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The influence of zwitterions on the partition of biomolecules in aqueous biphasic systems

Anusha Basaiahgari[a], Helena Passos[b], João A.P. Coutinho[b,*], Ramesh L. Gardas[a,*]

[a] Department of Chemistry, Indian Institute of Technology Madras, Chennai 600 036, India
[b] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

The use of hydrophilic zwitterionic compounds (ZI) to act as phase forming components of aqueous biphasic systems (ABS) has been attracting increased interest. Although previous works studied the phase behavior of ZI, there is still a lack of knowledge on the partition behavior of compounds in ZI-based ABS. This work reports a study on the influence of ZI structure for the extraction of 7 different biomolecules selected as molecular probes – 2 alkaloids (nicotine and caffeine), 2 phenolic compounds (gallic acid and vanillic acid) and 3 amino acids (*L*-tryptophan, *L*-tyrosine and *L*-phenylalanine). The partition and extraction efficiencies of these biomolecules were evaluated in ternary systems composed of hydrophilic ZI constituted by ammonium, imidazolium, pyridinium and piperidinium cationic and SO_3^- anionic groups, K_2CO_3 and water. The obtained results show that all the biomolecules preferentially partition to the ZI-rich phase, and the extent of their partition was closely related with the octanol–water partition coefficients of both biomolecules and ZI. It was demonstrated that the partition of biomolecules in ZI-based ABS is firstly ruled by hydrophobic/hydrophilic effects and only in some particular cases specific interactions between the biomolecule and the ZI affect their partition. Furthermore, despite the similarity between ZI and ionic liquids behavior as phase forming compounds of ABS for the extraction of biomolecules, ZI-based ABS presented a better performance in the extraction of phenolic compounds in alkaline environment.

1. Introduction

The study and development of new aqueous biphasic systems (ABS) has triggered a renewed interest among the research community for the use of liquid–liquid extraction (LLE) techniques in the separation and purification of value-added compounds [1]. As a part of research for the extraction of value-added chemicals from biomass residues, in the framework of biorefinery processes, it is of importance to develop cost-effective extraction and separation systems [2]. Among multiple factors that contribute to the success of the extraction and purification technologies, the proper choice of solvents is of high relevance since they have impact on the rate of mass transfer, on the stability of the target compounds, and in the economic and environmental feasibility of the whole process [3]. Thus, there is a challenge in the formulation of sustainable systems with appropriate solvents.

ABS are constituted by water in major proportions. These systems are nowadays considered as suitable alternatives to the conventionally used LLE systems since they fulfill the green chemistry principles by avoiding the use of conventional volatile organic solvents thereby minimizing their environmental impact. Conventional ABS are composed of either two polymers, a polymer and a salt or two salts resulting in two aqueous-rich phases in equilibrium [4]. Despite the “greener” character of these systems induced by their aqueous nature, conventional polymer-based systems present some issues, namely restricted polarity, high viscosity and consequent low phase separation rates [5].

Over the years, researchers have tried to develop novel ABS by using a variety of phase forming components that allow overcoming of drawbacks associated to the most conventional systems. In this context, ionic liquids (IL) were identified as a suitable alternative. Following the first work reported by Rogers and co-workers [6], several studies on the use of IL as phase forming components of ABS have been published in past decades [5]. IL are defined as salts constituted by asymmetrical cations and anions that present low melting points. Along with interesting properties, such as high chemical and thermal stability, tunability of their chemical structures and negligible vapor pressure, IL exhibit a wide solubility for compounds of variable polarities. These unique properties enabled them to be efficient phase forming components of ABS when combined with salts, polymers, amino acids,

* Corresponding authors.
E-mail addresses: jcoutinho@ua.pt (J.A.P. Coutinho), gardas@iitm.ac.in (R.L. Gardas).

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carbohydrates, inorganic acids among others [5,7–9]. Furthermore, IL-based ABS have shown an amazing potential for application in the extraction and separation of several varieties of compounds including amino acids, alkaloids, proteins, phenolic compounds, terpenoids, acids, metals etc [5,10–14].

Hydrophilic zwitterionic compounds (ZI), were recently proposed as phase forming compounds of ABS [15,16]. They are similar in behavior to IL, but are not composed of separated ions, rather they are constituted by covalently bonded cations and anions [17]. This type of chemical structure confers particular characteristics to ZIs; for example, despite their electric neutrality, these molecules present very high polarity induced by the coexistence of both opposite charges. These compounds are also well-known by their self-association ability and strong hydration, which allied with their non-toxicity and biodegradability, allowed their application in several fields, namely cosmetics, personal care and detergent industries [18,19]. Furthermore, their properties can be tuned by changes in their structures (modification of the cationic and anionic groups, the size of the spacer, addition or size change of hydrocarbons chains, etc.) allowing a large number of possibilities [19].

ZI use in the formation of ABS when mixed with salts and polymers conferred special properties to the systems that make them suitable alternatives for the development of novel extraction and separation processes. Ferreira et al. [16] showed that it is possible to tune the thermal behavior of ABS between upper critical solution temperature (UCST) and lower critical solution temperature (LCST) behavior by only changing the size of the alkyl chains that compose sulfobetaine-based ZI. These ABS allowed the separation of aromatic and aliphatic amino acids mixtures and opened the possibility to design more dynamic systems. The same authors [15] also demonstrated the potential application of ZI-polymer-based ABS as an integrated process in enzymatic catalysis, allowing both the recovery of the product and the reuse of the enzyme. Induced by these new findings, we synthesized novel hydrophilic ZI based on ammonium, imidazolium, piperidium and pyrrolidinium cations [20]. This previous work was focused on evaluating ZI phase behavior in combination with potassium-based salts and systems ability to partition caffeine, which was selected as a model alkaloid [20]. However, despite the increased interest in the application of ZI as phase forming compounds, the works reported up to now are not enough to provide broad knowledge on the influence of ZI nature on compounds partition in ZI-based ABS and how their ability is comparable to well-known IL-based ABS.

Aiming to explore the applicability of novel ZI-based ABS for the extraction and separation of a large range of compounds, it is here presented a study on the ability of ABS composed of hydrophilic ZI, potassium carbonate salt (K_2CO_3) and water to extract different types of biomolecules, namely 2 alkaloids (nicotine and caffeine), 2 phenolic compounds (gallic acid and vanillic acid) and three amino acids (*L*-tryptophan, *L*-tyrosine and *L*-phenylalanine). These compounds were selected as molecular probes, similarly to what was previously done in works about IL- and deep-eutectic-solvents-based ABS, since they serve as model compounds for a wide variety of value added compounds of interest present in biomass [21,22]. These biomolecules present different structures and polarities, which allow the evaluation of possible specific and non-specific interactions that may occur between biomolecules and phase-forming compounds.

2. Materials and methods

2.1. Materials

The chemical structures and properties, including octanol–water partition coefficient (K_{OW}) and water solubility, of the biomolecules here studied are reported in Table 1. In Table 2 are listed the ZI studied in this work along with their acronyms, structures and properties. The synthesis procedure for these ZI was previously described by us [20]. The reagents used in the ZI preparation were triethylamine (99.5 wt% pure), *N*-methylimidazole (99 wt% pure), *N*-vinylimidazole (99 wt% pure), *N*-methyl pyrrolidine (98 wt% pure), *N*-ethylpiperidine (98 wt% pure) and 1,4-butanesultone (99 wt% pure) all supplied by Sigma-Aldrich. Acetonitrile (99.5 wt% pure) and diethylether (98 wt% pure) from Sigma-Aldrich were used during synthesis procedure. ^1H and ^{13}C nuclear magnetic resonance (NMR) analysis was used to confirm ZI structure and purity. The water content of the ZI was determined by Karl Fischer titration, and these values were considered in the preparation of the ABS. The purity and water content determined for each ZI are presented in the Table S1.

Ternary systems studied in this work were prepared by mixing each ZI with dipotassium carbonate salt (K_2CO_3 of 99 wt% purity) supplied by Merck. Caffeine (98 wt% pure) from SRL, nicotine (99 wt% pure) from Fluka, gallic acid (99.5 wt%) from Merck, vanillic acid (97 wt% pure) from Sigma-Aldrich, and *L*-tryptophan (99 wt% pure), *L*-tyrosine (99 wt% pure) and *L*-phenylalanine (99 wt% pure) all supplied by Spectrochem, were used as biomolecules (Table 1).

2.2. Methods

2.2.1. Determination of tie-lines and tie-line length data

The binodal curves of ABS composed of ZI + K_2CO_3 + H_2O studied in this work were previously reported by us [20]. In order to select an appropriate mixture composition for biomolecules partition experiments, one tie-line (TL) was determined for each system. Moreover, to reduce the possible influence which could arise from the different compositions of the phases, the partition studies were performed in mixture points leading to similar tie-line length (TLL), ranging from 65 to 70. However, in the ABS composed of ViImC4S and C_2PipC4S it was not possible to prepare TLs with a length higher than 50 and 61, respectively, due to ZI solubility limitations. Thus, the mixture points selected for biomolecules partition experiments and TLs determination were 25 wt% of ZI + 25 wt% of K_2CO_3 + 50 wt% of H_2O for N_{222}C4S, C_1ImC4S and C_1PyrC4S ZI and 22 wt% of ZI + 25 wt% of K_2CO_3 + 53 wt% of H_2O for C_2PipC4S and ViImC4S ZI. The TLs were determined at (298.15 ± 1) K by a gravimetric method originally described by Merchuck et al. [25] and previously reported by us [20].

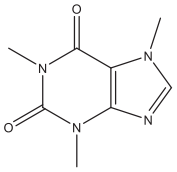
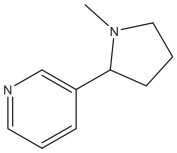
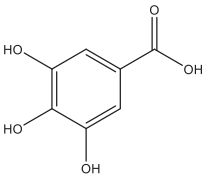
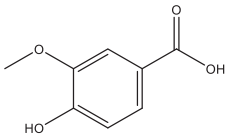
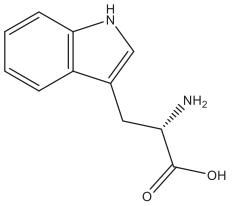
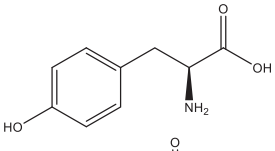
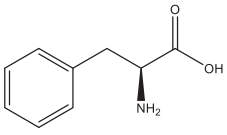
The pH of both top and bottom phases that compose each ZI-based ABS studied in this work were determined at (298 ± 1) K using a SevenExcellence™ pH/conductivity meter (Mettler Toledo (USA)).

2.2.2. Biomolecules partition in ZI-based ABS

An aqueous solution of each biomolecule was prepared at the following concentrations: 5.15×10^{-3} mol·L^{-1} of caffeine, 6.17×10^{-3} mol·L^{-1} of nicotine, 4.90×10^{-3} mol·L^{-1} of *L*-tryptophan, 0.55×10^{-3} mol·L^{-1} of *L*-tyrosine, 1.82×10^{-3} mol·L^{-1} of *L*-phenylalanine, 2.94×10^{-3} mol·L^{-1} of gallic acid and 2.97×10^{-3} mol·L^{-1} of vanillic acid. The ternary mixtures previously referred were gravimetrically prepared ($\pm 10^{-4}$ g) at (298 ± 1) K and atmospheric pressure. All phase forming components were weighed, vigorously stirred and then centrifuged for 30 min at 3500 rpm to ensure the complete phases separation and biomolecules partition. After the careful separation of both ZI- and salt-rich phases, the concentration of each biomolecule in each individual phase was determined through UV–Visible spectroscopy using a BioTeck Synergy HT microplate reader, at the following wavelengths: 273 nm for caffeine, 260 nm for nicotine, 279 nm for *L*-tryptophan, 275 nm for *L*-tyrosine, 258 nm for *L*-phenylalanine, 262 nm for gallic acid and 259 nm for vanillic acid. Biomolecules concentration was calculated using calibration curves previously established. In order to avoid possible interferences from the ZI and salt present in ABS phases, blank samples were prepared in the same mixture compositions by using pure water instead of aqueous solution of biomolecule.

Due to the high interferences derived from the ViImC4S ZI in the quantification of gallic acid and *L*-phenylalanine by UV–Visible

Table 1
Chemical structure and properties of the studied biomolecules [23,24].

	Biomolecule	Structure	log (K_{OW})	log (K_{OW}) at pH 12	s_{water} ($g{\cdot}L^{-1}$)[a]
ALKALOIDS	Caffeine (Caf)		−0.55	−0.55	21.6
	Nicotine (Nic)		1.16	1.16	miscible
PHENOLIC COMPOUNDS	Gallic acid (GA)		0.72	−6.66	11.9
	Vanillic acid (VA)		1.17	−3.93	1.5
AMINO ACIDS	*L*-Tryptophan (Tryp)		−1.09	−2.35	13.4
	L-Tyrosine (Tyr)		−1.49	−4.33	0.48
	L-Phenylalanine (Phe)		−1.18	−2.45	25.9

[a] at 298.15 K [24].

spectroscopy, the concentration of these biomolecules in the phases of ViImC4S-based ABS were determined by using high performance liquid chromatography (HPLC) with diode array detector (DAD). A HPLC-DAD apparatus (Shimadzu, model PROMINENCE) with an analytical C18 reversed-phase column (250 × 4.60 mm), Kinetex 5 µm C18 100 Å, from Phenomenex, was used. The mobile phase consisted of ultrapure H_2O (+0.05% trifluoroacetic acid (TFA)) as solvent A and methanol (+0.05% TFA) as solvent B, with the following gradient elution program: 0 min 15% B; 12 min 40% B; 14 min 74% B; 16 min 15% B; 25 min 15% B. This methodology was developed based on [26]. The flow rate used was 0.8 mL/min, with an injection volume of 20 µL. DAD was set at 258 and 262 nm for the quantification of *L*-phenylalanine and gallic acid, respectively. *L*-phenylalanine and gallic acid presented retention times of 7.8 and 4.8 min, respectively. Each sample was analyzed at least in duplicate. The column oven and the autosampler operated at 30 °C. Calibration curves were prepared using pure gallic acid and *L*-phenylalanine aqueous solutions.

All the experiments were performed in triplicate to determine the average partition coefficient (K), extraction efficiency percentage ($EE\%$) and the corresponding standard deviations (σ). Biomolecules partition coefficients (K_{Mol}) and extraction efficiency percentage ($EE_{Mol}\%$) were calculated according to the following equations:

$$K_{Mol} = \frac{[Mol]_{ZI}}{[Mol]_{Salt}} \quad (1)$$

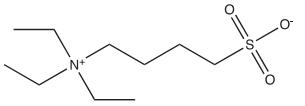
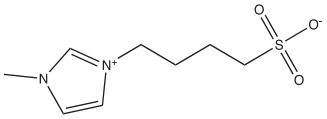
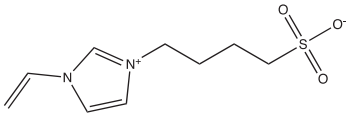
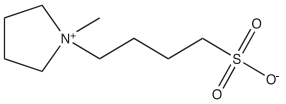
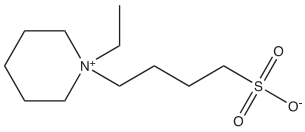
$$EE_{Mol}\% = \frac{m_{Mol}^{ZI}}{m_{Mol}^{ZI} + m_{Mol}^{salt}} \times 100 \quad (2)$$

where $[Mol]_{ZI}$ and $[Mol]_{salt}$ are biomolecule concentration in ZI- and salt-rich phase, respectively, and m_{Mol}^{ZI} and m_{Mol}^{salt} are the biomolecule weight (g) determined in the ZI-and salt-rich phase, respectively. The biomolecules weight in each phase was determined as the product of the biomolecule concentration and phase volume

3. Results and discussion

The demixing of two-aqueous phases, and consequent formation of an ABS, occurs due to the competition of two species for the formation of

Table 2
Name, acronym, structure and octanol–water partition coefficient (K_{OW}) of the ZI studied in this work.

Name	Acronym	Structure	log (K_{OW}) [23]
4-(triethylammonio)butane-1-sulfonate	$N_{222}C4S$		−1.28
4-(1-methylimidazolium-3-yl)butane-1-sulfonate	C_1ImC4S		−5.05
4-(1-vinylimidazolium-3-yl)butane-1-sulfonate	ViImC4S		−4.54
4-(1-methylpyrrolidinium-1-yl)butane-1-sulfonate	$C_1PyrC4S$		−1.95
4-(1-ethylpiperidinium-1-yl))butane-1-sulfonate	$C_2PipC4S$		−1.14

hydration complexes [5]. Despite the strong ability of ZI to interact with water [27,28], it was previously demonstrated that in ternary systems composed of ZI and salts, salts are capable of stronger hydration and are responsible for the salting-out effect and phase separation [16,20]. The liquid–liquid equilibrium of the ternary systems composed of C_1ImC4S, ViImC4S, $C_1PyrC4S$, $N_{222}C4S$ and $C_2PipC4S$ ZI + K_2CO_3 + H_2O were already reported [20]. The representation of the ternary phase diagrams can be found in the Fig. S1. In this previous study it was proposed that water solubility of ZI is related with their ability to induce phase separation, with ZI of higher water solubility being less capable to form ABS, and the following trend was found when ZI were mixed with K_2CO_3: C_1ImC4S < ViImC4S < $C_1PyrC4S$ < $N_{222}C4S$ ~ $C_2PipC4S$ [20].

Since the goal of the present work is to understand how biomolecules partition occurs in ZI-salt-based ABS, K_2CO_3 was selected as a moderate salting-out agent. Salts such as K_3PO_4, present a strong effect on the molecules partition due to their high charge density. This results in a strong ability to salt-out the compounds to the ZI-rich phases regardless the nature of ZI and, consequently, masking ZI influence on biomolecules partition.

Considering the phase diagrams previously reported [20], new TLs were determined for ZI + K_2CO_3 + H_2O ternary systems. To reduce the number of variables that may influence the partition of biomolecules, TLs of similar length were considered. The graphical representation of phase diagrams with selected mixture points as well as corresponding tie lines is presented in Fig. S2. The obtained results for the ZI- and salt-rich phases, along with the composition of mixtures points and TLL of the coexisting phases are presented in Table 3. As expected, for all studied systems it was observed the formation of a top phase rich in ZI while the bottom phase is rich in salt. The pH values of the phases of these systems are in the alkaline region (~12).

The effect of the ZI nature in the partition of biomolecules was here evaluated by measuring the partition coefficients of caffeine, nicotine, gallic acid, vanillic acid, *L*-tryptophan, *L*-tyrosine and *L*-phenylalanine in the biphasic systems reported in Table 3. The obtained results are presented in Figs. 1 to 4. Details on biomolecules partition coefficients and extraction efficiencies experimental data and respective standard deviations are given in Tables S2 and S3.

In Fig. 1, biomolecules partition coefficients are represented as function of their octanol–water partition coefficients (log) at pH 12 for each ABS. Since most of the studied biomolecules suffers speciation at the pH of the systems (pH 12), K_{OW} at this pH were estimated and used, instead of the usual octanol–water partition coefficients of neutral molecules. All K_{Mol} values are higher than 1, meaning that all biomolecules, independently of the type of ZI that compose the system, have a preferential partition to the ZI-rich phase. In what concerns extraction efficiencies (*cf.* Table S2), most of the biomolecules presented high EE_{Mol} % values (> 80%), while gallic acid showed a lower capability to be extracted to the ZI-rich phase (EE_{Mol} % ranges between 39 and 85 %). From Fig. 1, it is also possible to observe a trend valid for most systems: the higher is the octanol–water partition coefficient of the solute (at pH 12), the higher its partition for the ZI-rich phase. Gallic acid (K_{OW} (pH 12) = −6.66) is the biomolecule which presents the lower partition coefficients in all systems, while nicotine (K_{OW} (pH 12) = 1.16) is the compound with the higher capacity to partition to the ZI-rich phase, with the sole exception of the system composed of ViImC4S, in which *L*-tryptophan (K_{OW} (pH 12) = −2.35) presents the

Table 3
Experimental data for TLs, TLLs and phases pH for ABS composed of ZI + K_2CO_3 + H_2O, determined at (298.15 ± 1) K and atmospheric pressure.

ZI	Weight fraction percentage (wt %)								TLL
	$[salt]_M$	$[ZI]_M$	$[salt]_{salt}$	$[ZI]_{salt}$	pH_{salt}	$[salt]_{ZI}$	$[ZI]_{ZI}$	pH_{ZI}	
$N_{222}C4S$	25.01	25.00	48.48	0.05	11.8	3.86	47.48	12.4	65.12
C_1ImC4S	24.98	25.02	38.73	2.17	12.0	2.62	62.18	12.4	70.03
ViImC4S	21.99	22.01	31.00	6.91	11.8	5.19	50.17	12.1	50.37
$C_1PyrC4S$	24.95	25.00	39.93	2.11	12.0	1.52	60.80	12.6	70.15
$C_2PipC4S$	22.01	21.99	34.68	2.21	11.8	1.50	54.01	12.1	61.52

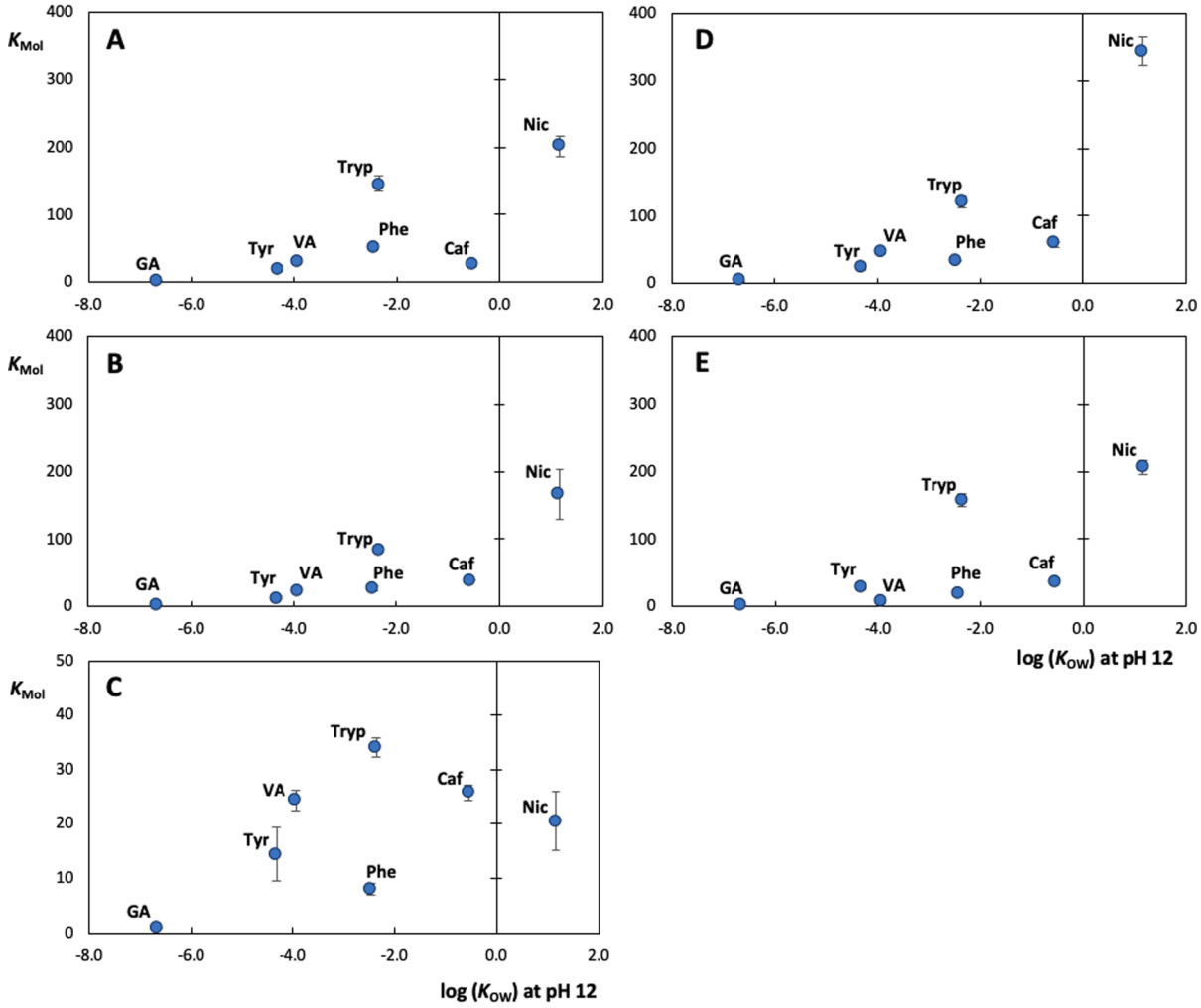


Fig. 1. Partition coefficient, K_{Mol}, of caffeine (Caf), nicotine (Nic), gallic acid (GA), vanillic acid (VA), L-tryptophan (Tryp), L-tyrosine (Tyr) and L-phenylalanine (Phe) as a function of their octanol–water partition coefficients, log(K_{OW}), at pH 12[23] in ABS composed of K_2CO_3 and the following ZI: (A) N_{222}C4S, (B) C_1ImC4S, (C) ViImC4S, (D) C_1PyrC4S and (E) C_2PipC4S.

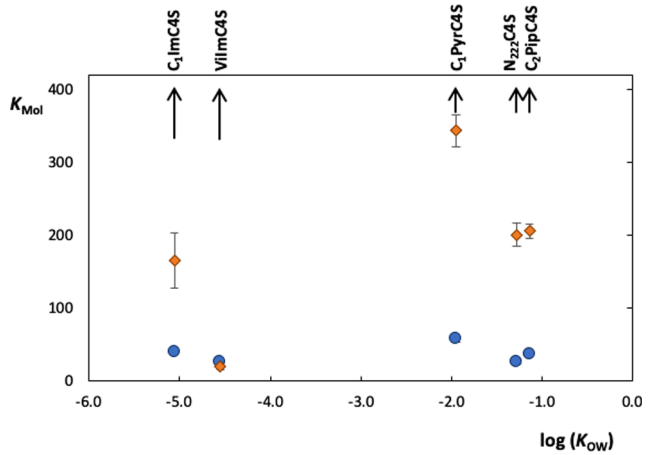


Fig. 2. Alkaloids partition as function of octanol–water partition coefficients (log) of ZI in ABS composed of ZI + K_2CO_3 + H_2O: caffeine (●) and nicotine (♦).

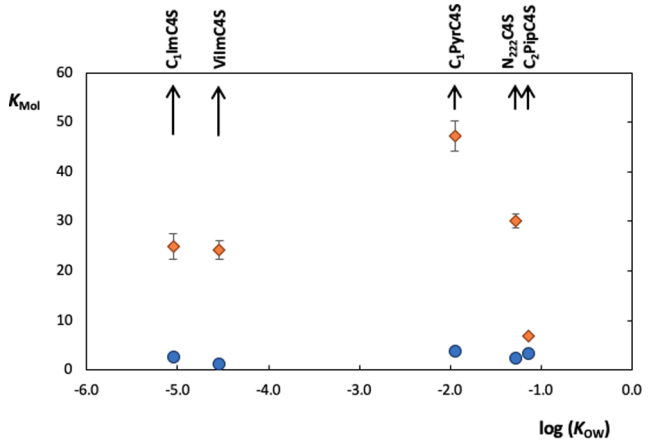


Fig. 3. Phenolic compounds partition as function of octanol–water partition coefficients (log) of ZI in ABS composed of ZI + K_2CO_3 + H_2O: gallic acid (●) and vanillic acid (♦).

highest value of K_{Mol}. These results are in good agreement with data previously reported for other types of ABS, including ZI- and IL-based ABS [16,20–22]. In fact, despite specific interactions that may occurs, it has been observed that the hydrophilic/hydrophobic nature of the solutes controls their preferential partition. In this work, the impact of the hydrophilic/hydrophobic nature of each solute is patent in the trends observed in Fig. 1, with all possible interferences which could arise from the different compositions of the phases being taken into account, since all ternary mixtures were prepared for TLs with similar

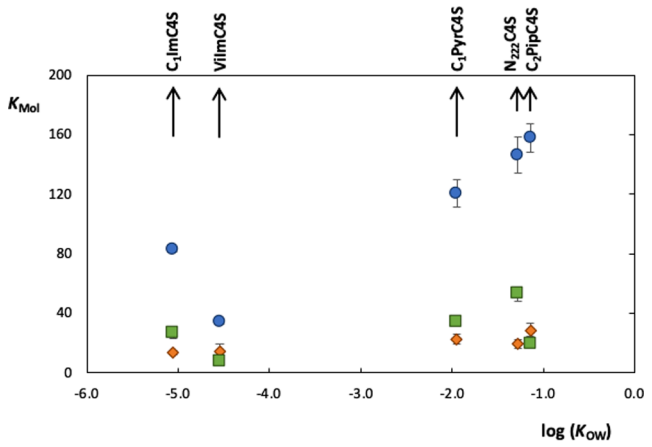


Fig. 4. Amino acids partition coefficients as function of octanol–water partition coefficients (log) of ZI in ABS composed of ZI + K_2CO_3 + H_2O: L-tryptophan (●), L-tyrosine (♦) and L-phenylalanine (■).

TLLs. Nevertheless, it is possible to observe some deviations to these trends, namely in the behavior of some biomolecules and in the ZI influence on biomolecules partition. These deviations will be discussed in detail below.

The lowest values of K_{Mol} were observed in the system composed of ViImC4S + K_2CO_3 + H_2O. While in the remaining systems the K_{Mol} reach values > 150, when the system is composed by the ZI ViImC4S, the partition coefficient values range only between 1.15 and 34. Furthermore, as shown by the data presented in Fig. 1C, this is the only system in which it is not possible to clearly distinguish a tendency between the K_{Mol} and log(K_{OW}) of the molecules as described above. Due to ZI solubility limitations this system is the one which has the smallest TLL – *cf.* Table 3. This may justify the lower partition of these biomolecules. It is well known that a lower TLL means a higher similarity between the phases that compose the system and a harder separation of the biomolecules between the phases [5,21]. This seems to have a higher impact on the partition of biomolecules with higher octanol–water partition coefficient, such as nicotine – *cf.* Fig. 1C.

To facilitate the interpretation and discussion of the behavior of each biomolecule, in Figs. 2–4 are presented the K_{Mol} values obtained for each family of biomolecules – alkaloids, phenolic compounds and amino acids – as a function of ZI log(K_{OW}). In Fig. 2 are presented the results obtained for the alkaloids nicotine and caffeine. Nicotine exhibited very high K_{Mol} values (higher than 100 in most systems), while caffeine presents relatively low partition to ZI-rich phase and with smaller variations with the type of ZI that compose the system (K_{Mol} range from 26 to 58). As previously referred, the differences observed between the two alkaloids can be explained based on their higher or lower affinity for the most hydrophobic phase. However, through the data presented in Fig. 2, it seems clear that the ZI hydrophobicity/hydrophilicity is not the only variable influencing the partition of the alkaloids to the ZI-rich phase. Considering nicotine, its favorable partition to ZI-rich phase follows the order: ViImC4S < C_1ImC4S < N_{222}C4S ~ C_2PipC4S < C_1PyrC4S, while caffeine presents the following trend: ViImC4S ~ N_{222} < C_2PipC4S ~ C_1ImC4S < C_1PyrC4S. Despite the differences, it is possible to conclude that the ZI influence, in general, it ismilar for both alkaloids. With the exception of ViImC4S-based ABS in which the low TLL may have a high impact on alkaloids partition, resulting in lower K_{Mol} values, C_1PyrC4S is the only ZI that is out of the expected trend. Nicotine has *N*-methylpyrrolidine ring linked to a pyridine group. The structural similarity between nicotine and the C_1PyrC4S ZI could result in specific interactions and justify the high ability of this system to extract nicotine. Furthermore, caffeine partition in systems composed of the same ZI and K_3PO_4 (a stronger salting-out agent than K_2CO_3) was previously evaluated by us [20]. In this previous work the system composed of C_1PyrC4S also presented the highest ability to extract caffeine to the ZI-rich phase, while N_{222}C4S showed the worst result [20]. It was proposed that this behavior was related with the absence of favorable π-π interactions between the biomolecule and the ammonium-based ZI. However, the results presented here suggest that, despite specific interactions have an important role in the biomolecules partition, several other effects can influence this behavior.

The results obtained for the partition of the phenolic compounds – gallic acid and vanillic acid – as a function of the log(K_{OW}) of each ZI, are presented in Fig. 3. Among all the families of biomolecules studied in this work, phenolic compounds were those that present the lower partition coefficients ranging between 6.92 and 47 and 1.15 to 3.7, for vanillic acid and gallic acid respectively. Vanillic acid differs from gallic acid on the number of hydroxyl groups present on the aromatic ring and it is comparatively more hydrophobic (*cf.* Table 1), justifying the higher K_{Mol} values obtained. It was previously reported that the pH of the medium has a significant impact on the partition of phenolic compounds due to their speciation [29]. At the alkaline pH of the studied ZI-based ABS, both phenolic compounds exist mostly in the form of their charged conjugated bases. It is expected that these charged species preferentially partition to the most hydrophilic phases in order to ensure effective solvation of these ions. This was previously reported by Cláudio et al. [29] in IL-based ABS. Even in presence of a strong salting-out agent such as K_3PO_4, gallic acid was extracted mainly to salt-rich phase (K < 1) in alkaline IL-based ABS [29]. However, here both phenolic compounds still present K_{Mol} > 1, meaning a preferential partition to the ZI-rich phase. These results suggest that ZI-based ABS could be a more suitable alternative to extract phenolic compounds in alkaline medium than IL-based ABS. Nevertheless, the obtained partition coefficients are still much lower for phenolic compounds when compared to the remaining biomolecules.

In what concerns the influence of ZI nature in the partition of the phenolic compounds, it is not possible to observe a well-defined trend in the data presented in the Fig. 3. Nevertheless, considering the hydrophilic character of phenolic compounds at alkaline pH, it is not expected to see an improvement on their partition to ZI-rich phase with the increase of ZI hydrophobicity (high log(K_{OW})). Thus, the decrease observed in vanillic acid partition coefficient in the systems composed of the ZI C_1PyrC4S, N_{222}C4S and C_2PipC4S could be related with its lower affinity for more hydrophobic phases.

In Fig. 4 are presented the experimental data obtained for the partition of the amino acids *L*-tryptophan, *L*-tyrosine and *L*-phenylalanine. As expected, and previously discussed, K_{Mol} values follow the trend K_{Try} > K_{Phe} > K_{Tyr} which is based on the differences in the hydrophobicity of these amino acids – *cf.* Table 1 and Fig. 1. From the data presented in Fig. 4 it is also clear that K_{Mol} of the amino acids increases with the hydrophobicity of ZI – higher log (K_{OW}) – meaning that amino acids partition in ZI-based ABS is by the hydrophobic/hydrophilic character of the ZI that compose the system. In fact, considering the partition coefficients obtained for *L*-tryptophan, it is possible to observe that this increases regardless ZI nature – aromatic, non-aromatic, quaternary ammonium, etc. – being mainly dependent on the log (K_{OW}) of the ZI. This is in good agreement with data previously reported for amino acids partition in polymer-based ABS, in which it was demonstrated that amino acids partition is mainly driven by hydrophobic effects [21,30]. Nevertheless, *L*-tryptophan showed exceptionally high K_{Mol} values resulting in its complete partition into the ZI-rich phase. Its partition was even higher than the partition of caffeine that present a high hydrophobic character (*cf.* Table 1), as can be observed in Fig. 1.

These results are also similar to what was previously reported for *L*-tryptophan extraction in ABS composed of IL and salts, [5,31] suggesting that, despite the structural differences between IL and ZI, there are some similarities on the way how both type of compounds driven the partition of biomolecules in ABS.

4. Conclusions

The ability of ABS composed of ZI and K_2CO_3 to partition 7 different biomolecules − 2 phenolic compounds, 2 alkaloids and 3 amino acids - was studied in this work. By a careful selection of the ternary mixture points used in the extractions (similar TLL) it was possible to evaluate the influence of ZI nature in the biomolecules partition without relevant interferences that could result from the differences observed in the systems phases composition. Furthermore, K_2CO_3 was selected as a moderate salting-out agent to avoid strong salting-out effects on the biomolecules partition. In all studied systems, partition coefficients were always higher than 1, meaning that all biomolecules present a favorable partition to the ZI-rich phase. The obtained results show that the biomolecules partition is mainly ruled by hydrophobic/hydrophilic effects and only in some cases specific interactions between the biomolecules and the ZI have some influence. Thus, the octanol–water partition coefficients of both biomolecules and ZI could be used in the explanation of the obtained results. In general the higher is the octanol–water partition coefficient of the biomolecules, considering their speciation at the pH of the studied systems (pH ∼ 12), the higher are their partition coefficients. Moreover, when in the presence of more hydrophobic biomolecules, namely nicotine and *L*-tryptophan, their partition increases with the K_{OW} of the ZI, while the opposite trend is observed with more hydrophilic compounds, such as phenolic compounds. Despite the structural differences between IL and ZI, the obtained results suggest that ZI behave in a similar way to IL, still conferring to the system phases some properties that make these systems suitable alternatives for the extraction of more hydrophilic compounds, such as phenolic compounds at alkaline conditions.

CRediT authorship contribution statement

Anusha Basaiahgari: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Helena Passos:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **João A.P. Coutinho:** Conceptualization, Resources, Supervision, Writing - review & editing, Funding acquisition. **Ramesh L. Gardas:** Conceptualization, Resources, Project administration, Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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