

Insight into the Interactions That Control the Phase Behaviour of New Aqueous Biphasic Systems Composed of Polyethylene Glycol Polymers and Ionic Liquids

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Abstract: New polyethylene glycol (PEG)/ionic liquid aqueous biphasic systems (ABS) are presented. Distinct pairs of PEG polymers and ionic liquids can induce phase separation in aqueous media when dissolved at appropriate concentrations. Phase diagrams have been determined for a large array of systems at 298, 308 and 323 K. A comparison of the binodal curves allowed the analysis of the tunable structural features of the ionic liquid (i.e., anionic nature, cationic core, cationic alkyl side chain length and functionalisation, and number of alkyl substituents in the cation) and the influence of the molecular weight of

the PEG polymer on the ability of these solutes to induce an ABS. It was observed that contrary to typical ABS based on ionic liquids and inorganic salts, in which the phase behaviour is dominated by the formation of the hydration complexes of the ions, the interactions between the PEG polymers and ionic liquids control the phase demixing in the polymer-type ABS studied herein. It is shown that both the ionic liquids and PEG polymers

can act as the salting-out species; that is, it is an occurrence that is dependent on the structural features of the ionic liquid. For the first time, PEG/ionic liquid ABS are reported and insight into the major interactions that govern the polymer/ionic liquid phase behaviour in aqueous media are provided. The use of two different nonvolatile and tunable species (i.e., ionic liquids and PEG polymers) to form ABS allows the polarities of the phases to be tailored. Hence, the development of environmentally friendly separation processes that make use of these novel systems is envisaged.

Keywords: biphasic systems • ionic liquids • phase diagrams • polyethylene glycol • salting-in/-out

Introduction

Aqueous biphasic systems (ABS) constitute a greener alternative to classical solvent-extraction approaches that make use of organic and volatile solvents. In an ABS, two aqueous phases coexist in equilibrium as a result of the dissolution, at appropriate concentrations, of suitable pairs of solutes (e.g., two polymers or a polymer and a salt) in water. Because both the phases are mainly composed of water (more than 80% on a molar basis), the native conformation and biological activity of the biomolecules in solution is preserved. Thus, ABS have been satisfactorily tested for over forty years in the separation of biological substances, such as proteins and blood cells.^[1–7] Nevertheless, these systems have also found application in other types of separations, for example, in the extraction of metal ions,^[8] and on the whole ABS strategies can be considered to be a more sustainable alternative to many separations carried out by traditional solvent extraction.

The first report of an ABS dates back from as early as the late 19th century, when Beijerinck mixed gelatin, agar, and water in certain proportions to obtain an aqueous system with two macroscopic phases.^[9] However, only during the recent decades, have ABS been consistently studied for separation purposes. After knowing for years about a series of

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different ABS created by two polymers and water (especially polyethylene glycol (PEG) and dextran), Albertsson reported in 1986 the possibility of forming an ABS between a polymer and an inorganic salt.^[10] More recently, other substances, such as proteins, surfactants, sugars, or amino acids, have been used as solutes for ABS formation in combination with either polymers or salts.^[11–15] Interestingly, the co-dissolution of two salts in water can also generate an ABS.^[16–20] In the latter case, one of the salts typically comprises one or both ions with a high-charge density (i.e., water/ion interactions are stronger than water/water interactions), and thus ions that are preferentially hydrated; whereas, the other salt comprises low-charge density ions (i.e., water/ion interactions are weaker than water/water interactions).^[21,22] The delocalisation of charge in the ions of the latter salt inherently implies a lower melting temperature of the salt itself, which commonly fits within the category of ionic liquids.

Ionic liquids have gained vast attention from academia and industry since the late 1990s as a result of a series of interesting features that they often exhibit.^[23] These typically include an extremely low vapour pressure, melting temperatures that are around or even well below room temperature, a wide liquid-temperature range, good chemical and thermal stabilities, the ability to solvate a broad range of compounds, and a considerable ability to tailor their properties by a judicious selection of the constituent ions.^[24,25] As a result of this set of characteristics, ionic liquids have been proposed, among many other applications, as alternative solvents to the conventional volatile organic solvents in separation and reaction processes.^[26–28] By virtue of their tunability, ionic liquids cover practically the whole hydrophilicity/hydrophobicity range.^[29,30] The existence of hydrophilic ionic liquids enables the possibility of their combined use with water and, in particular, as ABS-forming solutes. In fact, a series of advantages can be associated with the use of ionic liquids in ABS instead of conventional high-melting inorganic salts. For example, salt crystallisation problems can be fully avoided when using an ionic liquid which melts below room temperature. Even in the case of an ionic liquid that melts above room temperature, the presence of water will largely preclude its solidification, that is, the saturation levels of ionic liquids in aqueous solutions are usually significantly higher than those observed with common inorganic salts.^[31,32] Furthermore, the large number of available hydrophilic ionic liquids may enable the customisation of ABS with regard to specific separation or purification challenges. Polymer/polymer or polymer/conventional salt ABS display a limited range of polarities or hydrogen-bond basicity (and/or hydrogen-bond acidity) of their coexisting phases. Derivatisation of the polymer is a viable possibility to overcome this issue; still, it implies significant costs and makes the application more difficult. It has been shown recently that the use of ionic liquids as adjuvants permits the fine tuning of the characteristics of either the salt- or polymer-rich phase in a polymer/conventional salt ABS,^[33] thus allowing an efficient and tailored control of the partition coefficients and of

purification efficiencies achieved within these systems. A third advantageous aspect is that ionic liquids can optionally be designed to have a low corrosive character^[34] relative to the highly corrosive aqueous solutions of conventional inorganic salts, which largely hinders their use in industrial processes. Additionally, the undesirable handling of solids in a process plant may be decreased with the replacement of the traditional inorganic salt by an ionic liquid.

To date, the use of ionic liquids in ABS has been mainly confined to salt/salt ABS, in which one of the two salts is an ionic liquid, as described above. Nevertheless, the possibility of using ionic liquids in ABS of the polymer/salt type has remained largely unexplored. The idea of a polymer/ionic liquid-based ABS was present in some examples from patents in the mid-2000s,^[35,36] which, however, did not include experimental support for the related claims. Approximately at the same time, salting-in and -out effects of ionic liquids in polyethylene glycol (PEG) aqueous media were reported, thus tacitly anticipating that a proper selection of the polymer and ionic liquid could give rise to a polymer/ionic liquid ABS.^[37,38] Recently, polymer/ionic liquid ABS have been reported for a series of hydrophobic polypropylene glycol (PPG) polymers.^[39] Because PPG is a thermosensitive polymer, these novel ABS were proposed to have applications in the recycling and/or enrichment of hydrophilic ionic liquids from aqueous media.^[39] In spite of these preliminary efforts, no reports have been found regarding ABS composed of PEG polymers (less hydrophobic) and hydrophilic ionic liquids (highly polar fluids) that will allow the co-existing polarities of the phases to be tailored. Moreover, it should be remarked that the addition of PEG polymers to aqueous solutions of ionic liquids could also be also advantageous in the recovery/recycling of ionic liquids from aqueous media.

Herein, we report for the first time the formation of PEG/ionic liquid ABS and provide insight into the influence of both the structural features of the polymer and ionic liquid on the aqueous phase diagrams. These results allowed us to draw a conclusion about the main driving forces that control the phase behaviour of these systems. PEG has been selected because it is nontoxic, inexpensive, and biodegradable, and it has been widely used in ABS formulations with inorganic salts.^[40,41] Moreover, the molecular weight of PEG can be easily varied over a considerable range, thus enabling a certain tuning of its characteristics and of the phase diagrams of the ABS formed. In regard to the ionic liquid, a set of hydrophilic (or moderately hydrophobic) ionic liquids was selected, thus allowing a deep examination of the effect of several structural features related to the cationic or anionic nature and the length, number, or functionalisation of the cationic alkyl side chains on the formation and characteristics of the ABS. Phase diagrams were determined at 298, 308 and 323 K and atmospheric pressure.

Results and Discussion

Phase diagrams for the PEG/ionic liquid ABS were determined at 298, 308 and 323 K,^[42] and the corresponding binodal curves were successfully correlated by means of Equation (1),

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

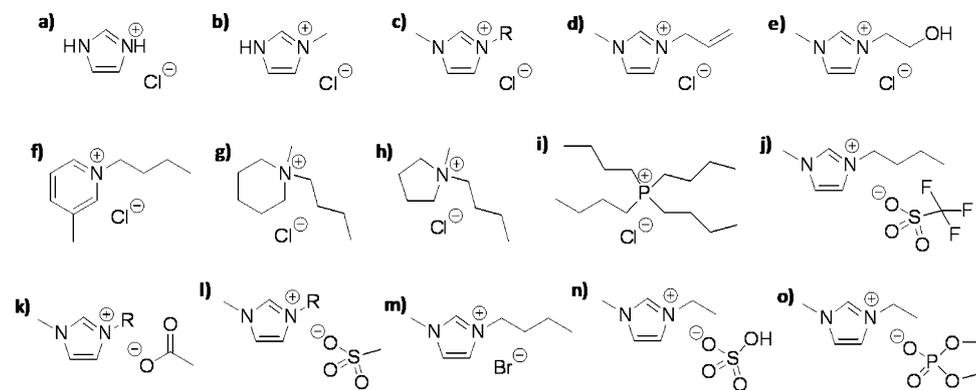
where A , B and C are the correlation constants and X and Y are the concentrations (in molality units) of ionic liquid and PEG, respectively (the fitted parameters are reported in the Supporting Information).

Several ionic liquids were investigated to access the influence of the anionic nature, cationic core, length and functionalisation of the cationic alkyl side chain, and the number of alkyl substituents on the phase-diagram behaviour. The ionic structures of these ionic liquids are shown in Scheme 1. A list that includes the definitions of their acronyms is provided in the Experimental Section.

PEG polymers of average molecular weight 1000, 2000, 3400 and 4000 g mol^{-1} were used to investigate the influence of the polymer in the ABS and are abbreviated as PEG 1000, PEG 2000, PEG 3400 and PEG 4000, respectively.

All binodal data are displayed in molality units, thus avoiding disparities in the ionic liquid-based ABS formation capability that could be a direct outcome of their distinct molecular weights (the detailed weight-fraction experimental data are listed in the Supporting Information).

Effect of the anion of the ionic liquid: In a typical PEG/inorganic salt ABS, the anion of the salt has a crucial impact on the phase behaviour relative to the influence of the cation. To analyse this effect in the PEG/ionic liquid ABS studied herein, aqueous systems formed by PEG 2000 and distinct ionic liquids with a common cation were investigated.



Scheme 1. Chemical structures of the studied ionic liquids. a) [im]Cl, b) [mim]Cl, c) [C_nmim]Cl (n = number of carbon atoms in the alkyl chain (R); n = 1, 2, 4, 6, or 8), d) [amim]Cl, e) [HOC_nmim]Cl, f) [C₄mpy]Cl, g) [C₄mpip]Cl, h) [C₄mpyr]Cl, i) [P₄₄₄₄]Cl, j) [C₄mim][CF₃SO₃], k) [C_nmim][CH₃CO₂] (n = 2 or 4), l) [C_nmim][CH₃SO₃] (n = 2 or 4), m) [C₄mim]Br, n) [C₂mim][HSO₄], o) [C₂mim][(CH₃)₂PO₄]. See the Experimental Section for the definition of the acronyms of the ionic liquids.

Specifically, two sets of ionic liquids were used: one with the [C₄mim]⁺ ion in common (i.e., [C₄mim]Cl, [C₄mim]Br, [C₄mim][CH₃CO₂], [C₄mim][CH₃SO₃], and [C₄mim][CF₃SO₃]) and the other with the [C₂mim]⁺ ion (i.e., [C₂mim]Cl, [C₂mim][CH₃CO₂], [C₂mim][CH₃SO₃], [C₂mim][HSO₄], and [C₂mim][(CH₃)₂PO₄]). The corresponding experimental binodal curves at 298 K and their correlation, derived by using Equation (1), are shown in Figures 1 and 2.

The ability of [C₄mim]-based ionic liquids to induce PEG-based ABS, as measured by the minimum required combined concentrations, follows the trend: [C₄mim][CH₃CO₂] ≈ [C₄mim][CH₃SO₃] > [C₄mim]Cl > [C₄mim]Br > [C₄mim][CF₃SO₃]; whereas for the [C₂mim]-based ionic liquids, the trend is as follows: [C₂mim][(CH₃)₂PO₄] > [C₂mim][CH₃CO₂] > [C₂mim][CH₃SO₃] > [C₂mim][HSO₄] > [C₂mim]Cl. The relative ability of an ionic liquid anion to induce the formation of a second liquid phase in a PEG/ionic liquid ABS follows the opposite order to that observed

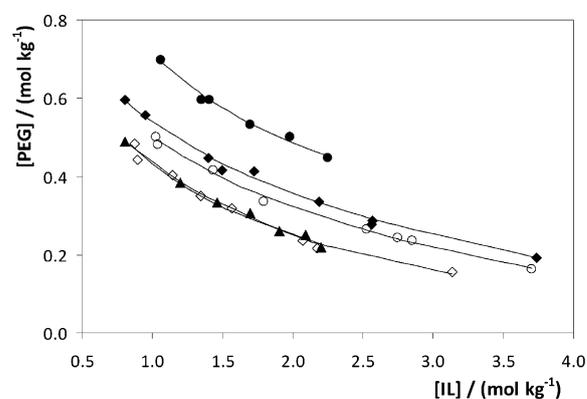


Figure 1. Experimental solubility data for PEG/ionic liquid ABS at 298 K to analyse the effect of the ionic liquid anion. PEG 2000/[C₄mim][CF₃SO₃] (●), PEG 2000/[C₄mim]Br (◆), PEG 2000/[C₄mim]Cl (○), PEG 2000/[C₄mim][CH₃SO₃] (▲) and PEG 2000/[C₄mim][CH₃CO₂] (◇). The lines correspond to the respective correlations derived from Equation (1).

for the K₃PO₄/ionic liquid ABS previously reported.^[19] These reverse sequences can be rationalised by taking into account the different salting-out aptitude of the distinct species solvated in aqueous media. When dealing with high-charge-density salts with an enhanced capacity for creating ion/water hydration complexes, such as K₃PO₄, the ability to induce ABS increases with the decrease in the affinity of the ionic liquid for water, namely, the ionic liquid is salted-out by the inorganic ions.^[19] On the

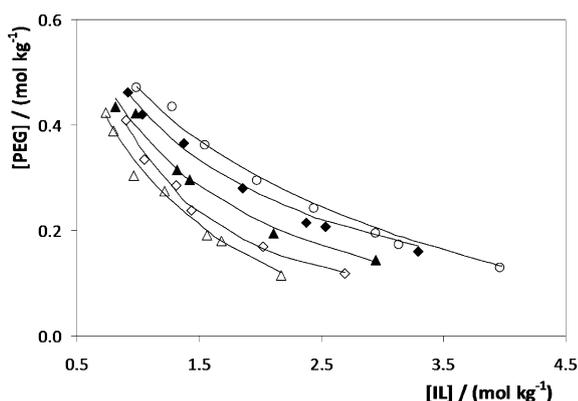


Figure 2. Experimental solubility data for PEG/ionic liquid ABS at 298 K to analyse the effect of the ionic liquid anion. PEG 2000/[C₂mim]Cl (○), PEG 2000/[C₂mim][HSO₄] (◆), PEG 2000/[C₂mim][CH₃SO₃] (▲), PEG 2000/[C₂mim][CH₃CO₂] (◇) and PEG 2000/[C₂mim][(CH₃)₂PO₄] (△). The lines correspond to the respective correlations derived from Equation (1).

contrary, in this study, when making mixtures of polymers and ionic liquids, the stronger agents for promoting ABS are ionic liquids that present an improved affinity for water and, hence, ionic liquid ions with a greater salting-out ability. This consequence comes directly from the PEG hydrophobic nature and the ease with which it is salted-out by charged species.

Regarding the sequence of the anions of the ionic liquid, it was verified that ionic liquids with a better ability to form ion/water complexes are more effective in promoting PEG/ionic liquid-based ABS. Indeed, the salting-out aptitude of an ion can be directly related to its Gibbs free energy of hydration (ΔG_{hyd}). Thus, the acetate anion ($\Delta G_{\text{hyd}} = 373 \text{ kJ mol}^{-1}$) is a stronger salting-out species, and therefore more prone to induce liquid/liquid demixing of PEG from aqueous media than, for example, the chloride or bromide anions ($\Delta G_{\text{hyd}} = 347$ and 321 kJ mol^{-1} , respectively).^[43]

Interestingly, the [CF₃SO₃]-based ionic liquid, one of the ionic liquids that is better at inducing the formation of the K₃PO₄/ionic liquid ABS,^[19] is the weakest species for salting-out the polymer when dealing with a PEG-based ABS. Besides the ionic liquid/water and PEG/water interactions that govern the phase behaviour in the systems presented above, we must be aware of the PEG/ionic liquid interactions that could additionally control the phase diagrams. In general, the larger the immiscibility between the ionic liquid and PEG polymer, the greater the ability of the ionic liquid to induce polymer separation from aqueous media. This trend correlates well with previously reported binary phase diagrams between poly(ethyl glycidyl ether) and several imidazolium-based ionic liquids.^[44] Watanabe and co-workers^[44] demonstrated that the solubility behaviour is largely dependent on the anionic nature of the ionic liquid. Fluorinated anions and/or anions with lower hydrogen-bond basicity are more miscible with the polymers investigated than anions with a higher aptitude to create ion/water complexes, such as acetate.^[44] As a consequence of this lower affinity be-

tween the acetate anion and polymers, the acetate-based ionic liquid is more efficient for the separation of the PEG aqueous phase than, for example, the [CF₃SO₃]-based ionic liquid (as experimentally observed in this study). Usually, strongly basic anions tightly interact with imidazolium cations and interrupt the hydrogen-bonding interactions between the PEG polymer and the aromatic cation of the ionic liquid, thereby lowering their miscibility.

Effect of the cationic core of the ionic liquid: Several PEG/ionic liquid ABS, chosen to evaluate the effect of different classes of ionic liquid cation in ABS formation, were studied. PEG 2000 was used as the common phase-forming polymer and five different chloride-based ionic liquids were investigated: [C₄mim]Cl, [C₄mpy]Cl, [C₄mpyr]Cl, [C₄mpip]Cl, and [P₄₄₄₄]Cl. The respective phase diagrams and the correlation of the experimental data derived by using Equation (1) are depicted in Figure 3.

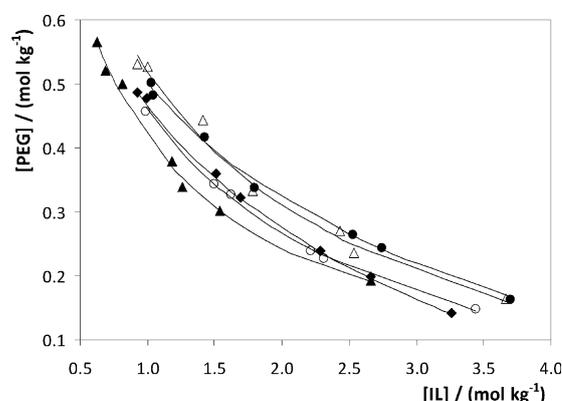


Figure 3. Experimental solubility data for the PEG/ionic liquid ABS at 298 K to analyse the effect of the ionic liquid cation core. PEG 2000/[C₄mim]Cl (●), PEG 2000/[C₄mpy]Cl (△), PEG 2000/[C₄mpyr]Cl (◆), PEG 2000/[C₄mpip]Cl (○) and PEG 2000/[P₄₄₄₄]Cl (▲). The lines correspond to the respective correlations derived from Equation (1).

The experimental results show that the ability of ionic liquids to induce PEG-based ABS follows the trend: [P₄₄₄₄]Cl > [C₄mpip]Cl ≈ [C₄mpyr]Cl > [C₄mpy]Cl ≈ [C₄mim]Cl. This ability correlates well with the relative hydrophobic character of the ionic liquid, with the most hydrophobic species showing cloud points at lower concentrations of ionic liquid (for a given concentration of PEG). The higher the affinity for water and/or the hydrophilic nature of the ionic liquid, the less effective such an ionic liquid is in promoting the PEG phase separation. As previously reported,^[45] imidazolium and pyridinium cations present stronger interactions with water (as a result of their aromatic character), which results in binodal curves that are more shifted to the top/right in the phase diagram. The quaternary phosphonium-based ionic liquid (with four alkyl chains) was found to be the strongest promoter of the formation of PEG/ionic liquid ABS among the ionic liquids studied as a result of the lower affinity of this ionic liquid for aqueous phases. The

phosphonium ionic liquid/water miscibility essentially derives from the hydrogen-bonding interactions of water with the chloride anion. This behaviour is in stark contradiction to the trend obtained for the anions discussed above, but the cationic pattern is in good agreement with previous reports in which ABS of imidazolium-, phosphonium-, and ammonium-based ionic liquids, in the presence of common inorganic salts, were investigated.^[17,20] This behavior occurs because the ABS formation for these systems is being driven by a different phenomenon from that discussed above for the effect of the anion of the ionic liquid. Here, the ABS formation is, as for the salt/ionic liquid ABS,^[17,20] a direct result of the salting-out aptitude of PEG over the moderate hydrophobic ionic liquids considered. PEG is preferentially hydrated and, thus, tends to salt-out the ionic liquids.

As for the anion effect discussed above, the interactions that occur between the PEG polymer and ionic liquids may also have an impact upon their phase separation. PEG is a polyether and, thus, hydrogen-bonding interactions are expected to occur with the aromatic pyridinium and imidazolium cations. The presence of π electrons in these ionic liquids corresponds to strong interactions with PEG. Hence, this reason could be the major factor for the lower ability of these ionic liquids to promote the PEG-based ABS, that is, the enhanced affinity between two compounds would require a higher quantity of both solutes for liquid–liquid demixing. Reports of results on the phase behaviour of binary systems composed of polyether compounds and ionic liquids^[44] corroborate the ability of the cation core to promote the PEG/aqueous phase separation observed here. Polyether compounds are more soluble in ionic liquids containing aromatic cations and immiscible in phosphonium- and ammonium-based ionic liquids, thus confirming that the intermolecular hydrogen-bonding between the polymers and acidic hydrogen atoms in the cation of the ionic liquid are crucial features for increasing their mutual miscibility.^[44] Hence, ionic liquid/PEG-based ABS are more readily formed when two poorly soluble species are present.

Effect of the alkyl chain length, number of alkyl groups, and functionalisation at the alkyl substituent in the cation of the ionic liquid: The family of 1-alkyl-3-methylimidazolium chloride ionic liquids was selected to study the effect of the length of the cationic alkyl substituent (which is one of the major structural features that allows the hydrophobicity of the ionic liquid to be tailored) on the PEG/ionic liquid/water phase diagrams. Moreover, imidazolium-based ionic liquids with no alkyl chains and only one alkyl group were additionally studied. Specifically, six ionic liquids were investigated: [im]Cl, [mim]Cl, [C₂mim]Cl, [C₄mim]Cl, [C₆mim]Cl, and [C₈mim]Cl. Again, PEG 2000 was used as the common polymer in all experiments. Figure 4 illustrates the experimental and correlated binodal curves for the various imidazolium chloride systems.

The aptitude of the ionic liquids to induce PEG-based ABS decreases on the following order: [C₂mim]Cl \approx

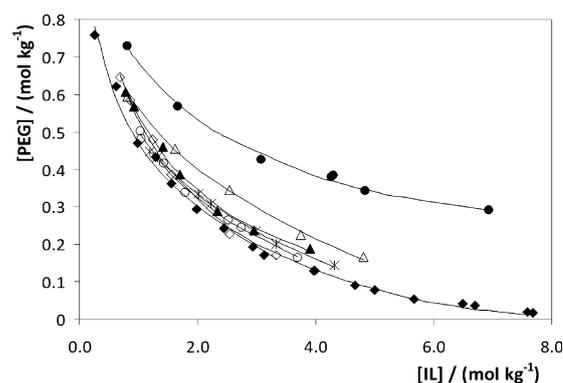


Figure 4. Experimental solubility data for the PEG/ionic liquid ABS at 298 K to analyse the effect of the length and number of the alkyl substituents in the ionic liquid cation. PEG 2000/[im]Cl (●), PEG 2000/[mim]Cl (△), PEG 2000/[C₁mim]Cl (*), PEG 2000/[C₂mim]Cl (◆), PEG 2000/[C₄mim]Cl (○), PEG 2000/[C₆mim]Cl (▲) and PEG 2000/[C₈mim]Cl (◇). The lines correspond to the respective correlations derived from Equation (1).

[C₈mim]Cl > [C₄mim]Cl \approx [C₆mim]Cl \approx [C₁mim]Cl > [mim]Cl > [im]Cl. It is easy to understand the lower ability of the monosubstituted, unsubstituted, and 1,3-dimethyl-based imidazolium ionic liquids to promote a biphasic system, given their higher hydrophilicity (high charge-density ionic liquids that resemble inorganic salts) according to the above discussion presented for the cation core. However, for the other bisubstituted imidazolium-based ionic liquids, more complex phenomena take place, and the trends in the phase behaviour need to be carefully examined.

Longer alkyl chains enhance the hydrophobic character of the ionic liquid and, therefore, decrease their affinity for water while increasing the ABS promoting ability, which was previously observed with ABS of the type K₃PO₄/ionic liquid.^[18] In PEG-based ABS, however, the opposite trend is observed for 1-alkyl-3-methylimidazolium chloride ionic liquids with hexyl or shorter alkyl chains (although the differences are small). This pattern is a direct consequence of the ionic liquid that acts, in this case, as the salting-out species.

Instead of the interactions of the solutes with water, the interactions between the PEG polymer and ionic liquids control the phase behaviour. As previously reported,^[46,47] an increase in the alkyl side chain of the cation enhances the solubility between the imidazolium-based ionic liquids and the PEG polymer. The stronger the cation/anion interaction, the lower the ability of the imidazolium-ring hydrogen atoms to hydrogen-bond with the PEG polymer.^[48] This trend is in close agreement with the data gathered with [C_nmim]Cl (with $n=2, 4$, and 6). The longer the alkyl side chain of the imidazolium cation, the higher the miscibility/affinity with PEG, thus leading to systems that require more ionic liquid to undergo liquid–liquid demixing in aqueous media.

The odd behaviour observed with the system containing [C₈mim]Cl implies that other factors besides those discussed above also take place. It should be reminded that this ionic liquid, contrary to those members of its homologous family

with shorter alkyl substituents, forms micelles in aqueous solution above a critical concentration^[49] and, therefore, more complex phenomena are involved. The fact that ionic liquids with long alkyl side chains tend to form phase-separated self-assembled structures corresponds to a decreased ability of [C₈mim]Cl to hydrogen bond with the PEG polymer. Hence, species that do not show a special affinity between themselves are more easily separated in aqueous solution.

From the gathered results of the effects of the ionic liquid cation core and alkyl side chain length, it seems that the formation of a PEG/ionic liquid-based ABS is not just a direct consequence of the ability of the ions to form hydration complexes, as typically happens for ABS induced by inorganic salts. Instead, a complex interplay of interactions between PEG/water, ionic liquid/water, and PEG/ionic liquid takes place. The imidazolium-based cations may form multiple hydrogen bonds between the various ring protons with even weak hydrogen-bond acceptors. Competition for the protons of the imidazolium cation among the anions and PEG molecules is consequently expected. The addition of hydrogen-bond acceptors (e.g., PEG polymers) to an ionic liquid system can bring about significant changes in the magnitude.^[50] From the results reported herein, it can be concluded that the hydrogen-bonding interactions that occur in ionic liquid/PEG pairs of solutes are also driving forces in the formation of the respective ABS.

Figure 5 shows the effect of the functionalisation of the alkyl substituents in the cation, in particular, the influence of the presence of a double bond or a hydroxyl group in the alkyl side chain of the imidazolium cation. The binodal curves for the systems with [amim]Cl and [HOC₂mim]Cl relative to those with [C₄mim]Cl and [C₂mim]Cl, with PEG 2000 as the common polymer, are examined.

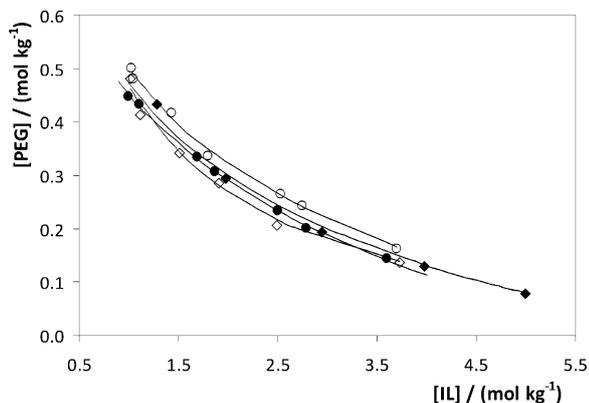


Figure 5. Experimental solubility data for the PEG/ionic liquid ABS at 298 K to analyse the effect of the functionalisation of the alkyl substituent in the ionic liquid cation. PEG 2000/[amim]Cl (●), PEG 2000/[HOC₂mim]Cl (◇), PEG 2000/[C₂mim]Cl (◆) and PEG 2000/[C₄mim]Cl (○). The lines correspond to the respective correlations derived from Equation (1).

The ability of the ionic liquids to promote ABS follows the order: [HOC₂mim]Cl > [C₂mim]Cl ≈ [amim]Cl >

[C₄mim]Cl. The presence of the hydroxyl group in [HOC₂mim]Cl shifts the binodal curve to the left relative to that of [C₂mim]Cl, which means an increased aptitude of the ionic liquid to promote the ABS. The presence of the double bond in [amim]Cl also enhances the ABS formation ability, which was observed by the comparison with the systems containing [C₂mim]Cl and [C₄mim]Cl. It should be reminded that the [amim]⁺ ion is the equivalent to the [C₃mim]⁺ ion with an additional double bond. As observed before, the trend in the ionic liquids ability to form ABS is the opposite of that observed for the K₃PO₄/ionic liquid ABS.^[18] These differences can be explained based on the increased ability of the functionalised ionic liquid ions to form hydration complexes and, hence, to act as stronger salting-out species.

Regarding the effect of all the structural features related to the cation of the ionic liquid, a general conclusion can be postulated: For PEG-based ABS containing the chloride-based ionic liquids (an anion that is in the middle rank of the Hofmeister series),^[21,22] the polymer tends to act as the privileged salting-out agent. Hence, hydrophobic ionic liquids are more readily salted out from the aqueous media and preferentially form ABS. However, if the increase in the hydrophobicity of the ionic liquid corresponds to a higher affinity for the PEG itself, the opposite behaviour is observed.

Effect of the molecular weight of PEG: The tunable character of the phase behaviour of the PEG/ionic liquid ABS is not exclusively derived from the manipulation of the ionic liquid structure. PEG also offers some degree of design by varying the length of its polymeric chains, that is, the average molecular weight. This effect was analysed by studying ABS formed by the codissolution of a given ionic liquid (i.e., [C₄mim]Cl) and PEG polymers of different molecular weights. Figure 6 depicts the binodal curves for several PEG/[C₄mim]Cl ABS in which the average molecular weight of the PEG was varied from 1000 to 4000 g mol⁻¹.

The influence of the length of the PEG chains on the phase diagrams is notorious, with the ability of the PEG polymer to induce ionic liquid-based ABS that decrease in the following order: PEG 4000 > PEG 3400 > PEG 2000 > PEG 1000. An analogous trend has been observed in conventional polymer/salt ABS.^[51–53] Hydrophobicity increases with the increase of the molecular weight of the PEG polymer, thus facilitating the formation of the ABS. Higher-molecular-weight PEG polymers present a lower affinity for water and are preferentially salted-out by the ionic liquid.

In the same line as the discussion previously presented, these trends also agree with the affinity/miscibility patterns observed in PEG/ionic liquid binary systems.^[47] Rodríguez et al.^[47] demonstrated that an increase in the molecular weight of the polymer leads to larger immiscibility gaps with chloride-based ionic liquids. Hence, higher-molecular-weight PEG polymers are less soluble in ionic liquids and are more readily separated from aqueous media.

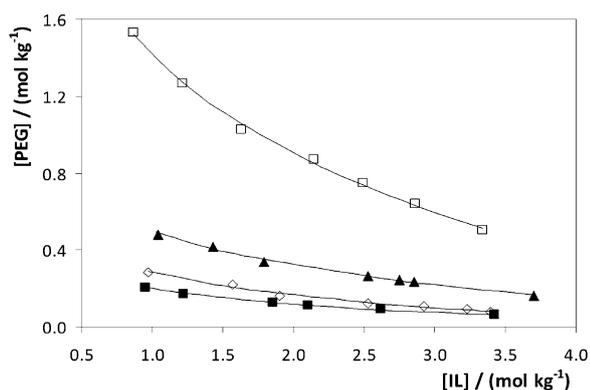


Figure 6. Experimental solubility data for the PEG/ionic liquid ABS at 298 K to analyse the effect of the molecular weight of the PEG. PEG 1000/[C₄mim]Cl (□), PEG 2000/[C₄mim]Cl (▲), PEG 3400/[C₄mim]Cl (◇) and PEG 4000/[C₄mim]Cl (■). The lines correspond to the respective correlations derived from Equation (1).

Effect of temperature: To gather information on the effect of temperature on the phase behaviour, additional PEG/ionic liquid/water phase diagrams were also determined at 308 and 323 K for two selected aqueous systems, namely, PEG 2000/[C₂mim]Cl and PEG 2000/[C₂mim][CH₃CO₂]. The results obtained are presented in Figures 7 and 8.

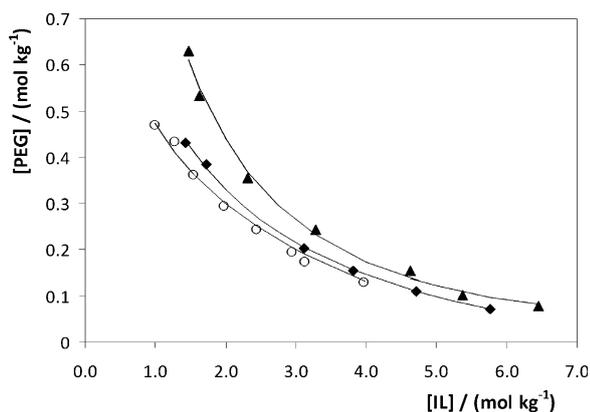


Figure 7. Experimental solubility data for the PEG 2000/[C₂mim]Cl ABS at 298 (○), 308 (◆) and 323 K (▲). The lines correspond to the respective correlations derived from Equation (1).

The binodal curves show that an increase in temperature decreases the immiscibility region. The pattern observed is in close agreement with that observed in ABS composed of ionic liquids and inorganic salts or ionic liquids and carbohydrates.^[54,55] Yet, an increase in temperature leads to larger immiscibility regimes in typical PEG/inorganic salt systems, whereas the opposite pattern is verified in polymer/polymer ABS.^[56,57] Therefore, the dependence on temperature of the phase-diagram behaviour for polymer/ionic liquid systems resembles that of polymer/polymer systems as a result of the lower charge-density of the ionic liquids relative to inorganic salts (and therefore their decreased salting-out aptitude). The trends obtained here are, however, a reflection of the

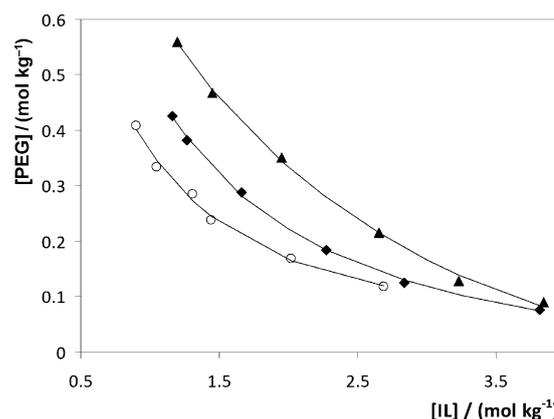


Figure 8. Experimental solubility data for the PEG 2000/[C₂mim][CH₃CO₂] ABS at 298 (○), 308 (◆) and 323 K (▲). The lines correspond to the respective correlations derived from Equation (1).

hydrophilic nature of the ionic liquids investigated and their capacity to create water/ion complexes. As a result, the patterns obtained in these ternary systems are a consequence of the typical behaviour from the upper critical solution temperature observed in binary mixtures composed of ionic liquids and water.^[45] The larger differences among the binodal curves at various temperatures for the acetate-based system relative to the chloride-based ABS are a direct result of the increased salting-out aptitude of the acetate anion, which is reproduced in a more pronounced dependence on temperature.

In summary, it can be stated that the PEG/ionic liquid ABS are more complex in their nature and dominating interactions than the ionic liquid/inorganic salt ABS previously studied.^[18,19] For the latter, as shown with the K₃PO₄/ionic liquid-based ABS,^[18,19] the phase separation is dominated by the salting-out ability of the inorganic salt, with the less-hydrophilic ionic liquids more prone to undergo ABS formation. In most examples of PEG/ionic liquid ABS, the ionic liquid acts instead as the salting-out species and, hence, they are preferentially formed by ionic liquids with an enhanced capacity to create ionic liquid/water complexes. This trend was verified to be always true when different ionic liquid anions are compared; yet, the situation was quite different when evaluating the effect of the cation core of the ionic liquid with more hydrophobic ions. The trend observed for the influence of the cation core on ABS formation does not follow the ability of the ions to form hydration complexes. Instead, this effect seems to be dominated by the PEG salting-out ability, with the more hydrophobic ionic liquids more able to form ABS, except for the cases in which the mutual solubilities of the PEG-ionic liquid control the system as observed with the increase of the cation alkyl chain length (verified with the [C_nmim]Cl ionic liquids). In general, for all the structural features evaluated, there is a close relation to the ionic liquid/PEG binary phase behaviour. Most inorganic salts are composed of simple and high charge-density ions; yet, when these are substituted by more complex and/or organic ions (as we do in most of the ionic

liquids investigated), the organic ion can itself influence the salting-in/-out behaviour through specific interactions that are established with the polymer and not just with water. In this context, the interactions that occur between the polymer and ionic fluid cannot be discarded and play a crucial role in the phase behaviour of PEG/ionic liquid-based ABS.

Conclusion

Novel aqueous biphasic systems of the polymer/ionic liquid type have been formed by codissolving ionic liquids and PEG polymers in appropriate concentrations in water. It was shown that the phase behaviour of the PEG/ionic liquid ABS can be tailored to a relevant extent by a judicious selection of the PEG molecular weight or of the chemical structure of the constitutive ions of the ionic fluid. A detailed understanding of the molecular-level interactions in the binary mixtures composed of ionic liquids and PEG polymers allowed us to show, for the first time, that the phase behaviour in PEG/ionic liquid ABS is not only dominated by the ability of the solutes to interact with water. Instead, it is a direct consequence of the favourable (or non-favourable) interactions that occur between the PEG polymer and ionic liquid to a large extent.

ABS composed of PEG polymers and ionic liquids may have great potential in the biotechnology field, in which the use of ionic liquids to substitute the high charge-density ions of inorganic salts can constitute an important improvement towards the extraction of biomolecules. Therefore, the use of these novel systems for extraction purposes is directly envisaged.

Experimental Section

PEG polymers of average molecular weights 1000, 2000, 3400, and 4000 g mol^{-1} (abbreviated as PEG 1000, PEG 2000, PEG 3400 and PEG 4000, respectively) were supplied by Fluka and used as received.

The ionic liquids studied were imidazolium chloride ([im]Cl), 1-methylimidazolium chloride ([mim]Cl), 1,3-dimethylimidazolium chloride ([C₂mim]Cl), 1-ethyl-3-methylimidazolium chloride ([C₂mim]Cl), 1-butyl-3-methylimidazolium chloride ([C₄mim]Cl), 1-hexyl-3-methylimidazolium chloride ([C₆mim]Cl), 1-methyl-3-octylimidazolium chloride ([C₈mim]Cl), 1-allyl-3-methylimidazolium chloride ([amim]Cl), 1-(2-hydroxyethyl)-3-methylimidazolium chloride ([HOC₂mim]Cl), 1-butyl-3-methylpyridinium chloride ([C₄mpy]Cl), 1-butyl-1-methylpiperidinium chloride ([C₄mpip]Cl), 1-butyl-1-methylpyrrolidinium chloride ([C₄mpyr]Cl), tetrabutylphosphonium chloride ([P₄₄₄₄]Cl), 1-butyl-3-methylimidazolium bromide ([C₄mim]Br), 1-ethyl-3-methylimidazolium acetate ([C₂mim][CH₃CO₂]), 1-butyl-3-methylimidazolium acetate ([C₄mim][CH₃CO₂]), 1-ethyl-3-methylimidazolium methanesulfonate ([C₂mim][CH₃SO₃]), 1-butyl-3-methylimidazolium methanesulfonate ([C₄mim][CH₃SO₃]), 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([C₄mim][CF₃SO₃]), 1-ethyl-3-methylimidazolium hydrogen sulfate ([C₂mim][HSO₄]) and 1-ethyl-3-methylimidazolium dimethylphosphate ([C₂mim][(CH₃)₂PO₄]). Their chemical structures are shown in Scheme 1. All ionic liquids were purchased from Iolitec, with the exception of [P₄₄₄₄]Cl, which was kindly supplied by Cytec Industries, Inc. All ionic liquid samples were dried under moderate temperature (343 K) and high vacuum conditions (<0.1 mbar), for a minimum of 48 h. The purity of the ionic

liquids was additionally checked by ¹H, ¹⁹F (whenever necessary), and ¹³C NMR spectroscopic analysis and was found to be >99 wt %, except for [P₄₄₄₄]Cl, which was >97 wt %.

The water content of all ionic liquids and polymers was measured by using Karl-Fischer titrations, and each value was individually taken into account for the calculation of the global compositions of the PEG + ionic liquid + H₂O mixtures prepared. In general, the water content in all ionic liquid samples after the drying procedure was found to be <100 ppm, whereas the water content in the polymers was <10000 ppm. Ultrapure water that was doubly distilled, passed through a reverse-osmosis system, and further treated with a Milli-Q plus water purification apparatus, was used.

The binodal curves in the phase diagrams were determined by means of the turbidometric titration method^[42] at atmospheric pressure and a constant temperature of 298.2 ± 0.5 K. Moreover, additional phase diagrams at 308.2 and 323.2 K were determined for selected systems within ±0.5 K by using a double-jacketed cell coupled to a Julabo circulator (model F25-HD). Different ternary mixtures (ca. 1 g) of known total composition, located in the biphasic region, were prepared inside closed glass tubes. Upon dilution with minimal dropwise additions of water, the test tubes were stirred for at least 12 h and allowed to settle for 24 h, which was found to be the common minimum time to reach the equilibrium in the different samples. These periods were particularly important as a result of the high viscosity of higher-molecular-weight PEG polymers that could provide erroneous results if not enough time for dissolution and phase separation was allowed. This process was repeatedly carried out until a limpid and monophasic solution was obtained. The additions were controlled gravimetrically, with an uncertainty of ±10⁻⁴ g. The total amount of water added was determined, and the final mass fraction of each component was calculated.

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- [1] V. P. Shanbhag, G. Johansson, *Biochem. Biophys. Res. Commun.* **1974**, *61*, 1141–1146.
- [2] G. Johansson, *Mol. Cell. Biochem.* **1974**, *4*, 169–180.
- [3] G. Johansson, *J. Chromatogr.* **1978**, *150*, 63–71.
- [4] V. P. Shanbhag, G. Johansson, *Eur. J. Biochem.* **1979**, *93*, 363–367.
- [5] L. Backman, *Eur. J. Biochem.* **1981**, *120*, 257–261.
- [6] D. Kessel, *Biochim. Biophys. Acta Gen. Subj.* **1981**, *678*, 245–249.
- [7] H. Walter, D. E. Brooks, D. Fisher, *Partitioning in Aqueous Two-Phase Systems: Theory, Methods, Uses, and Applications to Biotechnology*, Academic Press, Orlando, **1985**.
- [8] R. D. Rogers, A. H. Bond, C. B. Bauer, *Sep. Sci. Technol.* **1993**, *28*, 1091–1126.
- [9] M. W. Beijerinck, *Centr.-Bl. f. Bakter. u. Parasitenk.* **1896**, *2*, 698–699.
- [10] P.-Å. Albertsson, *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley, New York, **1986**.
- [11] X. Zeng, J. Quaye, K. Osseo-Asare, *Colloids Surf. A* **2004**, *246*, 135–145.
- [12] Y. Chen, S. Zhang, J. Zhang, Y. Zhang, *New type ionic liquid-sugar aqueous biphasic system*, CN 1751766A, **2006**.
- [13] J. S. Becker, O. R. T. Thomas, M. Franzreb, *Sep. Purif. Technol.* **2009**, *65*, 46–53.
- [14] F. Spyropoulos, A. Portscht, I. T. Norton, *Food Hydrocolloids* **2010**, *24*, 217–226.
- [15] M. Domínguez-Pérez, L. I. N. Tomé, M. G. Freire, I. M. Marrucho, O. Cabeza, J. A. P. Coutinho, *Sep. Purif. Technol.* **2010**, *72*, 85–91.

- [16] K. E. Gutowski, G. A. Broker, H. D. Willauer, J. G. Huddleston, R. P. Swatloski, J. D. Holbrey, R. D. Rogers, *J. Am. Chem. Soc.* **2003**, *125*, 6632–6633.
- [17] N. J. Bridges, K. E. Gutowski, R. D. Rogers, *Green Chem.* **2007**, *9*, 177–183.
- [18] C. M. S. S. Neves, S. P. M. Ventura, M. G. Freire, I. M. Marrucho, J. A. P. Coutinho, *J. Phys. Chem. B* **2009**, *113*, 5194–5199.
- [19] S. P. M. Ventura, C. M. S. S. Neves, M. G. Freire, I. M. Marrucho, J. Oliveira, J. A. P. Coutinho, *J. Phys. Chem. B* **2009**, *113*, 9304–9310.
- [20] C. L. S. Louros, A. F. M. Cláudio, C. M. S. S. Neves, M. G. Freire, I. M. Marrucho, J. Pauly, J. A. P. Coutinho, *Int. J. Mol. Sci.* **2010**, *11*, 1777–1791.
- [21] M. G. Freire, C. M. S. S. Neves, A. M. S. Silva, L. M. N. B. F. Santos, I. M. Marrucho, L. P. N. Rebelo, J. K. Shah, E. J. Maginn, J. A. P. Coutinho, *J. Phys. Chem. B* **2010**, *114*, 2004–2014.
- [22] M. G. Freire, P. J. Carvalho, A. M. S. Silva, L. M. N. B. F. Santos, L. P. N. Rebelo, I. M. Marrucho, J. A. P. Coutinho, *J. Phys. Chem. B* **2009**, *113*, 202–211.
- [23] P. S. Kulkarni, L. C. Branco, J. G. Crespo, M. C. Nunes, A. Raymundo, C. A. M. Afonso, *Chem. Eur. J.* **2007**, *13*, 8478–8488.
- [24] M. J. Earle, K. R. Seddon, *Pure Appl. Chem.* **2000**, *72*, 1391–1398.
- [25] J. F. Brennecke, E. J. Maginn, *AIChE J.* **2001**, *47*, 2384–2389.
- [26] S. T. Handy, *Chem. Eur. J.* **2003**, *9*, 2938–2944.
- [27] V. P. W. Böhm, W. A. Herrmann, *Chem. Eur. J.* **2000**, *6*, 1017–1025.
- [28] T. Welton, *Chem. Rev.* **1999**, *99*, 2071–2083.
- [29] J. G. Huddleston, A. E. Visser, W. M. Reichert, H. D. Willauer, G. A. Broker, R. D. Rogers, *Green Chem.* **2001**, *3*, 156–164.
- [30] M. Freemantle, *An Introduction to Ionic Liquids*, RSC, Cambridge, **2010**.
- [31] U. Domańska, M. Królikowski, A. Pobudkowska, T. M. Letcher, *J. Chem. Eng. Data* **2009**, *54*, 1435–1441.
- [32] U. Domańska, *Int. J. Mol. Sci.* **2010**, *11*, 1825–1841.
- [33] J. F. B. Pereira, A. S. Lima, M. G. Freire, J. A. P. Coutinho, *Green Chem.* **2010**, *12*, 1661–1669.
- [34] J. Qu, P. J. Blau, S. Dai, H. Luo, H. M. Meyer, J. J. Thuhan, *Wear* **2009**, *267*, 1226–1231.
- [35] J. Upfal, D. R. MacFarlane, S. A. Forsyth, *Solvents for use in the treatment of lignin-containing materials*, WO 2005/017252 A1, **2005**.
- [36] S. Dreyer, U. Kragl, *Verfahren zur Extraktion von Biomolekülen*, DE102007001347 A1, **2007**.
- [37] Z. P. Visak, J. N. Canongia Lopes, L. P. N. Rebelo, *Monatsh. Chem.* **2007**, *138*, 1153–1157.
- [38] J. N. Canongia Lopes, L. P. N. Rebelo, *Chim. Oggi* **2007**, *25*, 37–39.
- [39] C. Wu, J. Wang, Y. Pei, H. Wang, Z. Li, *J. Chem. Eng. Data* **2010**, *55*, 5004–5008.
- [40] J. Chen, S. K. Spear, J. G. Huddleston, R. D. Rogers, *Green Chem.* **2005**, *7*, 64–82.
- [41] P. Reutenauer, E. Buhler, P. J. Boul, S. J. Candau, J. M. Lehn, *Chem. Eur. J.* **2009**, *15*, 1893–1900.
- [42] *Aqueous Two-Phase Systems: Methods and Protocols (Methods in Biotechnology)* (Ed.: R. Hatti-Kaul), Humana Press, Totowa, **2000**.
- [43] Y. Marcus, *Ion properties*, Marcel Dekker, New York, **1997**.
- [44] K. Kodama, R. Tsuda, K. Niitsuma, T. Tamura, T. Ueki, H. Kokubo, M. Watanabe, *Polym. J.* **2011**, *43*, 242–248.
- [45] M. G. Freire, C. M. S. S. Neves, P. J. Carvalho, R. L. Gardas, A. M. Fernandes, I. M. Marrucho, L. M. N. B. F. Santos, J. A. P. Coutinho, *J. Phys. Chem. B* **2007**, *111*, 13082–13089.
- [46] H. Rodríguez, R. D. Rogers, *Fluid Phase Equilib.* **2010**, *294*, 7–14.
- [47] H. Rodríguez, M. Francisco, M. Rahman, N. Sun, R. D. Rogers, *Phys. Chem. Chem. Phys.* **2009**, *11*, 10916–10922.
- [48] A. M. Fernandes, M. A. A. Rocha, M. G. Freire, I. M. Marrucho, J. A. P. Coutinho, L. M. N. B. F. Santos, *J. Phys. Chem. B* **2011**, *115*, 4033–4041.
- [49] M. Blesic, H. M. Marques, N. V. Plechkova, K. R. Seddon, L. P. N. Rebelo, A. Lopes, *Green Chem.* **2007**, *9*, 481–490.
- [50] T. Singh, K. S. Rao, A. Kumar, *ChemPhysChem* **2011**, *12*, 836–845.
- [51] R. D. Rogers, J. Zhang, *J. Chromatogr. B: Biomed. Appl.* **1996**, *680*, 231–236.
- [52] M. E. Taboada, J. A. Asenjo, B. A. Andrews, *Fluid Phase Equilib.* **2001**, *180*, 273–280.
- [53] W. Zhi, J. Song, J. Bi, F. Ouyang, *Bioprocess Biosyst. Eng.* **2004**, *27*, 3–7.
- [54] Z. Du, Y. L. Yu, J. H. Wong, *Chem. Eur. J.* **2007**, *13*, 2130–2137.
- [55] Y. Chen, Y. Meng, S. Zhang, Y. Zhang, X. Liu, J. Yang, *J. Chem. Eng. Data* **2010**, *55*, 3612–3616.
- [56] H. D. Willauer, J. G. Huddleston, M. Li, R. D. Rogers, *J. Chromatogr. B: Biomed. Appl.* **2000**, *743*, 127–135.
- [57] D. Forciniti, C. K. Hall, M.-R. Kula, *Fluid Phase Equilib.* **1991**, *61*, 243–262.

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