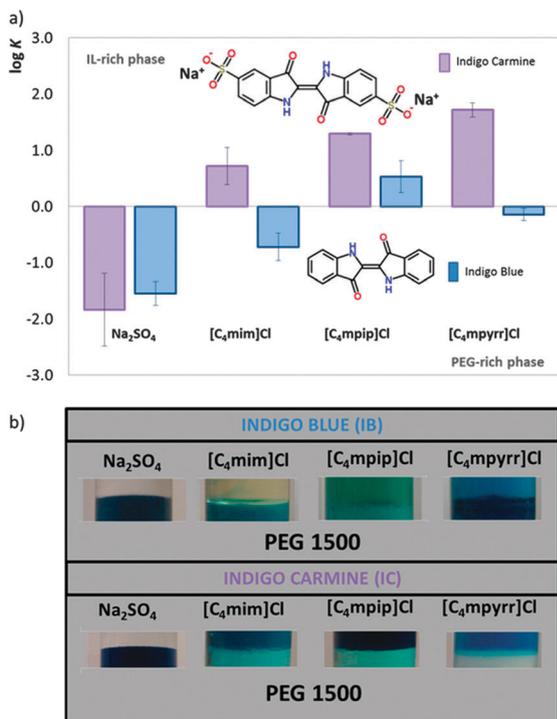


Fig. 3 (a) Log of the partition coefficients ($\log K$) of neutral IB and charged IC in several IL-based ABS at 323 K and in PEG 1500/Na₂SO₄ ABS. (b) The colour of IB and IC allows a visual inspection of the phase preferences. The molecular structures of IB and IC are provided in the inset.

exist as a cationic, zwitterionic, or anionic species depending on the pH. According to the results displayed in Fig. 2, at neutral pH, where CA is anionic (indicated as charged CA), a wide range of partition coefficients is observed within the various IL-PEG ABS; however, in all situations, anionic CA preferentially migrates to the IL-rich phase. This is a consequence of the negative charge of CA at this pH and its preference for the charged IL-rich phase. Therefore, in order to avoid the prevalence of electrostatic interactions, further experiments were conducted at a controlled pH (between 3 and 4) to maintain CA as a neutral species.

As depicted in Fig. 2, the partitioning of CA as a neutral molecule is remarkably different from that observed with its charged counterpart. Furthermore, with the traditionally studied polymer-polymer and polymer-salt systems, CA always migrates toward the PEG-rich phase – the most hydrophobic layer in both examples. On the other hand, this is not true for all of the PEG 1500/IL systems. CA still preferentially migrates to the PEG-rich phase in the [C₂mim]Cl- and [C₄mim]Cl-based ABS; nevertheless, this neutral dye actually prefers the IL phase when the more hydrophobic ILs, [C₄mpip]Cl and [C₄mpyrr]Cl, are used (Fig. 2). The most hydrophilic layer is the IL-rich phase in ABS composed of PEG 1500 and imidazolium-based ILs (aromatic fluids with higher affinity for water as discussed before). When moving towards more hydrophobic ILs, such as piperidinium- and pyrrolidinium-based compounds, the most hydrophobic phase becomes the polymer-rich one. This trend is in agreement with the discussion provided before regarding the hydrophobicity of

ILs and also revealed by the ILs aptitude to form ABS with PEG 1500.

CA has a high K_{ow} value reflecting its affinity for more lipophilic solvents.¹⁵ Hence, the inversion on the preferable migration of this probe dye clearly confirms an inversion on the relative phase polarities within the PEG 1500/IL-based ABS. It is therefore possible to tune the PEG-rich phase to be the more or less hydrophilic layer (simply by varying the IL cation).

In addition to the inversion of the migration pattern observed with the neutral CA, the effect of pH towards the partitioning behaviour should also be highlighted. In imidazolium-based systems, a reversal of the phase preference of the solute arises as a consequence of pH. An increase in the pH leads to a shift in the migration of the dye from the polymer- to the IL-rich phase. Visser *et al.*¹⁶ also demonstrated a reversible pH-dependent liquid-liquid partitioning of an indicator dye, thymol blue, but only with water immiscible ILs.

To further support the concept of switching the relative polarity of the phases of a PEG/IL ABS by choice of IL, the partition coefficients of Indigo Blue (IB) and Indigo Carmine (IC) were also determined. The partition coefficients and the visual appearance of the two dyes in each ABS are depicted in Fig. 3. The results obtained with a PEG 1500/Na₂SO₄ ABS are also shown for comparison. The details of the partition coefficient values, pH of the coexisting phases, and mixture compositions are shown in the ESI.†

The results illustrated in Fig. 3 were determined at a pH near 7 at which IB is mainly present as a neutral molecule and IC is in a salt form (see ESI† for the respective dissociation curves). While in a conventional system composed of PEG + inorganic salt, the partitioning of IB and IC to the PEG-rich phase is favoured, as was observed with CA, the IB partitioning pattern demonstrates that it is possible to manipulate and control the dye partition by a thoughtful selection of the IL. The inversion of the phase polarities is again confirmed by the reversal of the dyes' preferential partitioning. The partitioning of the IC salt, as previously observed for the charged CA, is different and reveals a preferential migration toward the charged IL-rich phase in all systems.

In summary, and in addition to the inversion on the polarities of the coexisting phases shown in Fig. 2 and 3, the wider hydrophilic-hydrophobic range that these new systems provide when compared with the traditional polymer-polymer and polymer-salt ABS should be remarked. Starting from the ABS formed with the inorganic salt, the scale on the phase polarities is largely increased with the new polymer-IL systems. Furthermore, the polarities of these coexisting phases can be fine-tuned by the choice of an appropriate IL. These features were confirmed with the migration of three probe dyes of different hydrophobicities and making use of their charged and neutral states. The inversion of the phase polarities was established by an observed shift in the dyes' preferential migration, *i.e.*, either towards the PEG- or the IL-rich phases. This versatility of PEG/IL-based ABS creates a plethora of new extraction and purification systems hitherto unknown.

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Notes and references

‡ ILS used: 1-ethyl-3-methylimidazolium chloride, [C₂mim]Cl; 1-butyl-3-methylimidazolium chloride, [C₄mim]Cl; 1-butyl-1-methylpiperidinium chloride, [C₄mpip]Cl; 1-butyl-1-methylpyrrolidinium chloride, [C₄mpyr]Cl.

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