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Aqueous Biphasic Systems Formed in (Zwitterionic Salt + Inorganic Salt) Mixtures

Abstract: The manuscript reports on a new class of aqueous biphasic systems (ABSs) formed in mixtures of inorganic salts (ISs) and zwitterionic salts (ZWSs). Aqueous ternary phase diagrams characterized by a binodal curve were determined for systems consisting of four ISs, K₃PO₄, K₂HPO₄, K₃HPO₄/KH₂PO₄, and K₂CO₃, and three structurally similar ZWSs differing in hydrophobicity. Comparison of phase behaviour of ABSs composed of ZWSs, ionic liquids (ILs) and zwitterions was provided. Potential of ZWSs based systems for extraction of aromatic molecules and amino acids, such as glycine, L-tryptophan, DL-phenylalanine, eugenol, and phenol was examined. Feasibility and limitations of isolation of products after partition and recovery of ZWS were discussed.

Keywords: Aqueous biphasic system, zwitterionic salt, amino acid, partition coefficient

Introduction

Aqueous biphasic systems were introduced by P.Å. Albertson as an alternative method to chromatography, suitable for large-scale isolation and purification of bio-molecules from fermentation and cell culture broth.¹,² This first generation of ABSs formed in polymer - IS mixtures or in mixtures of incompatible polymer pairs, as demonstrated by its inventor, has shown a great potential in separation and fractionation of cells, viruses, proteins, nucleic acids, and antigen-antibody.³ Experimental studies that followed established that partition of dissolved solutes in a complex mixture between two aqueous - rich phases in equilibrium is defined by multiple interactions of the added solute and the constituents of the ABS; these interactions can be electrostatic, hydrophobic, van der Waals, or hydrogen bond type. Since potential of ABSs was recognized, numerous methods and protocols for partition of soluble molecules, particulates, and proteins have been developed and well documented, which supported application of ABSs for product recovery in industrial separations and biotechnology.³,⁴

In the last decade, ILs emerged as new polymer - or IS - replacing components of ABSs, thus forming second generation of ABSs which offers solution for a relatively low polarity of polymer-constituting phase. Many possibilities of structural design of ILs aiming to achieve specific molecular interactions with dissolved solutes led to enhanced solvation capability in such systems by providing a suitable polarity range and/or predominant attractive interaction needed for improved extraction of targeted molecules. A pioneering work on IL - based ABSs published by Gutovski et al.⁵, was advanced by further studies and developments that resulted in a significant number of publications in recent years with an outstanding contribution coming from group of Coutinho and Freire.⁶-¹⁹ The most important findings on ILs - based ABSs are summarized in several reviews²⁰-²⁴ and a book²⁵.

ZWSs are hybrid materials, recently introduced by Blesic et al., which exhibit some characteristics of zwitterions, ISs and ILs.²⁶ They are comprised of cations and anions in which an additional zwitterionic moiety is embedded into either the cation or the anion. As a consequence of multiple charges present in their structures, ZWSs are characterized by high polarity and hydrophilicity, but also possibility of modulation and functionalization, good thermal stability, and non-toxicity for a range of studied microorganisms.²⁶, ²⁷ It was also reported that ZWSs engage in complex interactions with different type of dissolved organic molecules in aqueous solution, which enables them to be used either as solubility enhancers or phase separation promoters depending on structure of the dissolved solute.²⁷

In this work, we have studied phase behaviour and interactions in aqueous solutions of ZWSs and ISs. Ternary phase diagrams were determined for three structurally similar ZWSs, namely C₂S, C₃S, and C₄S (the structures presented in Figure 1), and four ISs, K₃PO₄, K₂HPO₄, K₃HPO₄/KH₂PO₄, and K₂CO₃. For the formed biphasic systems, we have studied the potential for extraction of L-tryptophan, DL-phenylalanine, eugenol, glycine, and phenol (Figure 1). Since a strong attractive interaction between phenol and ZWSs had been demonstrated previously, our initial investigations were on partition of phenol in ABSs consisting of ZWSs and ISs.²⁷ Afterwards, the study was extended by analysis of partition of eugenol, an important fragrance and flavor molecule, and three amino acids DL-phenylalanine, glycine, and L-tryptophan. Analysis of amino acid is significant because it could give an insight on interactions between ZWSs and proteins, and more complex biological molecules, since the most valuable technological application of ABS is expected to be recovery of biomolecules from feedstocks. The impact of factors such as hydrophobicity of ZWSs, concentration, and
type of salts, on the position of binodal curves, phase-splitting process, partition coefficient of dissolved solutes, and efficiency of extraction, were investigated.

Materials and Experimental

Synthesis and chemicals: Three sulfonate based ZWSs with ethyl, butyl, or hexyl chain attached to the cation, hence, characterized by different hydrophilicity (hydrophobicity) were synthesized according to procedure described previously. Their structures and abbreviations are given in the Figure 1. More details on the synthesis, and on purity of chemicals used in the synthesis and experiments, can be found in the ESI.

Phase diagrams: Binodal curves separating one and two-phase region were determined by titration method with visual detection of the solution turbidity. Highly concentrated (>40 wt%) solutions of ZWSs or ISs were gravimetrically prepared inside a cell using an analytical balance. Whilst providing continuous stirring, a solution of IS was added dropwise to concentrated solution of ZWS (or vice versa) until the first sign of turbidity appeared, which was taken as point of the liquid - liquid phase transition. It was followed by addition of water in order to re-reach homogeneous region of the measured phase diagram. After every addition of a compound into the cell, the mass of the added compound was accurately measured, and the two-step procedure was carried out repeatedly until a binodal curve was fully defined.

Partition of solutes: For determination of partition coefficient and efficiency of solute extraction in selected (ZWS + IS) systems the following concentration of solutes were prepared: phenol 1g dm\(^{-3}\), eugenol 0.32 g dm\(^{-3}\), L-tryptophan 1g dm\(^{-3}\), glycine 1g dm\(^{-3}\), and DL-phenylaniline 10 g dm\(^{-3}\). In order to analyse the effect of salt concentration on partition coefficient of a solute, biphasic (ZWS + IS) aqueous systems with the following ratio of molality ZWS : IS were prepared: 1.25m:1.25m, 1.5m:1m, and 1m:1.5m for system (ZWS+K\(_3\)PO\(_4\)), and 1.1m:1.5m for the system (ZWS+(K\(_2\)HPO\(_4\)+KH\(_2\)PO\(_4\))).

The partition coefficient was calculated as a ratio of a solute concentration in ZWS phase and IS phase:

\[
K_{\text{solute}} = \frac{[\text{solute}]_{\text{ZWS}}}{[\text{solute}]_{\text{inor. salt}}}
\]

Efficiency of extraction was calculated as:

\[
EE_{\text{solute}}(\%) = \frac{W_{\text{ZWS solute}}}{W_{\text{ZWS solute}} + W_{\text{inor. salt}}} \cdot 100
\]

where \(w_{\text{ZWS solute}}\) is mass of a solute in ZWS phase and \(w_{\text{inor. salt}}\) is mass of the solute in IS phase. The masses of the solutes in each phase were calculated based on their measured concentrations in both phases and volumes of the phases in equilibrium. Volumes of obtained coexisted phases were measured using a specially designed and accurately calibrated measuring cylinder. The volume of the measuring cylinder was 2ml with graduation of 0.02ml.

The following procedure was employed for preparation of mixtures of solutes in (ZWS+IS) aqueous systems: the aqueous solution of a solute was added to (ZWS+IS) system and then mixed for 3-4h to achieve an equilibrium distribution at room temperature. Afterwards, the mixture was left for 16h to separate into two phases and the samples of top and bottom phases were taken and diluted to appropriate concentrations suitable for absorbance measurement. Glycine concentration was determined using ninhydrin method previously described in literature. More details on the procedure are given in ESI.

Measurement of solute concentration: Agilent Technology Cary 60 UV-Vis spectrophotometer was used to determine the concentration of phenol, eugenol, phenylalanine, glycine, and tryptophan. The measurements were performed in duplicates or triplicates. A 1.00mm silica UV absorption cells was used in all measurements, except in the case of glycine where a 10mm cell was used. The instrument was calibrated using series of solute dilutions in Millipore water. The solutions for calibration were made by dilution of a stock solution of known concentration of the solute prepared in 0.25 dm\(^3\) volumetric flask. The absorbance was measured at wave length of 279 nm for L-tryptophan and eugenol, 258 nm for DL-phenylalanine, 270 nm for phenol, and 570 nm for glycine. Presence of ZWSs and ISs in samples had a slight interference with the measured absorbance. In order to minimise the error of a measurement caused by interference of the salts present in the solution, for every measured sample with a solute, an analogue sample without solute was prepared and used as a ‘blank’.

Back-extraction experiments: ZWS - rich aqueous phase enriched in a solute (phenol or eugenol) after partition in an ABS, was mixed with equivalent volume of ethyl acetate and mixed for 10 h to achieve an equilibrium. After separation
of the organic and aqueous layers, the concentrations of the solute in both layers were determined by UV spectroscopy following the above described procedure.

Fig. 1: The structures and abbreviations of ZWSs (A) and solutes (phenol (B), DL-phenylalanine (C), eugenol (D), L-tryptophan (F) and glycine (E)).

Results and discussion

Initially, phase behavior of (ZWS + IS) aqueous mixtures was studied in order to evaluate their ability to form ABSs. Ternary phase diagrams were determined for three structurally similar ZWSs, namely C₂S, C₄S, and C₆S (Figure 1), and four ISs, K₃PO₄, K₂HPO₄, K₂HPO₄/KH₂PO₄, and K₂CO₃. Concentrations in the ternary phase diagrams for mixtures of ZWS and inorganic kosmotropic salts in water are reported in units of molality and presented in Figure 2. Experimental data points for all measured binodal curves are reported in Table S2, ESI. In the presented phase diagrams biphasic region is located above binodal curves, while the area between binodal line and axes represents a homogeneous region.

In Figure 2a the influence of the anion of ISs on position of binodal lines and, consequently, its power to induce phase splitting was shown. It was found that the location of the binodal curves for different kosmotropic salts with common potassium cation, K₃PO₄, K₂HPO₄, phosphate buffer pH 7.5 (K₂HPO₄+KH₂PO₄), and K₂CO₃, was determined by strength of kosmotropicity of the IS anion. The kosmotropicity of an ion can be evaluated through several thermodynamic categories, such as Jones-Dole viscosity B - coefficient, structural hydration entropies, nuclear magnetic resonance B’ - coefficients, or limiting Walden product. According to data reported by Jenkins et al., the experimental values of B - coefficient of the anions PO₄³⁻ (B = 0.495 dm³ mol⁻¹), HPO₄²⁻ (B = 0.382 dm³ mol⁻¹), and
CO$_2^-$ (B = 0.294 dm$^3$ mol$^{-1}$) support the observed shift of the locus of binodal curves in the studied systems. Similar results were obtained in systems containing C$_2$S and C$_6$S, and reported in ESI (Figure S1 a, b). It is worth mentioning that tripotassium citrate and sodium sulphate salts were also tested in formation of ABS with ZWSs but showed no power to induce phase splitting in the studied concentrate range. Experimentally determined value of B - coefficient for the citrate anion which would enable evaluation of aforementioned trend is currently unavailable in the literature. It is often stated in recent literature that highly kosmotropic salts, such as K$_3$PO$_4$ in the process of phase separation will salt out chaotropic salts.\(^{15,19}\) Salting out could be manifested either through formation of second immiscible liquid phase or precipitation of a solid salt. Several publications explained the underlying phenomena of phase separation in systems consisting of hydrophilic imidazolium based ILs and ISs assuming that ions of a kosmotropic IS are entropically favorably hydrated in aqueous solution comparing to ‘chaotropic’ imidazolium or tetrabutylammonium ILs; it leads to their preferential exclusion from the solvation shell of the ILs and, consequently, to a local excess of water molecules nearby ILs, and eventual phase separation in the system.\(^{19}\) However, recent systematic analyses of experimental density and viscosity data in aqueous solutions of imidazolium based ILs, [C$_n$ mim]Cl (n = 0-8) including derivation of apparent molar volumes, standard partial molar volumes, and Jones-Dole viscosity B - coefficients, have shown that hydrophilic imidazolium ILs with alkyl side chain (n = 4, 6, 8) (typically used in ABS) behave as kosmotropic salts, while only ILs with the shortest side chain (n = 0, 1) can be considered chaotropic.\(^{32,34}\) Hydration behavior of ZWSs has not been reported in details so far and their kosmotropicity is yet to be quantified. However, literature describes strong hydrophobic hydration of tetralkylammonium cations which gives rise to the higher Jones-Dole B - coefficient than those that are typically found in aqueous solutions of ISs.\(^{32,35,36}\) Similar behavior can be expected in aqueous solution of ZWSs. Indeed, B - coefficients, which represent an indication of electrolyte kosmotropicity, have shown moderate positive values in aqueous ZWSs mixture, pointing out kosmotropic character of ZWSs (manuscript in preparation).

Figure 2b provides a comparison on the power of ZWSs in formation of ABS to other types of similar molecules with different degree of kosmotropicity reported in literature. Figure 2b depicts the binodal curves for several ILs (1-butyl-3-methylimidazolium chloride [bmim]Cl\(^{12}\), 1-ethyl-3-methylimidazolium chloride [emim]Cl\(^{12}\), 1-butyl-3-methylimidazolium bromide [bmim]Br\(^{12}\), and benzyltrimethyl ammonium chloride [TMBA]Cl\(^{29}\), a zwitterion (N,N,N-triethyl-3-sulfonyl-1-propaneammonium)\(^{39}\), and ZWSs (C$_2$S and C$_6$S) in mixtures with a common IS, K$_3$PO$_4$. It can be noticed that both C$_2$S and C$_6$S undergo phase splitting process at lower concentration range than those reported for ILs [bmim]Cl\(^{12}\), [emim]Cl\(^{12}\), [bmim]Br\(^{12}\), or [TMBA]Cl\(^{29}\). It means that these ZWSs can be considered stronger phase splitting agents than reported ILs. On the contrary, the phase behavior of the ZWSs was very similar to behavior of the zwitterion, N,N,N-triethyl-3-sulfonyl-1-propaneammonium, documented by Ferreira et al.\(^{30}\)

**Fig. 3:** Phase diagram of aqueous ternary systems of (+C$_2$S, ● C$_3$S, ○ C$_6$S) with a) K$_3$HPO$_4$ and b) (K$_2$HPO$_4$+KH$_2$PO$_4$).

In Figure 3a and b the effect of hydrophobicity of ZWSs on their ability to form ABS is shown. The aqueous mixture of C$_2$S, C$_3$S, and C$_6$S in presence of K$_3$HPO$_4$ (Figure 3a), phosphate buffer (K$_3$HPO$_4$+KH$_2$PO$_4$)(Figure 3b), K$_3$PO$_4$ and K$_2$CO$_3$ (ESI, Figure S2, a and b) were prepared and their phase behavior analyzed. The most hydrophobic ZWS, C$_6$S forms ABS in the lowest salt concentration range, while C$_3$S requires a high salt concentration for the start of de-mixing process in all IS solutions. Similarly, it was previously reported that an increase of alkyl chain in molecule of IL\(^9\) and tetralkylammonium zwitterion\(^{30}\) promotes phase separation in ABSs. It is well known that increasing the alkyl chain...
length of tetraalkylammonium ions, i.e. increasing the ion size, will result in an increase of kosmotropicity, as revealed through values of the B - coefficients and the structural entropies.\textsuperscript{33, 35, 37} As aforementioned, kosmotropicity of imidazolium based ILs follows the same trend.\textsuperscript{34} The hydrophobic hydration of the extended alkyl chain(s) results in the increased order in the surrounding water and thus, a negative entropy change upon hydration and enhanced kosmotropicity.\textsuperscript{32, 38} This points to the conclusion that an increase of kosmotropicity of ionic species (ISs, ILs, zwitterions or ZWSs) that constitute an ABS facilitates de-mixing process and liquid-liquid separation.

![Graph showing partition coefficients of solutes](Fig. 4)

Once the potential of ZWSs to form ABS was established, their ability to extract small molecules was studied. A large number of studies on extraction ability of ILs in both ABSs and liquid-liquid extraction systems with organic solvents, were focused on aromatic molecules.\textsuperscript{7, 16, 20, 24, 39-41} Aromatic molecules are often chosen as partitioning solutes because of the targeted π-π interaction with the aromatic ring of imidazolium and pyridinium ILs. It is considered that the π-π interaction is sufficiently strong to overcome the contribution coming from H-bonding or electrostatic interaction, and all-important for separation of the aromatic solutes.\textsuperscript{20} However, despite the lack of aromatic ring, ZWSs have demonstrated ability of strong attractive interaction with aromatic phenol molecule manifested through a huge reduction of upper critical solution temperature in phenol - water mixture.\textsuperscript{27} In this work, we investigated whether similar electrostatic interactions between two positive charges in ZWS cation and π electrons of aromatic ring of dissolved solutes can selectively drive the extraction of aromatic molecules from their complex mixtures.

In Figure 4 the partition coefficients obtained for the four solutes, namely, phenol, eugenol, DL-phenylalanine, and L-tryptophan in solutions of C\textsubscript{2}S, C\textsubscript{4}S, C\textsubscript{6}S and K\textsubscript{3}PO\textsubscript{4} are presented. The molar ratio of ZWS : IS was 1.5:1. It was found that increase of hydrophobicity of ZWSs significantly improves the partition coefficients for all studied solutes. The partition coefficients of all studied solutes in the solution of C\textsubscript{2}S were relatively low, around 10. In the presence of C\textsubscript{4}S the partition coefficients of phenol, eugenol, and DL-phenylalanine were close to 20, and they increased in mixtures of C\textsubscript{6}S. The highest partition coefficient was recorded for phenol and L-tryptophan in the system containing C\textsubscript{6}S. The high partition coefficient of L-tryptophan can be explained by its hydrophobicity which favors less polar environment as those provided by more hydrophobic ZWSs. The higher distribution of phenol in ZWS-rich phase compared to eugenol and DL-phenylalanine, we believe, is result of a specific attractive interaction between phenol molecule and ZWSs. Currently, neutron-scattering data on this system are being analyzed by our group to get a deeper insight on nature of this interaction. It should be noticed that C\textsubscript{6}S displays a hydrotropic behavior and it was used in the study at a concentration higher than its critical hydrotrope concentration.\textsuperscript{27} It means that the segregated alkyl chains of C\textsubscript{6}S could create a non-polar environment and accommodate hydrophobic molecules improving their solubility. Similarly, it was reported that partition coefficient of tryptophan increased with the increase of alkyl chain length of the [C\textsubscript{n}mim]Cl ILs.\textsuperscript{11} It emphasizes the importance of hydrophobic interaction in partition of the studied solutes. In general, partition of molecules in ABSs can be driven by multiple factors such electrochemical interaction, hydrophobicity and molecular size of the solute, and specific affinity between solute and component constituents of the ABS. However, it would be very difficult to accurately state which of these factors had a dominant impact reflected on the values of distribution coefficient of the studied structurally similar aromatic molecules.
Partition coefficients of L-tryptophan and DL-phenylalanine in ABSs formed in systems with diverse ISs and a range of hydrophilic ILs have been studied thoroughly in literature.\textsuperscript{9, 11, 29, 42} However, a straightforward comparison of published results is not possible, because the reported measurements are performed at different salts concentrations and different mutual ratios of concentrations IS : IL. The Figure 5a shows that the ratio of molar concentrations of ZWSs and IS in ABSs has a significant impact on the partition coefficients. The partition coefficients and volumes of both phases are two factors which define the efficiency of extraction. The Figure 5a shows that the increase of molar ratio K$_3$PO$_4$ : C$_6$S in solution from 1:1.5 to 1.5:1 has resulted in a significant enhancement of partition coefficient of dissolved L-tryptophan. Much milder increase of the partition coefficient was found upon the increase the starting concentration of tryptophan from 0.4 to 1 g dm$^{-3}$. The efficiency of extraction was not, however, significantly affected by the change of the concentration ratio; the values of 98.5% and 98.6% were measured in systems with 1.5:1 and 1:1.5 concentration ratios, respectively. Figure 5b presents the partition coefficients determined for eugenol and DL-phenylalanine in the ABSs consisting of C$_2$S, C$_4$S, C$_6$S, and K$_3$PO$_4$ in mixtures with molar ratio ZWS: K$_3$PO$_4$ = 1:1.5. Similarly, the partition coefficients are roughly 2.5-3 times as high as in the systems with molar ratio 1.5:1 (Fig. 4). Again, the partition coefficients of both molecules were enhanced by the increasing the alkyl chain length in ZWSs. The lower partition coefficients found in system containing DL-phenylalanine in comparison to eugenol and L-tryptophan system, are probably a consequence of its higher hydrophilicity and better accommodation within polar environment.

\textbf{Fig. 5:} Partition coefficients of a) L-tryptophan as a function of concentration ratio, $r$, of K$_3$PO$_4$ : C$_6$S, for concentration of tryptophan $1 g dm^{-3}$, $0.7 g dm^{-3}$, $0.4 g dm^{-3}$. Phase diagram showing composition of the mixtures prepared in the given concentration ratio, $r$, and corresponding tie - lines is provided in ESI, Figure S3. b) DL-phenylalanine and eugenol for molar ratio ZWS : K$_3$PO$_4$ = 1 : 1.5, in solutions of C$_2$S, C$_4$S, and C$_6$S.

K$_3$PO$_4$ is a common salting out agent with powerful kosmotropic and salting-out properties broadly studied in formation of ABSs with polymers, ILs\textsuperscript{15} and zwitterions\textsuperscript{30}. However, the highly basic character of its aqueous solution (pH ≈11) can be unsuitable for extraction of many pH-sensitive molecules, particularly biomolecules, but also plant-derived phe-
nolic compounds because of their degradation under basic conditions. Therefore, we have explored possibility of extraction the phenol, glycine, eugenol, and L-tryptophan using phosphate buffer system at pH 7.5. The amino acids used herein at pH 7.5 should stay in their anionic form, since their isoelectric points are below 6.0. The obtained partition coefficients of studied solutes are presented in Figure 6. Obviously, the partition coefficients of phenol and L-tryptophan have much lower values, while partition coefficient of eugenol still shows a moderately high value, comparing to values recorded in the systems with K$_3$PO$_4$. Interestingly, the partition coefficient of the most hydrophilic amino acid studied, glycine, is 0.12, suggesting that glycine predominately stays in IS-rich phase. Ferreira et al. similarly reported that in ABSs with hydrophobic zwitterions and K$_3$PO$_4$ glycine preferentially separated in more hydrophilic IS-rich layer.

In numerous scientific reports a lot of attention was given to high partition coefficients of solutes achievable by employment of IL environment in ABSs. However, in chemical engineering practice high partition coefficients are a desirable, but not an essential requirement, since processes with unfavorable partition coefficient in a single extraction step are commonly improved by continuous operation design. On the contrary, problems related to solute recovery caused by creating a strong interaction solute - IL and inability to selectively back-extract solute were often neglected. Even though the product recovery is a critical step in design of an extraction process, only small number of publications tackled the problem of solute recovery from IL phase and recycling of ILs. ZWSs are class of compound which are readily soluble in water phase (similar to ISs), and practically insoluble in almost all common organic solvents. This property can be advantageous for process of recovery and isolation of extraction product. In that sense, ZWSs can be superior to ILs in the stage of product recovery. We have studied possibility of back-extraction of eugenol and phenol from C$_2$S-rich aqueous layer using a relatively benign organic solvent, ethyl acetate. It was found that 91% of phenol and 78% eugenol can be recovered in one extraction step. The process can be further improved by multiple-step extraction or continuous extraction unit design. Figure S4 ESI presents a proposed process design for product isolation with recycling of ZWS and organic solvent used in ABS. However, it should be mentioned that many biological molecules denature in non-aqueous media or have a very low miscibility in organic solvents. Even though literature reports a process design for product recovery which consists of multiple ABSs, the proposed approach generates additional inorganic waste and leaves the extracted product in mixture with an IS (NaCl) and, as such, might not be appealing for industrial applications. It leads to conclusion that for a real breakthrough in application of ABS-based bio-separation it is crucial to develop methods for efficient recovery of large amounts of extracted compounds and recycling of salt solutions at relatively low costs.

**Conclusion**

We have studied aqueous ternary phase diagrams for systems consisting of ISs and ZWSs differing in hydrophobicity. The potential of ZWSs based systems for extraction of aromatic molecules and amino acids was examined. Even though the values of partition coefficients in ZWS-based systems might be lower than in organic solvent-water systems, or IL-based ABSs, ZWSs have demonstrated potential to selectively extract aromatic compounds, as shown on the example aromatic and non-aromatic amino acids. Furthermore, since ZWSs are strong salting-out agents for many polar and nonpolar organic molecules, they can be potentially used for extraction of phenolic compounds from complex mixtures under neutral pH conditions. Additionally, their insolubility in organic solvents enables recovery of the compound by back-extraction using a range of organic solvents with low or partial miscibility with water, like ethyl acetate. The lack of toxicity against microorganisms makes ZWSs potential candidates for enhanced productivity in ABSs which integrate simultaneous product extraction and bioconversion. However, further studies are essentially required to develop knowledge on mechanisms of solutes' partitioning and selectivity of extraction from complex mixtures, and to address feasibility of product recovery and recyclability of ABS constituents from solution.

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