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

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The ability of water-soluble ammonium-based zwitterions (ZIs) to form aqueous biphasic systems (ABS) in the presence of aqueous solutions of salts is disclosed here. 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 ZI alkyl chain 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. An example is given regarding their ability to selectively separate mixtures of aromatic and aliphatic amino acids in a single-step.

The use of ionic liquids (ILs) in liquid–liquid extractions has been a hot topic of research in the past few 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 ILs remarkable alternatives over volatile organic solvents. Furthermore, the possibility of changing the IL properties by the structural design of both the cation and anion is considered one of their most interesting characteristics, allowing us to tailor these fluids for specific applications.

Recently, it was proposed that the IL properties could be further fine-tuned by using IL mixtures.² However, whenever IL-containing systems lead to the formation of more than one phase, IL mixtures may lead to a different partitioning of their ions between the coexisting phases, leading therefore to changes in the compositions and properties of these phases.³ To overcome some of these unwanted trends, 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's ability to dissolve and extract proteins, several authors^{3,5,6} addressed the study of ZI applications in liquid–liquid extractions. In these studies,^{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 evaluated here regarding their ability to form aqueous biphasic systems (ABS) with conventional salts. ABS were first reported in the 50s as more benign liquid–liquid separation processes for biomolecules due to their water-rich environment.⁹ These systems consist of two immiscible aqueous-rich phases, that are formed 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, 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 an object of a great deal of work; yet, only a limited number of systems have been identified.^{6,12,16}

Herein we study the ability of water-soluble ammonium-based ZIs to form ABS by mixing them with aqueous solutions

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of salts. 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 evaluate their thermal behaviour. Five ammonium-based ZIs with different alkyl side chain lengths (Fig. 1) were synthesized and used in the formation of ABS. The definition of the ZI acronyms is provided as a footnote. § The detailed synthetic procedure for their preparation is given in the ESI. † The chemical structure and purity of the ZIs were confirmed by ^1H NMR and elemental analysis, and their thermal properties were determined by DSC and TGA (cf. the ESI †).

The phase diagrams of ternary mixtures constituted by each ZI (Fig. 1), each salt (K_3PO_4 , $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$, K_2CO_3 , K_2HPO_4 and KH_2PO_4), and water were initially determined at 25 °C to address the ability of water-soluble ammonium-based ZIs to form ABS. Fig. 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

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

Fig. 2 depicts the solubility curves for systems composed of $\text{N}_{555}\text{C3S}$, 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 the ZI and/or salt required to induce the ABS or two-phase formation. From the gathered results, and at the molality of the ZI at which it equals the molality of salt in each binodal curve (i.e., $[\text{ZI}] = [\text{salt}]$), the ability of the potassium-based salt anions to induce the formation of ABS follows the order: $\text{PO}_4^{3-} > \text{C}_6\text{H}_5\text{O}_7^{3-} > \text{CO}_3^{2-} > \text{HPO}_4^{2-} \gg \text{H}_2\text{PO}_4^-$. This salt anion trend is in good agreement with the Hofmeister series,¹⁷ and with the 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 form hydration complexes and to induce the salting-out of the more hydrophobic ZIs.

The phase diagrams obtained with the salts $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ and $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$, and KH_2PO_4 and NaH_2PO_4 , with a given ZI, allow us to address the ability of salt cations 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 $\text{N}_{555}\text{C3S}$ -based ABS. However, when a more hydrophilic ZI is used, such as $\text{N}_{333}\text{C3S}$, the salting-out ability of the citrate-based salt cations follows the order: $\text{Na}^+ > \text{K}^+$, in good agreement with trends observed for IL-based ABS.¹⁸ This trend was also verified with phosphate-based salts, in which only NaH_2PO_4 was able to induce phase separation with $\text{N}_{333}\text{C3S}$ – cf. the ESI. †

Fig. 3 depicts the influence of the ZI alkyl chain length on the formation of ABS with a given salt (K_3PO_4). The solubility curves show a strong dependency on the ZI alkyl side chain length – the effect is even more pronounced than that verified with the salt ions. At the solubility curve, namely when $[\text{ZI}] = [\text{salt}]$ (mol kg^{-1}), the tendency of ZIs to form ABS by the addition of K_3PO_4 follows the order: $\text{N}_{555}\text{C3S} > \text{N}_{333}\text{C3S} > \text{N}_{222}\text{C3S} > \text{N}_{111}\text{C4S} > \text{N}_{111}\text{C3S}$. The longer the cation/anion alkyl chains of the ZI, representing an increase of the ZI hydrophobicity and capability for being salted-out, the better is their ability to undergo liquid-liquid demixing in the presence of aqueous solutions of salts. This trend also supports the loss in the ability to form ABS with the salts of weaker salting-out strength displayed by ZIs with smaller alkyl side chains – 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

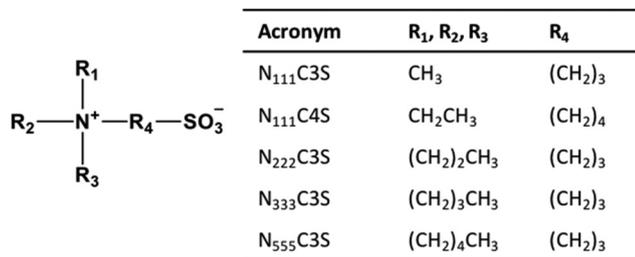


Fig. 1 Chemical structures and acronyms of the ZIs used.

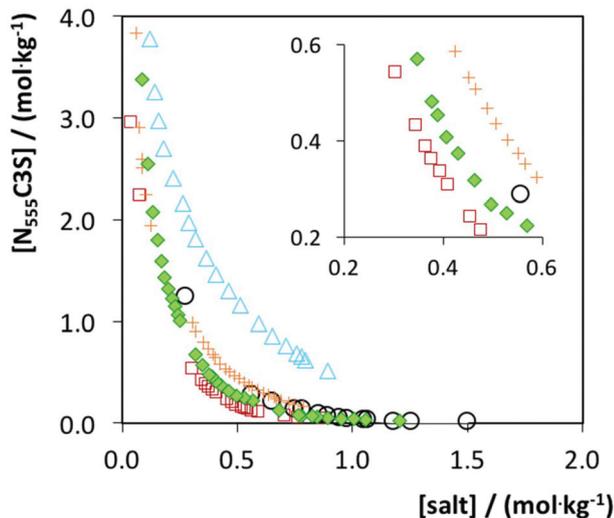


Fig. 2 Salt anion effect in the phase diagrams of ternary systems composed of water, $\text{N}_{555}\text{C3S}$ and potassium-based salts at 25 °C: K_3PO_4 (□), $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ (◆), K_2CO_3 (○), K_2HPO_4 (+) and KH_2PO_4 (△).

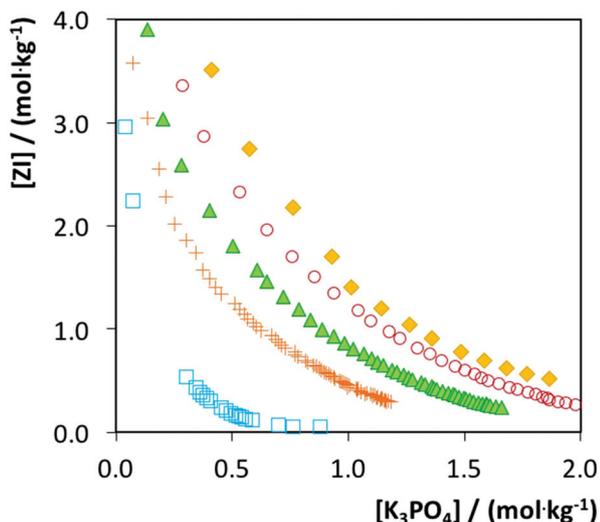


Fig. 3 ZI alkyl chain length effect in the phase diagrams of ternary systems composed of water, K_3PO_4 and ZIs at 25 °C: $N_{555}C3S$ (\square), $N_{333}C3S$ ($+$), $N_{222}C3S$ (\blacktriangle), $N_{111}C4S$ (\circ) and $N_{111}C3S$ (\blacklozenge).

25 °C discussed above. The detailed experimental procedure, weight fraction data, and the representation of the phase diagrams for the remaining ZIs are given in the ESI.†

Fig. 4 depicts the temperature dependency of the $N_{555}C3S$ -, $N_{333}C3S$ - and $N_{111}C3S$ - K_3PO_4 -based ABS phase diagrams. Remarkably, within the studied series of ZIs, a change in the temperature dependency was observed. For the $N_{555}C3S$ -based ABS, an increase in temperature enlarges the two-phase region (Fig. 4A), whereas the opposite effect is observed with $N_{111}C3S$ (Fig. 4C). In this line, a negligible effect of temperature occurs in the $N_{333}C3S$ -based ABS (Fig. 4B). It was previously shown that $N_{555}C3S$ presents a LCST phase transition behaviour with water³ and, consequently, the phase's 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 contrary, when more hydrophilic ZIs, such as $N_{222}C3S$, $N_{111}C3S$ and $N_{111}C4S$, are used, an increase in temperature decreases the two-phase region of the respective ABS (Fig. 4C and additional data in the ESI†), demonstrating the presence of the UCST-type phase behaviour in systems dominated by non-directional interactions. For ZIs of intermediate alkyl chain length, such as $N_{333}C3S$, the temperature seems to have a negligible impact upon the binodal curves, with their overlapping at different temperatures – Fig. 4B. In summary, as the ZI hydrophobicity decreases, by the decrease of their alkyl side chain length, a shift in the dominant interactions between the ZIs and water occurs, from directional hydrogen bonding to non-directional interactions, therefore allowing the design of the thermal behaviour of ZI-based ABS.

Upon the establishment of the temperature dependency of the studied ABS, 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 $N_{555}C3S$ (30 wt% ZI + 4 wt% K_3PO_4) or $N_{111}C3S$ (19.5 wt% ZI + 19.5 wt% K_3PO_4). For the $N_{555}C3S$ + K_3PO_4 + 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 phase separation occurs, resulting in a top ZI-rich phase and a bottom salt-rich phase. If the temperature is decreased again to 25 °C, the initial monophasic system is recovered. Since $N_{111}C3S$ -based ABS present the opposite temperature dependency behaviour, the formation of two phases occurs at lower temperatures (25 °C) that disappear upon heating up to 45 °C. The thermoreversible behaviour of the studied systems is illustrated in Fig. 5, for both $N_{555}C3S$ - and $N_{111}C3S$ -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 phase when the phase separation occurs.

The potential application of the studied thermoreversible systems was finally investigated to separate mixtures of amino acids that could exist in protein hydrolysates or in fermenta-

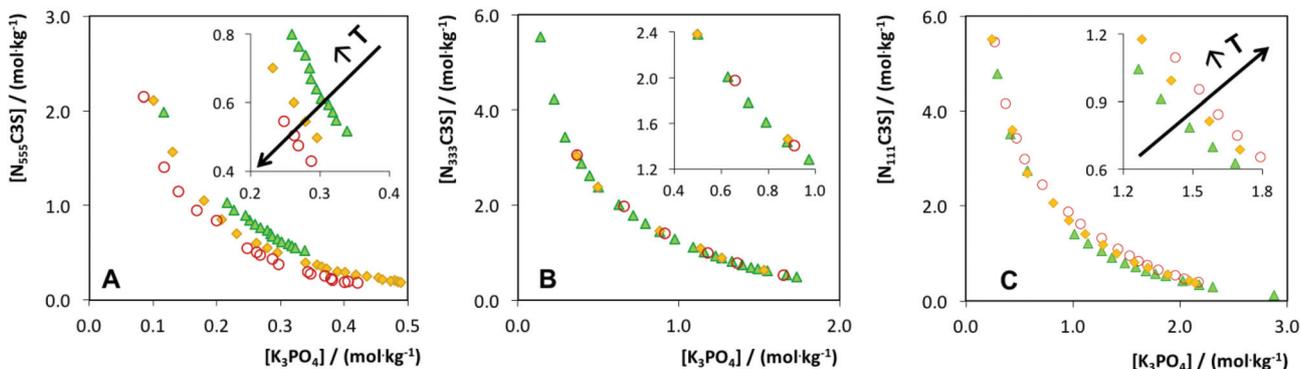


Fig. 4 Temperature (T) effect in the phase diagrams of ternary systems composed of ZI + K_3PO_4 + water at 25 °C (\blacktriangle), 35 °C (\blacklozenge), and 45 °C (\circ): (A) $N_{555}C3S$, (B) $N_{333}C3S$, and (C) $N_{111}C3S$.

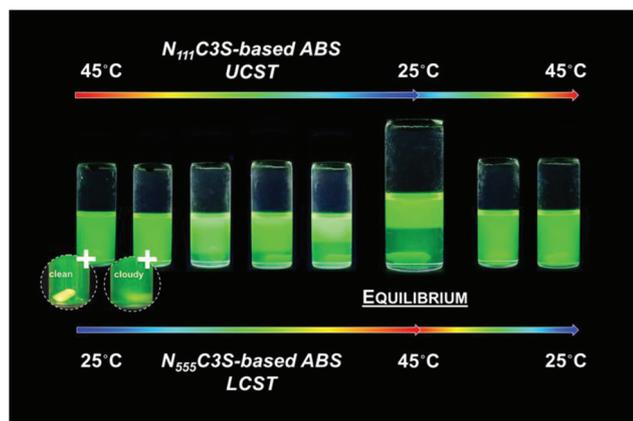


Fig. 5 Thermoreversible behaviour of ZI-based ABS.

tion broths. As an example of these types of mixtures, aromatic and aliphatic amino acids, namely *L*-tryptophan (Try) and glycine (Gly), were studied. The previously described ABS mixture compositions were used. Details regarding the quantification of both amino acids are given in the ESI.† The obtained results are depicted in Fig. 6. Data are shown in percentage extraction efficiencies ($EE_{AA}\%$) of each amino acid for each phase. $EE_{AA}\%$ corresponds to the percentage ratio between the amount of each amino acid in a given phase and that in the total mixture.

In general, there is a high impact of the ZI alkyl chain length on the separation performance of the studied ABS. In the $N_{111}C3S$ -based ABS both amino acids preferentially partition to the ZI-rich phase, with extraction efficiencies ranging between 72% and 86%. However, in the $N_{555}C3S$ -based ABS, glycine partitions to the opposite phase ($EE_{AA}\% = 67\%$), the salt-rich phase instead of the ZI-rich phase, while the phase to which *L*-tryptophan partitions and its extraction extent ($EE\% = 84\%$) are almost not affected. The trends obtained are in good agreement with the hydrophilic/hydrophobic character of each amino acid ($\log(K_{ow})_{Try} = -1.09$ and $\log(K_{ow})_{Gly} = -3.41$, with

K_{ow} defined as the octanol–water partition coefficient).²⁰ With the increase in the ZI alkyl chain length there is an increase in the hydrophobicity of the corresponding phase; therefore, an inversion of the partition trend of the most hydrophilic amino acid occurs, preferentially migrating to the more hydrophilic salt-rich phase. The selectivities of the $N_{111}C3S$ - and $N_{555}C3S$ -based ABS for the separation of *L*-tryptophan from glycine are 2.4 and 6.8, respectively, meaning that an increase in the ZI alkyl chain length improves (3-fold increase) the selectivity of ABS. In summary, the separation of aromatic (*L*-tryptophan) and aliphatic (glycine) amino acids can be achieved in a single-step using adequate ZI-based ABS, further supporting the tailoring ability of the investigated liquid–liquid systems.

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 chain length, while allowing their tuning according to the specific requirements of a given separation process, as demonstrated with their use in the separation of mixtures of amino acids. These reversible ZI-based ABS occur at temperatures close to room temperature, avoiding additional energetic consumptions or the 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.

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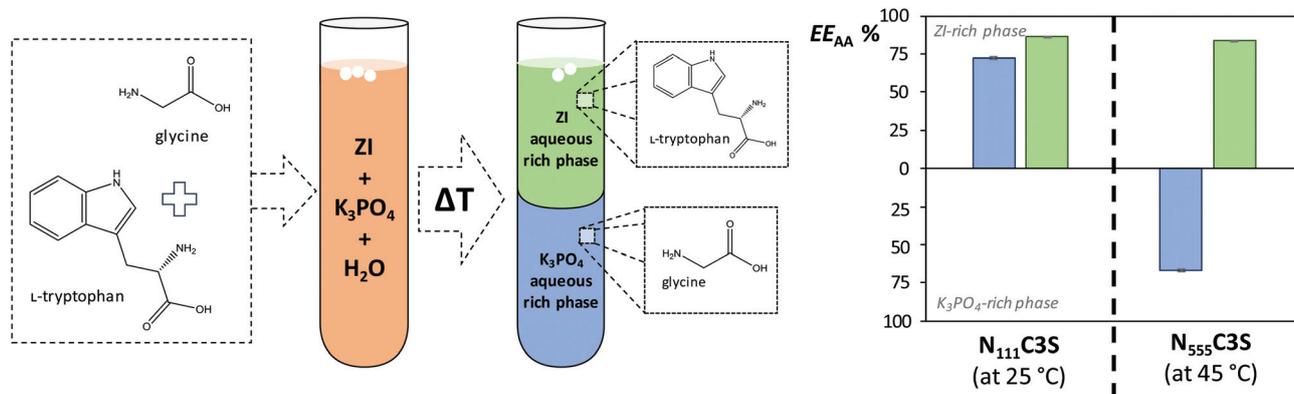


Fig. 6 Partition of *L*-tryptophan (Try) and glycine (Gly) in $N_{111}C3S$ - and $N_{555}C3S$ -based ABS formed at 25 °C and 45 °C, respectively: $EE_{Try}\%$ (green bars), $EE_{Gly}\%$ (blue bars).

Notes and references

§ 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).

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