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Triple salting-out effect: a required phenomenon in the formation of ionic-liquid-based aqueous multiphase systems

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Abstract

Novel aqueous multiphase systems (MuPS) formed by quaternary mixtures composed of cholinium-based ionic liquids (ILs), polymers, inorganic salts and water are here reported. The influence of several ILs was studied, demonstrating that a triple salting-out is a required phenomenon to prepare MuPS. The respective phase diagrams and “tie-surfaces” were determined, followed by the evaluation of the effect of temperature. Finally, it is shown the remarkable ability of IL-based MuPS to selectively separate a complex mixture of dyes.

Keywords

aqueous multiphase systems; dyes; ionic liquids; salting-out; selective separation

Separation processes based on three-liquid-phase systems are promising approaches for the isolation of different compounds present in complex mixtures, allowing their simultaneous separation amongst the different phases in a single-step.[1] These systems are usually prepared by the addition of an organic solvent to a polymer-salt-based aqueous biphasic system (ABS), resulting in the formation of an organic-solvent-rich top phase, a polymer-rich middle phase and a salt-rich bottom phase. Furthermore, due to their compositions and the chemical nature of each phase, the coexisting phases present a wide range of polarities and highly distinct chemical properties. This is one of the main reasons behind the considerably higher selectivities displayed by three-liquid phase systems when compared to conventional water-oil liquid-liquid systems and ABS.[2]

In 2012, Mace et al.[3] introduced the concept of aqueous multiphase systems (MuPS), *i.e.*, systems composed of three or more aqueous-rich phases, without organic solvents employed. The authors[3] reported the formation of more than 300 MuPS by mixing

different polymers and surfactants in aqueous solutions. Following their approach, Liang et al.[4] presented an in-depth study on an aqueous four-phase system constituted by sodium dodecyl sulphate (SDS), dodecyltrimethylammonium bromide (DTAB), polyethylene glycol (PEG) with a molecular weight of 6000 mol·g⁻¹, and NaBr. In this work, the phase compositions and their properties, as well as the partition of xylene orange between the coexisting phases, were studied and reported.[4] Other authors demonstrated that MuPS present characteristics useful for specific applications, such as in the separation of nanoparticles by rate-zonal centrifugation,[5] or even in the treatment of cellular components of human blood for medical purposes.[6] Nevertheless, the molecular-level knowledge behind the formation of MuPS is still very limited, and the mechanisms associated with the multiple phases' separation were not fully disclosed in these pioneering works.[3–6] Furthermore, the phase-forming components proposed for the creation of MuPS were always polymers, surfactants and polysaccharides of high molecular weights, resulting in highly viscous aqueous phases and further difficulties in the phases' separation and mass transfer phenomena.

The application of ionic liquids (ILs) as phase-forming components of ABS has been a hot topic of research in the past years.[7,8] Their unique properties, such as their high solvation ability for a large range of compounds and the possibility of tuning their properties by the correct choice of both the cation and anion, makes IL-based ABS valuable in processes of extraction and separation of a wide range of compounds.[9] Furthermore, these types of systems present additional and outstanding advantages when compared to the more traditional polymer-based systems, such as low viscosity, quick phase separation, and high extraction efficiencies for the most diverse biomolecules,[7] contributing to the development of more cost-effective processes. Although not investigated to date, these advantages could be transposed to MuPS if ILs could be used as phase-forming components. Thus, in this work, we evaluate the possibility of using ILs for the formation of MuPS, address their phase diagrams and the molecular-level mechanisms behind the observed phase transitions, and evaluate their efficacy in separation processes.

Quaternary mixtures of cholinium ([N₁₁₁(2OH)])-based ILs – cholinium butanoate, ([N₁₁₁(2OH)][But]), cholinium propanoate ([N₁₁₁(2OH)][Pro]), cholinium lactate ([N₁₁₁(2OH)][Lac]), cholinium acetate ([N₁₁₁(2OH)][Ace]), cholinium glycolate ([N₁₁₁(2OH)][Gly]), cholinium dihydrogenphosphate ([N₁₁₁(2OH)][DHP]), and cholinium chloride ([N₁₁₁(2OH)]Cl) – PEG with a molecular weight of 600 mol·g⁻¹ (PEG 600), potassium phosphate (K₃PO₄), and water were used to prepare MuPS. The mixtures able to form three-phase systems (or not) are identified in Table 1, complemented with the information on the ability of the same ILs to form ABS (two-phase systems) with PEG 600 or K₃PO₄. Further details on the experimental procedure adopted are given in the Supporting Information. It should be remarked that [N₁₁₁(2OH)]Cl and [N₁₁₁(2OH)][DHP] do not fall within the IL category if their melting temperatures are considered as a threshold (> 100 °C). However, when dealing with IL-based ABS and related aqueous systems the phenomenon is more intricate and does not depend only on the melting temperature of each salt.[10,11] Therefore, and for a matter of simplicity, all the investigated cholinium-based salts will be described as ILs and K₃PO₄ as the (inorganic) salt.

With the exception of $[N_{111}(2OH)][Pro]$ and $[N_{111}(2OH)][But]$, all the studied $[N_{111}(2OH)]$ -based ILs are able to form three-phase systems when mixed (in correct proportions) with aqueous solutions of PEG 600 and K_3PO_4 – cf. Table 1. These data reveal that the ability of an IL to form a three-phase system is related with its ability to form ABS with the other two solutes, *i.e.*, with the salt or the polymer. These results are in good agreement with the criteria used by previous authors,[3–6] who suggested that if the aqueous mixtures composed of the solutes A/B, B/C and A/C are able to form ABS, aqueous mixtures composed of A/B/C will result in the formation of three-phase systems. However, and despite the apparent validity of this criterion, its application requires previous knowledge on the pairs of solutes able to form ABS, resulting in a hard and time-consuming procedure. To overcome this drawback, it is crucial to understand the molecular-level mechanisms behind the formation of MuPS to be able to predict which mixtures allow their formation.

The demixing of two aqueous phases, and consequent formation of an ABS, occurs when two water-soluble solutes are mixed above certain concentrations and/or temperature in an aqueous solution. These solutes compete for the formation of hydration complexes and the phase-separation occurs.[7,10,14] For example, in systems constituted by $[N_{111}(2OH)]$ -based ILs and K_3PO_4 , the liquid-liquid demixing results from the salting-out effect of the salt over the IL, *i.e.*, high-charge density salt ions are preferentially hydrated leading to the exclusion of the less hydrophilic IL to a second liquid phase.[12,13] Similarly, for systems composed of polymers and salts, such as $PEG\ 600 + K_3PO_4 + H_2O$ and $PEG\ 600 + [N_{111}(2OH)]$ -based ILs + H_2O , there is a salting-out effect of the salt (or IL) over the polymer and the phases demixing occurs.[10] Thus, when a quaternary mixture is prepared using three solutes able to induce ABS formation when mixed as pairs, all these interactions and preferential hydration will occur, resulting in the formation of three distinct aqueous phases: an IL-rich phase, a salt-rich phase and a polymer-rich phase.

A schematic representation of the salting-out effects identified in the quaternary mixtures under study is presented in Figure 1. The vertices of the triangles represent the three solutes that compose the aqueous quaternary mixture, while the edges represent the ternary mixtures (two solutes and water) that are, or not, able to form an ABS. In the cases where phase separation occurs, the arrows indicate the direction of the salting-out effect. As discussed above, and as represented in Figure 1A, when all solutes that compose the quaternary system are able to induce a two-phase separation when mixed in pairs in aqueous media, three salting-out effects occur simultaneously in the mixture – the K_3PO_4 salting-out effect over $[N_{111}(2OH)]Cl$ and PEG 600, and the $[N_{111}(2OH)]Cl$ salting-out over PEG 600 – and a MuPS is obtained. This behavior is transversal to the quaternary mixtures composed of all the $[N_{111}(2OH)]$ -based ILs able to form MuPS – cf. Table 1. However, for the cases where there is only two salting-out effect occurring – K_3PO_4 salting-out effect over both PEG 600 and $[N_{111}(2OH)][Pro]$, and no salting-out effect of the IL over the polymer – it is impossible to form a MuPS (Figure 1B). This last example occurs for the ILs $[N_{111}(2OH)][Pro]$ and $[N_{111}(2OH)][But]$ which present favorable IL–PEG interactions,[10] not allowing their exclusion to different phases and further formation of a MuPS. It is thus clear that a triple salting-out effect is the crucial phenomenon which rules the formation of three-phase systems.

To gather a better understanding on the three phases separation in IL-based MuPS, the system composed of $[N_{111}(2OH)]Cl + PEG\ 600 + K_3PO_4 + H_2O$ was studied in detail. First, the surfaces limiting the monophasic, biphasic, triphasic, and solid-liquid regions were established (Figure 2). Details on the experimental procedure applied and equilibrium surfaces data obtained are given in the Supporting Information. In Figure 2A it is possible to distinguish a surface connecting the binodals of the ternary phase diagrams composed of $[N_{111}(2OH)]Cl + K_3PO_4 + H_2O$ (blue dots) and $PEG\ 600 + K_3PO_4 + H_2O$ (green dots), limited in the bottom by the $[N_{111}(2OH)]Cl + PEG\ 600 + H_2O$ system (red dots). All quaternary mixtures prepared at concentrations above this surface will result in homogeneous solutions – monophasic region – while, below the surface, all mixtures are within the multiphasic region. Thus, this surface is the phase boundary, whereas the three-phase region is within the multiphase region delimited by the phase boundary. In Figure 2B it is shown that the three-phase region is considerably smaller than the biphasic region and is limited in the bottom by the solid-liquid region, in which the solutes are not completely soluble and a solid-phase appears – *cf.* Figure 2B.

The composition of the coexisting phases of two quaternary mixtures were analytically determined, namely 30.70 wt % of $[N_{111}(2OH)]Cl + 29.64$ wt % of $PEG\ 600 + 9.86$ wt % of $K_3PO_4 + 29.81$ wt % of H_2O and 22.61 wt % of $[N_{111}(2OH)]Cl + 21.36$ wt % of $PEG\ 600 + 22.63$ wt % of $K_3PO_4 + 33.40$ wt % of H_2O , mainly to infer the phase composition and if ions exchange between the phases occurs. Details on the experimental procedure are given in Supporting Information. The obtained results are depicted in Figure 2C through the representation of “tie surfaces”.

The detailed composition of the coexisting phases is given in the Supporting Information. As expected, each phase is composed of water, $[N_{111}(2OH)]Cl$, $PEG\ 600$ and K_3PO_4 . However, each aqueous phase is richer in one of these three components. In the two mixture points studied, the top phases are rich in $[N_{111}(2OH)]Cl$, the middle phases are mostly composed of $PEG\ 600$, and the bottom phases are mainly constituted by the inorganic salt. The quantification of each ion (of the salt and IL) in each phase was also carried out, demonstrating that the ion exchange between the phases is negligible in these systems – detailed data are given in the Supporting Information.

The temperature effect on the formation of MuPS was also evaluated. The surface that limits the biphasic and the triphasic regions was determined at 45 and 65 °C, and compared to the surface previously determined at 25 °C. The obtained results are represented in Figure 3 for a phase diagram cut at 0.85 mol of $[N_{111}(2OH)]Cl$ *per* mol of $[N_{111}(2OH)]Cl + PEG\ 600$, where it is shown that an increase in the temperature leads to an increase of the three phases region, *i.e.*, is favorable for the phase demixing. This behavior is observed in the entire range of the phase diagram – *cf.* the Supporting Information. The reported behavior is similar to that observed in polymer-salt-based ABS in which the systems temperature dependency is dominated by hydrogen-bonding interactions occurring between the polymer and water.[15]

Aiming at assessing the application of the studied systems in separation processes, the ability of IL-based MuPS for the selective separation of a mixture of three dyes – sudan III, pigment blue (PB) 27 and tartrazine (E102) – was evaluated. Details on the experimental

procedure used are given in the Supporting Information. The macroscopic appearance, as well as the extraction efficiencies of the $[N_{111}(2OH)][Ace]$ -based MuPS for dyes, are presented in Figure 4A. Remarkably, the investigated system allows the separation of the 3 dyes by the three phases with an extraction efficiency of 93% for one of the dyes and complete separation of the other two. Sudan III, which has a less polar character ($\log(K_{ow}) = 7.47$), [16] is completely extracted to the more hydrophobic PEG 600-rich phase (purple-dyed phase). On the other hand, charged species, such as PB27 and E102 ($\log(K_{ow}) < 0$) [16] partition preferentially to the more charged and polar phases, such as the IL- (yellow dyed phase) and salt-rich phases (blue dyed phase). The extraction efficiencies of the remaining MuPS for the three dyes are shown in Figure 4B. The same remarkable separation ability was obtained, with the complete extraction of Sudan to the PEG-rich phase, the complete extraction of PB27 to the salt-rich phase, and a more dependent extraction efficiency for the E102 dye according to the IL chemical nature (decreasing with the IL hydrophilic character).

In summary, the formation of novel IL-based MuPS was demonstrated. A large number of novel MuPS composed of cholinium-based ILs, K_3PO_4 , PEG 600, and water were investigated, allowing the conclusion that the formation of three aqueous phase systems is ruled by the requirement of a triple salting-out effect. Moreover, an increase in temperature is favorable for the formation of IL-based MuPS. Due to the use of ILs as phase-forming components, these systems are of low viscosity and display an improved separation performance, as demonstrated here with a mixture of 3 dyes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.
Schematic representation of the salting-out effect in quaternary mixtures composed of K_3PO_4 , PEG 600, water and (A) $[N_{111}(2OH)]Cl$ or (B) $[N_{111}(2OH)][Pro]$.

Figure 2.

Phase diagram of MuPS composed of $[N_{111}(2OH)]Cl + PEG\ 600 + K_3PO_4 + H_2O$. **(A)** Phase boundary between the monophasic and multiphasic regions – ternary phase diagrams composed of $[N_{111}(2OH)]Cl + K_3PO_4 + H_2O$ (blue dots), $PEG\ 600 + K_3PO_4 + H_2O$ (green dots), and $[N_{111}(2OH)]Cl + PEG\ 600 + H_2O$ (red dots), and quaternary mixtures composed of $[N_{111}(2OH)]Cl + PEG\ 600 + K_3PO_4 + H_2O$ (black dots). **(B)** Limits between the biphasic and triphasic regions (pink dots), and the triphasic and solid-liquid regions (yellow dots), inside the phase boundary (grey surface). **(C)** “Tie surfaces” determined in the mixture points 30.70 wt % of $[N_{111}(2OH)]Cl + 29.64$ wt % of $PEG\ 600 + 9.86$ wt % of $K_3PO_4 + 29.81$ wt % of H_2O (“tie surface” 1 - orange) and 22.61 wt % of $[N_{111}(2OH)]Cl + 21.36$ wt % of $PEG\ 600 + 22.63$ wt % of $K_3PO_4 + 33.40$ wt % of H_2O (“tie surface” 2 - green), inside the phase boundary. Legend: MR – monophasic region, BR – biphasic region, TR – triphasic region, SLR – solid-liquid region, MP – mixture points. Different perspectives of phase diagram are given in Supporting Information.

Figure 3.

Temperature effect in the three phase region of the MuPS composed of $[N_{111}(2OH)]Cl + PEG\ 600 + K_3PO_4 + H_2O$ - phase diagram cut at 0.85 mol of $[N_{111}(2OH)]Cl$ *per* mol of $[N_{111}(2OH)]Cl + PEG\ 600$: 25 °C (■), 45 °C (●), and 65 °C (▲).

Figure 4.

Dyes mixture separation in MuPS composed of [N₁₁₁(2OH)]-based IL + PEG 600 + K₃PO₄ + H₂O. **(A)** Selective extraction of (i) sudan, (ii) E102 and (iii) PB 27 in [N₁₁₁(2OH)][Ace]-based MuPS; **(B)** IL anion effect on the selective extraction of E102 dye between the IL- and polymer-rich phases.

Table 1

Identification of mixtures able (✓) or not able (✗) to form ABS or MuPS with aqueous solutions of PEG 600 and/or K₃PO₄.

[N ₁₁₁ (2OH)]-based IL	ABS		MuPS
	PEG 600[10]	K ₃ PO ₄	PEG 600 + K ₃ PO ₄
[N ₁₁₁ (2OH)]Cl	✓	✓[12]	✓
[N ₁₁₁ (2OH)][Ace]	✓	✓[12]	✓
[N ₁₁₁ (2OH)][Lac]	✓	✓[a]	✓
[N ₁₁₁ (2OH)][Gly]	✓	✓[a]	✓
[N ₁₁₁ (2OH)][DHP]	✓	✓[a]	✓
[N ₁₁₁ (2OH)][Pro]	✗	✓[13]	✗
[N ₁₁₁ (2OH)][But]	✗	✓[13]	✗

^[a]Phase diagrams determined in this work (experimental data are provided in the Supporting Information).