A Triple Salting-Out Effect is Required for the Formation of Ionic-Liquid-Based Aqueous Multiphase Systems

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

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-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, because of their composition 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 ABSs.[2]

In 2012, Mace et al.[3] introduced the concept of aqueous multiphase systems (MuPSs), that is, systems composed of three or more aqueous phases, without volatile organic solvents employed. The authors[3] reported the formation of more than 300 MuPSs 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 g mol\(^{-1}\), and NaBr. In this work, the composition of the phases and their properties, as well as the partition of xylene orange between the coexisting phases, were studied and reported.[4] Other authors have demonstrated that MuPSs present characteristics useful for specific applications, such as 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 MuPSs is still very limited, and the mechanisms associated with the multiple phases' separation were not fully elucidated in these pioneering works.[1–4]

Furthermore, the phase-forming components proposed for the creation of MuPSs 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 ABSs has been a hot area of research.[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 ABSs valuable in the extraction and separation of a wide range of compounds.[9] Furthermore, these types of systems present additional 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 MuPSs if ILs could be used as phase-forming components. Thus, in this work, we evaluate the possibility of using ILs for the formation of MuPSs, 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 ([N\(_{112026}\)])-based ILs (cholinium butanoate, [N\(_{112026}\)][But]; cholinium propanoate, [N\(_{112026}\)][Pro]; cholinium lactate, [N\(_{112026}\)][Lac]; cholinium acetate, [N\(_{112026}\)][Ace]; cholinium glycolate, [N\(_{112026}\)][Gly]; cholinium dihydrogenphosphate, [N\(_{112026}\)][DHP]; and cholinium chloride, [N\(_{112026}\)][Cl]), PEG with a molecular weight of 600 g mol\(^{-1}\) (PEG 600), potassium phosphate (K\(_2\)PO\(_4\)), and water were used to prepare MuPSs. 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 K$_3$PO$_4$. Further details on the experimental procedure are given in the Supporting Information. [N$_{1112021}$]Cl and [N$_{1112021}$]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 ABSs and related aqueous systems, the phenomenon is more complex and does not depend only on the melting temperature of each salt.[10,11] Therefore, for simplicity, all the investigated cholinium-based salts will be described as ILs and K$_3$PO$_4$ as the (inorganic) salt.

With the exception of [N$_{1112021}$][Pro] and [N$_{1112021}$][But], all the studied [N$_{1112021}$]-based ILs are able to form three-phase systems when mixed (in correct proportions) with aqueous solutions of PEG 600 and K$_3$PO$_4$, see Table 1. These data reveal that the ability of an IL to form a three-phase system is related with its ability to form ABSs with the other two solutes, that is, 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 ABSs, 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 ability of the pairs of solutes to form ABSs, resulting in a difficult and time-consuming procedure. To overcome this drawback, it is crucial to understand the molecular-level mechanisms behind the formation of MuPSs 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] Several pairs of solutes, such as polymers, salts, ILs, carbohydrates, and amino acids, among others, can be used in ABS formation and, depending on the selected pair, the same solute can act as the salting-out or the salted-out species. For instance, in systems constituted by [N$_{1112021}$]-based ILs and K$_3$PO$_4$, the liquid–liquid demixing results from the salting-out effect of the salt over the IL, that is, 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$_3$PO$_4$–H$_2$O and PEG 600/[N$_{1112021}$]-based ILs/H$_2$O, the salt (or IL) are the preferentially hydrated species, thus inducing the exclusion of the polymer to a second aqueous phase—salting-out effect of the salt (or IL) over the polymer.[10] When a quaternary mixture is prepared using three solutes that are able to induce ABS formation when mixed as pairs in aqueous media, all these salting-out phenomena 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, whereas the edges represent the ternary mixtures (two solutes and water) that are, or are not, able to form an ABS. In the cases in which phase separation occurs, the arrows indicate the direction of the salting-out effect. As discussed above, and as represented in Figure 1A, when all the 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$_3$PO$_4$ salting-out over [N$_{1112021}$]Cl and PEG 600 and the [N$_{1112021}$]Cl salting-out over PEG 600—and a MuPS is obtained. This behavior is common to the quaternary mixtures composed of all the [N$_{1112021}$]-based ILs able to form MuPSs (see Table 1). However, for the cases in which there are only two salting-out effects occurring—the K$_3$PO$_4$ salting-out over both PEG 600 and [N$_{1112021}$][Pro] and no salting-out of the IL over the polymer—it is impossible to form MuPSs (Figure 1B). This last example occurs for the ILs [N$_{1112021}$][Pro] and [N$_{1112021}$][But], which present favorable IL–PEG interactions,[10] avoiding the salting-out of the IL over the polymer and, consequently, preventing their exclusion to different phases and further formation of MuPSs. This result suggests that a triple salting-out effect is required for the formation of triphasic systems.

This hypothesis was additionally confirmed with other polymers and salts. It was previously reported that [N$_{1112021}$]–[Pro] and [N$_{1112021}$][But] are able to salt-out polypropylene glycol (PPG) with an average molecular weight of 400 g mol$^{-1}$ and consequently induce the formation of an ABS.[15] In fact, by replacing PEG 600 by PPG 400, the formation of a MuPS ([N$_{1112021}$][Pro] or [N$_{1112021}$][But])/PPG 400/K$_3$PO$_4$/H$_2$O, 23/
Three-phase compositions of IL-based MuPSs were also investigated, namely Na$_2$SO$_4$, that is not able to form ABs with the investigated ILs and for which the formation of a MuPS was not observed. All of these results support the idea that a simultaneous triple-salting out effect is required to create triphasic systems and that all the three water-soluble solutes are relevant for MuPS formation.

To achieve a better understanding of the triple-phase composition of IL-based MuPSs, the system composed of [N$_{111206}$]Cl/PEG 600/K$_3$PO$_4$/H$_2$O was studied in detail. The surfaces limiting the monophasic, biphasic, triphasic, and solid–liquid regions were established (Figure 2). Details on the experimental procedure and equilibrium surfaces data obtained are given in the Supporting Information. In Figure 2A it is possible to distinguish a surface connecting the binodal curves of the ternary phase diagrams composed of [N$_{111206}$]Cl/K$_3$PO$_4$/H$_2$O (blue dots) and PEG 600/K$_3$PO$_4$/H$_2$O (green dots), limited in the bottom by the [N$_{111206}$]Cl/PEG 600/H$_2$O system (red dots). All quaternary mixtures prepared at concentrations above this surface will result in homogeneous solutions—monophasic region; whereas, below the surface, all mixtures are within the multiphasic region. This surface is the phase boundary, whereas the three-phase region is within the multiphasic 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 (see Figure 2B).

The composition of the coexisting phases of two quaternary mixtures were also analytically determined for the following mixtures: [N$_{111206}$]Cl/PEG 600/K$_3$PO$_4$/H$_2$O (30.70/29.64/9.86/29.81 wt %) and [N$_{111206}$]Cl/PEG 600/K$_3$PO$_4$/H$_2$O (22.61/21.36/22.63/33.40 wt %). Details on the experimental procedure and results are given in the Supporting Information. The obtained results are depicted in Figure 2C as “tie surfaces”. As expected, each phase is composed of water, [N$_{111206}$]Cl, PEG 600, and K$_3$PO$_4$. 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$_{111206}$]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 MuPSs was further 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 presented in the Supporting Information. It is observed that an increase in the temperature leads to an increase of the three-phase region, that is, is favorable for the phase demixing. This behavior is observed over the entire range of the phase diagram. The reported behavior is similar to that observed in polymer-salt-based ABs in which the system’s temperature dependency is dominated by hydrogen-bonding interactions occurring between the polymer and water.$^{[16]}$

To assess the application of the studied systems in separation processes, their ability for the selective separation of a mixture of three textile dyes—sudan III, Prussian blue pigment (PB) 27, and chloranilic acid—was evaluated. More than 1 million tons of dyes are produced by year, and 10–15% of them are discharged by textile industries into aqueous effluents leading to both environmental and economic concerns.$^{[17]}$ In this context, environmental regulations are becoming increasingly stricter in regards to the removal of dyes from aqueous effluents. Despite the large number of biological, physical, and chemical processes proposed for the removal of dyes from water and wastewater, most of them display major drawbacks, such as a high cost, low efficiency,
and labor-intensive operations. Therefore, the MuPSs studied in this work may contribute to the development of alternative and cost-effective removal strategies, which from a circular economy point of view may allow not only the treatment of dye-containing wastewater but also the recovery and reuse of these materials inside the industrial processes and the reuse of the final products. Details on the experimental procedure and results are given in the Supporting Information. The macroscopic appearance, as well as the extraction efficiencies of the [N$_{112024}$][Ace]-based MuPSs for dyes, are presented in Figure 3A. Remarkably, the investigated system allows the separation of the three dyes by the three phases, with an extraction efficiency of 65% for one of the dyes and the complete separation of the other two. Sudan III, which has a less polar character (log($K_{ow}$) = 7.47), is completely extracted into the more hydrophobic PEG 600-rich phase (purple-dyed phase). On the other hand, charged species, such as PB27 and chloranilic acid (log($K_{ow}$) < 0), partition preferentially into the more charged and polar phases, the IL- and salt-rich phases (pink- and blue-dyed phases, respectively). A remarkable separation performance was also obtained with the remaining MuPSs, with the complete extraction of Sudan into the PEG-rich phase, the complete extraction of PB27 to the salt-rich phase, and a partial extraction of the chloranilic acid dye with an efficiency that depended on the IL chemical structure (Figure 3B). The extraction of sudan III and chloranilic acid through the application of IL-based ABBs was previously reported. However, both dyes partitioned preferentially into the same phase of the system, and consequently, their simultaneous extraction and separation is not possible. Thus, MuPSs appear as an alternative for the separation of textile dyes.

The use of MuPSs for the separation of other types of (bio)molecules, namely amino acids, phenolic compounds and alkaloids was additionally investigated (see the Supporting Information). Not only molecules with very distinct size and chemical structure but also molecules of high chemical structure similarity can be separated with the investigated systems. Overall, these data support the versatility of IL-based MuPS applications in separation processes.

In summary, the formation of novel IL-based MuPSs was demonstrated in this work. A large number of novel MuPSs composed of cholinium-based ILs, salts, polymers, and water were investigated, allowing the conclusion that the formation of three-aqueous-phase systems is ruled by a simultaneous triple salting-out effect. Due to the use of ILs as phase-forming components, these systems are of low viscosity and display an improved separation performance for a wide variety of compounds.

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### Conflict of interest

The authors declare no conflict of interest.

### Keywords:

aqueous multiphase systems · dyes · ionic liquids · salting-out effect · separations

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