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Enhanced separation performance of aqueous biphasic systems formed by carbohydrates and tetraalkylphosphonium- or tetraalkylammonium-based ionic liquids

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Abstract

Aiming at establishing more effective and sustainable separation processes, herein we propose the use of carbohydrates combined with tetraalkylphosphonium- and tetraalkylammonium-based ionic liquids (ILs) to form aqueous biphasic systems (ABS). The formation of ABS composed of non-aromatic and non-fluorinated ILs with carbohydrates is here shown for the first time. These novel systems are competitive extraction platforms when compared against more conventional ABS formed by ILs and salts or fluorinated ILs and carbohydrates. Finally, it is shown that these systems can be efficiently recovered and reused.

During the past decade there was a significant effort on the study of ionic-liquid-based aqueous biphasic systems (IL-based ABS) as alternative separation platforms.¹ Although ABS composed of polymers and/or salts are known since the 50's², the use of ILs in ABS has led to a wide range of new liquid-liquid systems with remarkable extraction efficiencies and enhanced selectivity, as a result of the ILs tunable features.³ A large number of works describing the application of these systems in the separation of amino acids⁴, proteins^{5, 6}, pharmaceuticals^{7, 8} persistent pollutants⁹, among others, from model solutions to real complex media, is currently available. ABS are water-rich systems that allow the replacement of the commonly used volatile organic solvents in liquid-liquid extractions.¹ This is important when dealing with biologically active compounds with interest to the food and pharmaceutical industries¹⁰, as well as when considering some environmental and health hazards of volatile organic solvents.

Albeit ABS have been described as benign and biocompatible alternative separation systems, most of the studied IL-based ABS are formed by pairing imidazolium ILs and inorganic

salts¹, which may rise some biodegradability and toxicity concerns. This fact triggered the research on ILs derived from natural sources and/or by combining ILs with polymers, carbohydrates or amino acids, aiming at creating more bio- and environmentally-friendly ABS.^{11–16} In particular, ABS composed of ILs and carbohydrates have been previously reported.^{16–18} These studies were focused on offering a greener character to ABS by the use of carbohydrates, non-toxic and renewable compounds, instead of high charge density inorganic salts. While promising advances towards more sustainable separation techniques have been achieved, all of these studies employed ILs comprising aromatic cations and fluorinated anions. These fluoride-based ILs are more expensive, some are non-water-stable¹⁹, and in general they are environmentally persistent.²⁰ The use of ILs with fluorinated anions, usually paired with imidazolium- and pyridinium-based cations, is however required to have more hydrophobic ILs able to undergo liquid-liquid demixing in presence of weak salting-out species, such as carbohydrates. In this context, there is still the need to find new ILs that could replace the commonly used ILs by others of a more sustainable nature. To the best of our knowledge, there are no reports in the literature regarding the use of ILs with higher biodegradability, lower toxicity and lower cost²¹, such as tetralkylphosphonium- and tetralkylammonium-based paired with non-fluorinated anions, to form ABS with carbohydrates.

Focused on addressing this challenge, novel ABS composed of carbohydrates and tetralkylphosphonium- and tetralkylammonium-based ILs are herein studied. The carbohydrates investigated comprise: (i) monosaccharides, such as D-(-)-fructose, D-(+)-glucose, xylose, and D-(+)-mannose; (ii) disaccharides, such as D-(+)-maltose, and sucrose; and (iii) polyols, such as maltitol, xylitol, and D-sorbitol. These were combined with aqueous solutions of [P₄₄₄₄]Br, [P₄₄₄₁][MeSO₄], [P₄₄₄(OC₂)]Br, [P₄₄₄₄]Cl, [N₄₄₄₄]Br, and [N₄₄₄(OC₂)]Br, aiming at forming novel ABS. The description and chemical structures of the carbohydrates and ILs investigated are displayed Fig. 1. Not all the combinations tested lead to the formation of ABS - a detailed list of the combinations tested is given in the ESI†. To identify the ILs and carbohydrates compositions able to form ABS, and to employ these systems in separation and purification processes, the respective phase diagrams, tie-lines, and tie-line lengths, were determined at 25 °C. To address the effect of temperature in their liquid-liquid demixing ability, phase diagrams of the ABS formed by [P₄₄₄₄]Br + D-(-)-fructose + water were also determined at 15, 35 and 45 °C. Details on the materials and experimental procedure adopted are given in the ESI†. The ILs and carbohydrates pairs able to form ABS are represented in Fig. 2, together with the respective ternary phase diagrams and fitted solubility curves (*cf.* the ESI† with detailed information). Mixtures above the solubility curves form a two-phase system, while mixture compositions below the curve are monophasic.

From Fig. 2 it is clear that there is a set of non-aromatic and non-fluorinated ILs able to form ABS with carbohydrates, including more benign ILs, namely analogues to glycine-betaine ([N₄₄₄(OC₂)]Br), a naturally occurring and low cost amino acid. Glycine-betaine can be found in sugar beet molasses (up to 27 wt%), after the extraction of sucrose, and is still an underexploited by-product of the sugar industry.²² Therefore, the identified ABS formed by the glycine-betaine IL analogue and carbohydrates can also be envisioned as possible

separation strategies in this type of industry, while allowing the use of glycine-betaine and their derivatives as food supplements,²³ and in cosmetic lotions and formulations.²⁴

In aqueous media, the competition between the IL ions and carbohydrates for water solvation is dominated by the carbohydrates that will thus act as salting-out species, further leading to the exclusion of the IL to a second aqueous phase. This mechanism is supported by the trend obtained, in which the more hydrophilic carbohydrates (i.e. those with more –OH groups and with higher ability for hydrogen-bonding with water) combined with more hydrophobic ILs ($[P_{4444}]Br > [P_{444}(OC_2)]Br > [P_{4441}][MeSO_4] \sim [N_{4444}]Br > [N_{444}(OC_2)]Br > [P_{4444}]Cl$, as defined by their hydrogen-bond basicity²⁵) are more prone to form ABS. Taking into account the solubility curves obtained with fructose (Fig. 2), $[P_{4444}]Br$ is the most effective IL to create ABS. In fact, this IL can form ABS with monosaccharides, disaccharides and polyols. Compared to $[P_{4444}]Br$, $[P_{4444}]Cl$ has an anion of higher hydrogen-bond basicity, and thus with more affinity for water, being less prone to be salted-out. Therefore, this IL does not form ABS with any of the studied carbohydrates. In the same line, $[P_{4441}][MeSO_4]$, comprising a shorter alkyl chain and an anion of higher hydrogen-bond basicity than Br^- , is less susceptible to be salted-out by carbohydrates and to form ABS. Comparing the pairs $[N_{444}(OC_2)]Br$ vs. $[N_{4444}]Br$ and $[P_{444}(OC_2)]Br$ vs. $[P_{4444}]Br$, it is observed a decrease on the efficiency to form ABS when additional ester groups are present (with a higher aptitude to hydrogen-bond with water). In summary, the lower the IL hydrophilic nature or affinity for water, the higher it is its ability to form ABS with carbohydrates - following the trend observed in IL-salt ABS, in which salts also act as salting-out species.¹

The phase diagrams of the ABS formed by $[P_{4444}]Br + D(-)$ -fructose were determined at different temperatures (from 15 to 45 °C), with an almost negligible decrease on the liquid-liquid demixing ability as temperature increases. The corresponding data are shown in the ESI†. This trend could be seen as a major advantage when designing these systems for scaled-up strategies in terms of energetic inputs.

Previous works focused on ABS composed of a wide variety of carbohydrates and ILs constituted by fluorinated anions and imidazolium¹⁷ or pyridinium¹⁸ cations. Although these ILs are highly distinct from those investigated in this work, a similar trend for the carbohydrates salting-out ability was identified. The comparison of the phase diagrams obtained in this work with those previously reported with other ILs is shown in the ESI†. In general, disaccharides are better salting-out agents than polyols, and the former are better salting-out agents than monosaccharides (this trend is more clear in molality units, as shown in the ESI†, since discrepancies arising from the different carbohydrates molecular weights are avoided). As previously identified¹⁷, two features of the carbohydrates seem to rule their salting-out ability: the number of –OH groups and their relative positions at C(2) and C(4). It has been shown that the carbohydrates hydrogen-bonding ability with water depends on the spacing and orientation of their polar groups, in which the C(4) axial/equatorial ratio has a significant impact. In this work, we have additionally observed that the ability of carbohydrates to create ABS with ILs closely follows the carbohydrates octanol-water partition coefficients (given in the ESI†).

We have here identified, for the first time, a set of non-aromatic and non-fluorinated ILs prone to form ABS with carbohydrates, contributing towards the development of more sustainable IL-based ABS. Taking advantage of their more attractive features, the possible application of these systems in separation processes of interest to the food, nutraceutical and pharmaceutical industries was then ascertained. These systems were evaluated in terms of their extraction performance for antioxidants, namely vanillic (VA), gallic (GA) and syringic (SA) acids. All extractions experiments were carried out at two mixture compositions in the biphasic regime: 35 wt% of carbohydrate + 40 wt% of [P₄₄₄₄]Br and 50 wt% of carbohydrate + 25 wt% of [P₄₄₄₄]Br, [P₄₄₄₁][MeSO₄] or [P₄₄₄(OC₂)]Br, allowing us to address the effects of both the mixture composition and IL nature. In all the investigated ABS, the sugar-rich aqueous phase corresponds to the bottom phase (as confirmed by us by conductivity), while the upper phase is mostly composed of IL and water. Details on the experimental procedure and detailed data are given in the ESI†.

Fig. 3 depicts the extraction efficiencies (*EE*%) of the investigated IL-based ABS for the 3 antioxidants (phenolic acids), as well as their chemical structures. Extraction efficiencies correspond to the percentage weight amount of each phenolic acid in the IL-rich phase in respect to that present in both phases. Results given in partition coefficients (concentration of each phenolic acid in the IL-rich phase in respect to that in the carbohydrate-rich phase) are provided in the ESI†.

With the novel systems identified in this work, single-step extraction efficiencies of all antioxidants to the IL-rich phase ranging between 65 and 99% were obtained. Two trends on the performance of the studied ABS to extract phenolic acids were however observed: gallic acid > vanillic acid > syringic acid (with phosphonium-based ILs) and vanillic acid > syringic acid > gallic acid (with ammonium-based ILs). According to the chemical structures displayed in Fig. 3, it is patent that all antioxidants have a similar structure that differs in the substituents of the aromatic ring. With the ammonium-based ILs, the trend obtained follows the antioxidants logK_{ow} (octanol-water partition coefficient) values (provided in Fig. 3). For these systems, the differences in hydrophobicity between the two phases seem to dominate the partition trend. Nevertheless, an inversion on the trend for gallic acid, the phenolic acid with a higher number of hydroxyl groups, was observed in phosphonium-based ABS, being this antioxidant that most extensively partitions into the IL-rich phase. In this case, specific interactions between gallic acid and the IL must be taking place, which given their chemical structures must correspond to hydrogen-bonding and dispersive-type interactions, and since electrostatic interactions do not play a role as discussed below.

Most of the studied systems are acidic in nature - *cf.* the ESI† with the pH values of the ABS coexisting phases - with pH values below the phenolic acids dissociation constants²⁶, meaning that antioxidants are being extracted in their protonated (neutral) form to the IL-rich phase. This fact suggests that electrostatic interactions are not taking place and are not responsible for the preferential migration of phenolic acids to the IL-rich phase.

Overall, ABS formed by [P₄₄₄₄]Br and [N₄₄₄₄]Br perform better for the extraction of all antioxidants to the IL-rich phase than the respective [P₄₄₄(OC₂)]Br and [N₄₄₄(OC₂)]Br

counterparts, where due to their higher polarity a small decrease on the extraction efficiencies is observed. An increase in the hydrophobicity of ILs seems to improve the extraction performance. On the other hand, when comparing all the [P₄₄₄₄]Br-based ABS, there is not a significant impact of the carbohydrate nature on the systems extraction efficiency.

An additional factor that can influence the extraction performance of IL-based ABS systems is temperature.¹ However, in the studied systems, temperature, at least between 15 and 45 °C, has a negligible effect, leading to similar extraction efficiencies – *cf.* the ESI†. This trend is in accordance with the almost negligible effect of temperature on the phase diagrams behaviour discussed above, thus not leading to changes in the phase's compositions.

Aiming at developing sustainable separation processes, we finally addressed the recovery of the studied phenolic acids from the IL-rich phase, and the phase reuse for two consecutive recycles. We carried out the recovery of phenolic acids by solid-phase extraction (SPE), using previously identified effective cartridges for phenolic acids.^{27, 28} The retention mechanisms of the used cartridges depend on van der Waals forces, hydrogen-bonding and dipole-dipole interactions.²⁸ Experimental details are given in the ESI†. We successfully eluted the IL and retained the phenolic acids by adsorption onto the column, which is finally recovered by a final elution step. With the [P₄₄₄₄]Br + D-(-)-fructose ABS, we were able to recover between 98% and 100% of the initial content of gallic, syringic and vanillic acids at the IL-rich phase. The IL-rich solution was then subjected to evaporation under vacuum at room temperature, up to constant weight, and then used in the formation of new ABS and their extraction performance addressed. The IL loss in each step is below 5 wt%, although we do believe that this value can be decreased by working with higher volumes of each phase. In the two subsequent extraction studies with the recovered IL, extraction efficiencies for gallic acid higher than 95% were always obtained (shown in the ESI†), confirming that the IL can be successfully recovered and reused without losses on the systems extraction performance.

The remarkably high extraction efficiencies achieved with the novel systems shown in this work are not common in ABS composed of ILs and carbohydrates. This is a main result of the weak salting-out ability of the sugar, in which only very hydrophobic compounds, *e.g.* β-carotene, were previously observed to extensively partition to IL-rich phases.¹⁷ To assert the higher potential of the studied ILs as novel phase-forming components of ABS for extraction purposes, the extraction abilities of ABS formed by imidazolium- and fluorinated-based ILs and carbohydrates ([C₄mim][CF₃SO₃] + D-sorbitol and [C₄mim][CF₃SO₃] + sucrose)¹⁷ were additionally investigated, for the same phenolic acids and similar mixture compositions. An ABS formed by ILs and salts ([P₄₄₄₄]Br + C₆H₅K₃O₇) was also investigated for comparison purposes (mixture composition: 25 wt% [P₄₄₄₄]Br + 38 wt% C₆H₅K₃O₇). The results obtained are depicted in Fig. 3. Curiously, with the systems formed by imidazolium-based ILs and carbohydrates, all phenolic acids preferentially partition to the carbohydrate-rich phase (*EE*% < 50%) – the opposite trend to that observed in ABS formed by phosphonium-/ammonium-based ILs. These data further support the idea that electrostatic interactions are not relevant since [C₄mim][CF₃SO₃]-based ABS display a

higher pH (*cf.* the ESI⁺), and yet the charged phenolic acids still prefer to partition towards the low ionic strength phase (carbohydrate-rich).

Previous works have reported on the extraction of antioxidants using ABS formed by imidazolium-based ILs and inorganic salts.^{29, 30} In some of these works it has been observed a significant effect of the pH of the medium and electrostatic interactions²⁸, thus allowing the design of switchable separation systems³¹. To further clarify if the pH plays a significant role on the type of systems studied herein, the separation of the three antioxidants with the ABS composed of [P₄₄₄₄]Br + D-(-)-fructose and [P₄₄₄₄]Br + D-sorbitol at two additional pH values (5 and 6) were investigated – data shown in the ESI⁺. Although a small increase in the antioxidants partition to the IL-rich phase occurs as the pH decreases, the extraction efficiencies obtained with [P₄₄₄₄]Br-based ABS are still significantly higher than those obtained with ABS formed by ILs bearing imidazolium cations and fluorinated anions, and always preferentially migrating to the IL-rich phase (*EE*% > 50%).

According to Fig. 3, high extraction efficiencies (from 87 to 99%) were obtained with IL-salt-based ABS, demonstrating the preferential partition of phenolic acids to the IL-rich phase – an expected trend given the strong salting-out strength of the citrate-based salt used. ABS composed of polyethylene glycol (PEG), Na₂SO₄, and ILs as additives have been also reported in the literature in what concerns their ability to extract antioxidants.²⁹ For all systems it was observed the preferential partition of antioxidants to the PEG-rich phase (*EE*% > 90%), a result of the strong salt salting-out effect.²⁹ However, all these values are similar to those obtained with ABS formed by phosphonium- and ammonium-based ILs and carbohydrates, reinforcing the remarkable and competitive extraction performance of this set of more benign and lower ionic strength ABS.

All studies reported hitherto on the development of sustainable IL-based ABS by the use of carbohydrates focused on ILs constituted by aromatic cations combined with fluorinated anions - a crucial requirement given the weak salting-out of carbohydrates.^{17–20} Herein, we demonstrate the possibility of creating more sustainable ABS formed by carbohydrates and phosphonium- and ammonium-based ILs. Furthermore, these novel ABS display an outstanding performance to extract moderately hydrophilic compounds, such as phenolic acids, an unexpected behavior when compared with systems formed by carbohydrates and ILs previously reported.^{17–20} The novel systems disclosed in this work are competitive in terms of extraction performance, even when compared with ABS formed by ILs and strong salting-out salts. Finally, we demonstrated that phenolic acids can be recovered from the IL-rich phase, and that the employed IL can be recovered and reused with no losses on the ABS extraction performance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Chemical structures of the studied carbohydrates (CHs) and ionic liquids (ILs) able to form ABS.

Figure 2.

A-Phase diagrams of ABS composed of IL + D-(-)-fructose + H₂O at 25 °C: [P₄₄₄₄]Br (♦); [P₄₄₄(OC₂)]Br (▲); [P₄₄₄₁][MeSO₄] (■); [N₄₄₄₄]Br (◇); [N₄₄₄(OC₂)]Br (△). **B**-Phase diagrams of ABS composed of [P₄₄₄₄]Br + carbohydrate + H₂O at 25 °C: sucrose (■); D-(-)-fructose (♦); D-sorbitol (●); xylitol (▲); maltitol (*).

Figure 3.

Extraction efficiencies ($EE\%$) of the studied systems for phenolic acids at 25 °C. ■■■ correspond to gallic acid (GA), syringic acid (SA), and vanillic acid (VA), respectively. Chemical structures and $\log(K_{ow})$ values of the studied antioxidants are also presented.