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Designing the thermal behaviour of aqueous biphasic systems composed of ammonium-based zwitterions

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Abstract

The ability of water-soluble ammonium-based zwitterions (ZIs) to form aqueous biphasic systems (ABS) in presence of salts aqueous solutions is here disclosed for the first time. These systems are thermoreversible at temperatures close to room temperature and further allow the design of their thermal behavior, from an upper critical solution temperature (UCST) to a lower critical solution temperature (LCST), by increasing the ZIs alkyl chains length. The investigated thermoreversible ABS are more versatile than typical liquid-liquid systems, and can be applied in a wide range of temperatures and compositions envisaging a target separation process.

The use of ionic liquids (ILs) in liquid-liquid extractions has been a hot topic of research in the past decades.¹ Their unique properties, namely negligible vapour pressures, high thermal and chemical stabilities, and high solvation ability for a large range of compounds, make of ILs remarkable alternatives over volatile organic solvents. Furthermore, the possibility of changing the ILs properties by the structural design of both the cation and anion is considered one of their most interesting characteristics, allowing to tailor these fluids for specific applications.

Recently, it was proposed that the design of ILs properties could be fine-tuned by using ILs mixtures.² However, whenever IL-containing systems lead to the formation of more than one phase, ILs mixtures may lead to a different partitioning of their ions between the coexisting phases, leading therefore to changes in the composition of the phases and respective properties.³ To overcome some of these unwanted consequences, some authors^{3–6} proposed the use of zwitterions (ZIs), compounds where the cation and the anion are covalently tethered, instead of ILs. These ion pairs remain covalently linked even after adding strong acids, such as lithium salts⁷ and Brønsted acids.⁸

Since 2012, when Ohno and co-workers⁴ proposed the use of ZIs as additives in IL-water systems to improve the water content in hydrophobic IL-rich phases, and consequently to increase the IL-phase ability to dissolve and extract proteins, several authors^{3,5,6} addressed the study of ZIs applications in liquid-liquid extractions. In these works,^{3–6} ZIs with a high hydrophobic character were investigated, whereas more hydrophilic ZIs have been described as unable to induce phase separation with water, even by temperature changes. These compounds, that did not seem very attractive for use in liquid-liquid extractions from aqueous media, are here evaluated regarding their ability to form aqueous biphasic systems (ABS) with conventional salts. ABS were first reported in the 50's as more benign liquid-liquid separation processes for biomolecules due to their water-rich environment.⁹ These systems consist in two immiscible aqueous-rich phases, that are created by the mixture of two polymers, a polymer and a salt or two salts in aqueous media.¹⁰

In the past few years, the research on dynamic and reversible biphasic systems involving ILs or ZIs has attracted much attention towards the development of novel and more efficient separation processes, either by changes in pH,¹¹ temperature^{3,6,12–14} or CO₂/N₂ addition.¹⁵ Systems displaying an UCST- or LCST-type phase behaviour can be used to move between monophasic and biphasic regimes by inducing temperature-dependent phase transitions, shown to be highly advantageous for the separation of proteins,¹² metals¹³ and catalysts.¹⁴ However, most of the phase transitions in these systems occur at temperatures far from room temperature, or are confined to narrow mixture compositions. Therefore, the design of novel systems with UCST- or LCST-type phase behaviour occurring at temperatures close to room temperature has been object of a great deal of work; yet, only a limited number of systems has been identified.^{6,12,16}

Herein we study the ability of water-soluble ammonium-based ZIs to form ABS by mixing them with salts aqueous solutions. Since the cation and anion of ZIs are covalently tethered, there is no ion exchange between the coexisting phases. The effect of temperature on the respective phase diagrams was also appraised to infer on their thermal behaviour. Five ammonium-based ZIs with different alkyl side chains length (Fig. 1) were synthesized and used in the creation of ABS. The definition of the ZIs acronyms is provided as a footnote[‡]. The detailed synthetic procedure for their preparation is given in the ESI. The structure and purity of the ZIs were confirmed by ¹H NMR and elemental analysis, and their thermal properties were evaluated by DSC and TGA (*cf.* the ESI).

The phase diagrams of ternary mixtures constituted by each ZI (Fig. 1), salts (K₃PO₄, K₃C₆H₅O₇, K₂CO₃, K₂HPO₄ and KH₂PO₄), and water, were initially determined at 25 °C to infer on the ability of water-soluble ammonium-based ZIs to create ABS. Figure 2 displays an example of the ternary liquid-liquid phase diagrams obtained. Further details on the experimental procedure adopted, as well as the detailed experimental weight fraction data and the representation of the remaining phase diagrams, are given in the ESI. All solubility

[‡]ZIs used: *N,N,N*-tripentyl-3-sulfonyl-1-propaneammonium (N₅₅₅C3S); *N,N,N*-tripropyl-3-sulfonyl-1-propaneammonium (N₃₃₃C3S); *N,N,N*-triethyl-3-sulfonyl-1-propaneammonium (N₂₂₂C3S); *N,N,N*-trimethyl-3-sulfonyl-1-propaneammonium (N₁₁₁C3S); *N,N,N*-trimethyl-4-sulfonyl-1-butaneammonium (N₁₁₁C4S).

curves are represented in molality units in order to better interpret the salting-in/-out effects obtained, while avoiding the effect of the different species molecular weights.

Figure 2 depicts the solubility curves for systems composed of N₅₅₅C3S, potassium-based salts and water, allowing the evaluation of the salt anion effect on the formation of ZI-based ABS. The solubility curves represent the limit between the monophasic and biphasic regimes, in which mixture compositions above the solubility curve result in biphasic liquid-liquid systems, and those below fall in the monophasic region. The larger the monophasic region of each phase diagram the higher is the amount of ZI and/or salt required to induce the ABS or two-phase formation. From the gathered results, and at the molality of ZI at which it equals the molality of salt in each binodal curve (*i.e.*, [ZI] = [salt]), the ability of the potassium-based salt anions to induce the formation of ABS follows the order: PO₄³⁻ > C₆H₅O₇³⁻ > CO₃²⁻ > HPO₄²⁻ >> H₂PO₄⁻. This salt anions trend is in good agreement with the Hofmeister series,¹⁷ and with previously reported ranks for IL-based ABS.¹⁸ This trend indicates that potassium-based salts act as salting-out species. In the studied systems composed of salts with high charge density ions and ZIs, the former are more able to create hydration complexes and to induce the salting-out of the more hydrophobic ZIs.

The phase diagrams obtained with the salts Na₃C₆H₅O₇ and K₃C₆H₅O₇, and KH₂PO₄ and NaH₂PO₄, with a given ZI, allow us to address the salt cations ability to promote the formation of ZI-based ABS. The respective phase diagrams and detailed experimental weight fraction data are reported in the ESI. The results obtained demonstrate that the salt cation has no significant effect on the binodal curves of N₅₅₅C3S-based ABS. However, when a more hydrophilic ZI is used, such as N₃₃₃C3S, the salting-out ability of the citrate-based salts cations follows the order: Na⁺ > K⁺, in good agreement with trends observed for IL-based ABS.¹⁸ This trend was also verified with phosphate-based salts, in which only NaH₂PO₄ was able to induce phase separation with N₃₃₃C3S – *cf.* the ESI.

Figure 3 depicts the influence of the ZIs alkyl chain length in the formation of ABS with a given salt (K₃PO₄). The solubility curves show a strong dependency on the ZI alkyl side chains length – effect even more pronounced than that verified with the salt ions. At the solubility curve, namely when [ZI] = [salt] (mol·kg⁻¹), the tendency of ZIs to form ABS by the addition of K₃PO₄ follows the order: N₅₅₅C3S > N₃₃₃C3S > N₂₂₂C3S > N₁₁₁C4S > N₁₁₁C3S. The longer the cation/anion alkyl chains of the ZI, representing an increase of the ZI hydrophobicity and easiness for being salted-out, the better it is their ability to undergo liquid-liquid demixing in presence of salts aqueous solutions. This trend also supports the loss in the ability of ZIs with smaller alkyl side chains to form ABS with salts of weaker salting-out strength – *cf.* the ESI.

After appraising the possibility of forming ZI-salt-based ABS, the temperature effect on this type of systems was studied. Additional phase diagrams were determined at 35 and 45 °C, and compared with the solubility curves obtained at 25 °C discussed above. K₃PO₄ was used since it is the only salt able to induce the formation of ABS with all ZIs under study. The detailed experimental procedure, weight fraction data, and the representation of the phase diagrams for the remaining ZIs, are given in the ESI.

Figure 4 depicts the temperature dependency of the N₅₅₅C3S-, N₃₃₃C3S- and N₁₁₁C3S-based ABS phase diagrams. Remarkably, within the studied series of ZIs, a change in the temperature dependency was observed. For N₅₅₅C3S-based ABS, an increase in temperature enlarges the two-phase region (Fig. 5A), whereas the opposite is observed with N₁₁₁C3S (Fig. 5C), and a negligible effect of temperature occurs in the N₃₃₃C3S-based ABS (Fig. 5B). It was shown that N₅₅₅C3S presents a LCST behaviour with water³ and, consequently, the phases separation is favoured at higher temperatures – a phenomenon that seems to prevail in the respective ABS. This behaviour is similar to that reported for polymer-salt ABS,¹⁹ in which the temperature dependency is dominated by hydrogen-bonding interactions between the polymer and water. On the opposite, when more hydrophilic ZIs are used, such as N₂₂₂C3S, N₁₁₁C3S and N₁₁₁C4S, an increase in temperature decreases the two-phase region of the respective ABS (Fig. 5C), being the UCST-type phase behaviour usually observed in systems dominated by non-directional interactions. For ZIs of intermediate alkyl chains length, such as N₃₃₃C3S, the temperature seems to have a negligible impact upon the binodal curves, with their overlapping at different temperatures – Fig. 5B. In summary, as the ZIs hydrophobicity decreases, by the decrease of their alkyl side chains length, a shift in the dominant interactions between the ZIs and water occurs, from directional hydrogen bonding to non-directional coulombic interactions, allowing therefore the design of the thermal behaviour of ZI-based ABS.

Upon the establishment of the temperature dependency of the studied ABS and their thermal behaviour designer ability, their temperature-reversible behaviour was further appraised. A mixture point between the solubility curves at 25 and 45 °C was prepared for systems composed of N₅₅₅C3S or N₁₁₁C3S. For the N₅₅₅C3S + K₃PO₄ + water ABS, a mixture point between these two solubility curves results in a homogeneous solution at 25 °C – *cf.* Fig 4A. However, when the temperature increases to 45 °C the phase separation occurs, resulting in a top ZI-rich phase and in a bottom salt-rich phase. If the temperature is decreased again to 25 °C, the initial monophasic system is recovered. Since N₁₁₁C3S-based ABS presents the opposite temperature dependency behaviour, the formation of two phases occurs at lower temperatures (25 °C) that disappear on heating up to 45 °C. The thermoreversible behaviour of the studied systems is illustrated in Fig. 5, for both N₅₅₅C3S- and N₁₁₁C3S-based ABS. A luminescent molecule – fluorescein – was added to each system to highlight the phase separation phenomenon by a change in temperature. Fluorescein partitions almost completely to the ZI-rich phases when the phases separation occurs.

In summary, it was here demonstrated that water-soluble ZIs can form ABS with aqueous solutions of salts, and that their thermoreversible behaviour can be designed by playing around with the ZI alkyl chains length, while allowing their tuning according to specific requirements of a given separation process. These reversible ZI-based ABS occur at temperatures close to room temperature, avoiding additional energetic consumptions or thermal degradation of some target products. Furthermore, the temperature range of operation can be selected based on the ternary mixture compositions to fit the requirements of a specific process and is not restricted to fixed temperatures imposed by the thermodynamic nature of binary liquid-liquid systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Acronym	R ₁ , R ₂ , R ₃	R ₄
N ₁₁₁ C3S	CH ₃	(CH ₂) ₃
N ₁₁₁ C4S	CH ₂ CH ₃	(CH ₂) ₄
N ₂₂₂ C3S	(CH ₂) ₂ CH ₃	(CH ₂) ₃
N ₃₃₃ C3S	(CH ₂) ₃ CH ₃	(CH ₂) ₃
N ₅₅₅ C3S	(CH ₂) ₄ CH ₃	(CH ₂) ₃

Fig. 1.
Chemical structures and acronyms of the ZIs used.

Fig. 2. Salt anion effect in the phase diagrams of ternary systems composed of water, $N_{555}C3S$ and potassium-based salts at 25 °C: K_3PO_4 ($\square$), $K_3C_6H_5O_7$ ($\blacklozenge$), K_2CO_3 ($\circ$), K_2HPO_4 ($+$) and KH_2PO_4 ($\triangle$).

Fig. 3.
ZIs alkyl chains length effect in the phase diagrams of ternary systems composed of water, K_3PO_4 and ZIs at 25 °C: $N_{555}C3S$ ($\square$), $N_{333}C3S$ (+), $N_{222}C3S$ ($\blacktriangle$), $N_{111}C4S$ ($\circ$) and $N_{111}C3S$ ($\blacklozenge$).

Fig. 4.

Temperature (T) effect in the phase diagrams of ternary systems composed of ZI + K_3PO_4 + water at 25 °C ($\blacktriangle$), 35 °C ($\blacklozenge$), and 45 °C ($\circ$): (A) $N_{555}C3S$, (B) $N_{333}C3S$, and (C) $N_{111}C3S$.

Fig. 5.
Thermoreversible behaviour of ZI-based ABS.