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**PAPER**

# Aqueous biphasic systems composed of a water-stable ionic liquid + carbohydrates and their applications†

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A water-stable ionic liquid, 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4mim][CF_3SO_3]$, is herein proposed to be used in the formation of aqueous biphasic systems (ABS) with a large range of mono- and disaccharides, as well as polyols. Binodal curves, tie-lines, and densities and viscosities of the co-existing aqueous phases, were determined for each ternary system. The proposed systems are low-viscous offering enhanced features over conventional polymer-based ABS. In addition, the partitioning of model biomolecules, such as L-tryptophan, caffeine, and β-carotene, was further investigated to ascertain the applicability of such ABS. These systems are particularly interesting in the recovery of bioactive products from natural sources, while the availability of carbon-based sources to cells constitutes a major advantage in separations from fermentative media. Moreover, the use of carbohydrates in ionic-liquid-based ABS constitutes a step forward along the biorefinery concept envisaging sustainable conversions of biomass into a broad spectrum of bio-based products.

## Introduction

Sustainability concerns have led to intensive research activity in unconventional solvents and extraction techniques. Aqueous biphasic systems (ABS) have shown to be an alternative, efficient, and clean approach for the separation and purification of a broad array of biomolecules through their partitioning between two aqueous liquid phases.[1,2] Due to the inherent aqueous environment, ABS are recognized as biocompatible systems for cells, cells organelles and biologically active substances in downstream processing.[2–4] Three main types of ABS, depending on their chemical composition, are known: water + polymer + polymer, water + polymer + salt, and water + salt + salt.

Recent advances in ABS have suggested ionic liquids (ILs) as viable alternatives to polymeric-rich phases.[5] Gutowski *et al.*[5] have shown that imidazolium-based ionic liquids were able to form ABS in the presence of salting-out inducing inorganic salts, such as $K_3PO_4$. Subsequently, several works have emerged in the literature reporting novel phase diagrams for IL + water + inorganic salt systems.[6–8] The focal interest in ionic liquids to be used in ABS conveys on their generally unique combination of physicochemical properties (negligible volatility, non-flammability, high thermal and chemical stability, and large liquid temperature range) which have contributed to their common acceptance as relatively benign solvents.[9–12] Nevertheless, the major advantage of using ionic liquids in ABS is their solvation/extraction ability. The adequate design of the ionic liquid cation/anion combinations allows the tailoring of the co-existing phases' polarities, and thus to optimize the extraction efficiencies of diverse biomolecules and added-value products.[13–20] Hitherto, biomolecules such as amino acids, proteins, enzymes, antibiotics, phenolic compounds, alkaloids, steroids, saccharides and terpenoids have been studied as partitioning solutes in IL-based ABS.[5,13–27]

Besides the large interest devoted to IL-based ABS formed by the addition of inorganic salts,[5,15–27] the use of carbohydrates to replace those highly charged systems might lead to improved biotechnological routes. For instance, the use of $K_3PO_4$ as salting-out inducing agent (or similar salting-out salts) inserts potassium and phosphate ions into the aqueous medium, complicating thus the recycling process of ionic liquids as they also dissociate in aqueous solution. In addition, the use of $K_3PO_4$ also creates a system with a highly alkaline medium that may be deleterious to a number of pH sensitive biomolecules or to microorganisms. On the other hand, carbohydrates are non-charged, biodegradable, nontoxic, and a renewable feedstock. The mono and disaccharides are polyhydroxy aldehydes or ketones with a high affinity for water and salting-out aptitude (several –OH groups with dual donor/acceptor character that are involved in hydrogen bonding). The hydrogenated forms of such aldoses and ketoses are known as polyols. Sorbitol,

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for instance, has the same linear structure as the chain form of glucose, whereas the aldehyde (–CHO) group is replaced by a $–CH_2OH$ group, enhancing thus its affinity for water. Therefore, common carbohydrates are good candidates for the development of novel IL-based ABS. Combining the use of non-volatile and tailored ionic liquids with renewable bio-sourced organic compounds (carbohydrates) may lead to greener processes overall.[28]

Previous carbohydrate-IL-based ABS have been reported;[29–33] yet, such works merely focused on the phase diagrams behaviour and on the possibility of recycling or concentrating ionic liquids from aqueous solutions. Nonetheless, the use of biomass for the production of energy and added-value compounds is a major path towards a sustainable development. Recently, it was demonstrated that ionic liquids are adequate media for the dissolution of carbohydrates, allowing a better performance of biorefinery platforms.[34–36] The enhanced solubilisation of carbohydrates in ionic liquids has enabled a broad number of chemical derivatizations.[37–40] Appropriate IL-based ABS can stabilize enzymes without disrupting their native structure (or without interacting with their active sites).[27] In this context, IL-carbohydrate-based ABS offer the opportunity to combine purification processes with improved performance of some enzymes in ionic media,[41,42] as well as in the development of advanced materials or alternative energy. Nevertheless, the development of IL-carbohydrate-based ABS with the purpose of extracting/purifying biomolecules, as well as potential media for biorefinery transformations, remain unexplored fields.

In this work, novel IL-carbohydrate-based ABS are reported making use of a water-stable ionic liquid – 1-butyl-3-methylimidazolium trifluoromethanesulfonate ($[C_4mim][CF_3SO_3]$) – combined with a broad panel of structurally iterated carbohydrates (monosaccharides, disaccharides, and polyols). The phase diagrams, tie-lines, tie-line lengths, and densities and viscosities of both co-existing phases, were determined at 298 K.

With the goal of seeking these novel IL-carbohydrate-based ABS as potential extractive media, both from biomass matrices and from biorefinery processes, three representative (model) biomolecules were herein screened *via* their partitioning between the two aqueous phases. Caffeine and β-carotene were chosen as bioactive substances belonging to the alkaloids and terpenoids families. These compounds are usually present in biomass and have multiple therapeutic effects and pharmacological activities.[43–45] L-Tryptophan is an essential amino acid to humans required for the production of the neurotransmitter serotonin, and can be produced by fermentative processes.[46]

## Results and discussion

### Phase diagrams and tie-lines

Novel ternary phase diagrams were determined for the common ionic liquid $[C_4mim][CF_3SO_3]$, water and distinct carbohydrates. The $[C_4mim][BF_4]$ + glucose + water ternary system was used to validate the experimental procedure adopted.[30] Good agreement was observed between literature data[30] and the results collected in this work (see ESI†).

In the presence of weaker salting-out agents (carbohydrates instead of inorganic salts) the capability of forming IL-based ABS is largely reduced since a strong salting-in ionic liquid is required. The formation of carbohydrate and ionic liquid aqueous systems is a reflection of the competition between the carbohydrate and the ionic liquid ions in the creation of hydration complexes.[47,48] Therefore, the ability of a specific ion to be preferentially hydrated largely depends on its hydrogen-bond accepting strength. Previously, we have shown[16] that the ionic liquids capability to undergo through ABS is favoured by low hydrogen-bond basicity ($\beta$) values. These values reflect the thermodynamic tendency of a substance to act as a hydrogen-bond acceptor. Although several ionic liquids were evaluated (1-butyl-3-methylimidazolium chloride, 1-hexyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium methylsulfate, 1-butyl-3-methylimidazolium hydrogensulfate and 1-butyl-3-methylimidazolium dicyanamide) they were not found to be able to induce macroscopic liquid–liquid demixing in the presence of carbohydrates concentrated solutions. Neither the increase of the length of the cation side alkyl chain[15] nor ionic liquid anions with higher values of $\beta$ (up to $[CF_3SO_3]^-$) are sufficiently strong salting-in agents able to form carbohydrate-IL-water biphasic systems. Only $[C_4mim][BF_4]$ ($\beta$ = 0.55)[49] and $[C_4mim][CF_3SO_3]$ ($\beta$ = 0.57)[49] were shown to undergo phase separation in the presence of aqueous solutions of carbohydrates. Indeed, with the information gathered hitherto, it can be anticipated that there are no additional hydrophilic imidazolium-based ionic liquids (by change of the ionic liquid anion) capable of inducing phase separation using carbohydrates aqueous solutions. Other anions with lower values of $\beta$[49] are $[PF_6]^-$ and $[(CF_3SO_3)_2N]^-$; yet, the $[C_nmim][PF_6]$ and $[C_nmim][(CF_3SO_3)_2N]$ ionic liquids are not completely miscible with water at room temperature, and two aqueous-rich phases biocompatible for cells, cells organelles and biologically active substances could not be attained.[50,51] Only fluoride-based ionic liquids with higher affinity for water ($[BF_4]$- and $[CF_3SO_3]$-based ionic liquids) are capable of inducing liquid–liquid phase separation in the presence of aqueous solutions of carbohydrates.

Albeit $[C_4mim][BF_4]$ can form carbohydrate-based ABS, it was previously shown that $[BF_4]$-based ionic liquids are not water stable.[52] $[BF_4]$-based ionic liquids suffer hydrolysis in contact with water releasing fluoridric acid, even at moderate temperatures, while the hydrolysis extent was shown to increase with the cation side alkyl chain length.[52] Therefore, $[C_4mim][CF_3SO_3]$ is here proposed as a novel and water-stable ionic liquid capable of forming ABS in the presence of a large range of carbohydrates (monosaccharides, disaccharides and polyols). The chemical structures of the carbohydrates evaluated are shown in Fig. 1 and 2.

Ternary phase diagrams of $[C_4mim][CF_3SO_3]$ + several carbohydrates + water, at 298 K and atmospheric pressure, were determined in order to infer on the carbohydrates potential to induce ABS. Binodal curves for each carbohydrate + IL + $H_2O$ system are displayed in Fig. 3 to 5. Fig. 3 presents the phase diagrams for the monosaccharides (D-(+)-glucose, D-(+)-mannose, D-(−)-fructose, D-(+)-galactose, D-(+)-xylose, D-(−)-arabinose and L-(+)-arabinose) and Fig. 4 for the disaccharides (D-(+)-maltose and sucrose) evaluated. Fig. 5 depicts the polyols (D-maltitol, xylitol and D-sorbitol) effect in the phase diagrams behaviour.

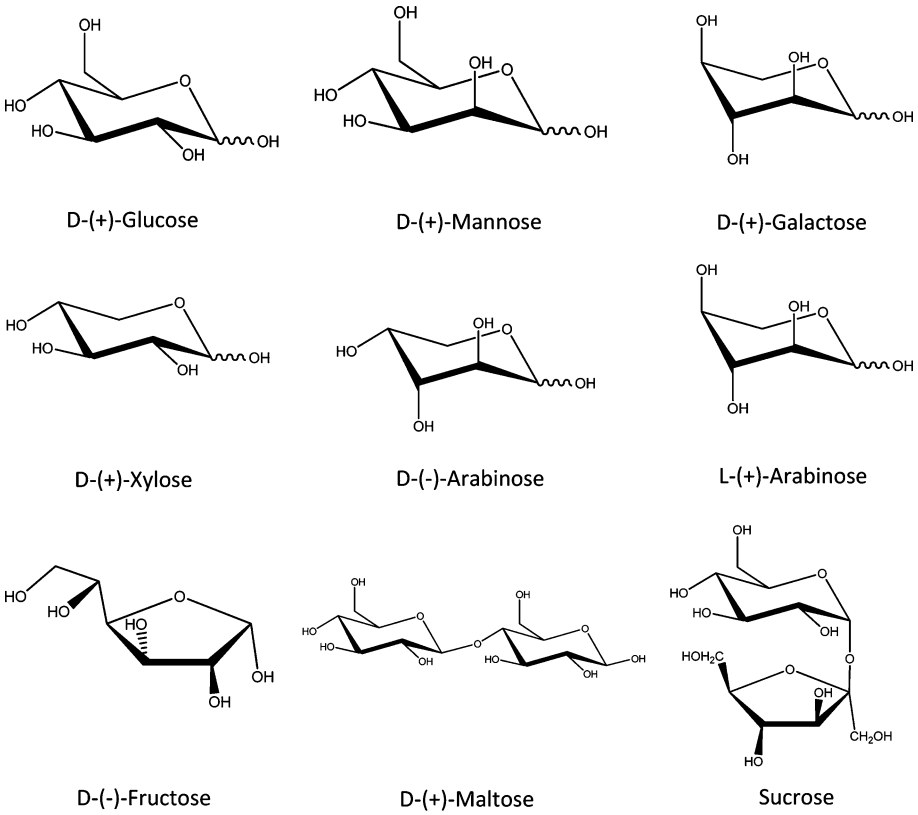


**Fig. 1** Chemical structures of the monosaccharides and disaccharides studied.

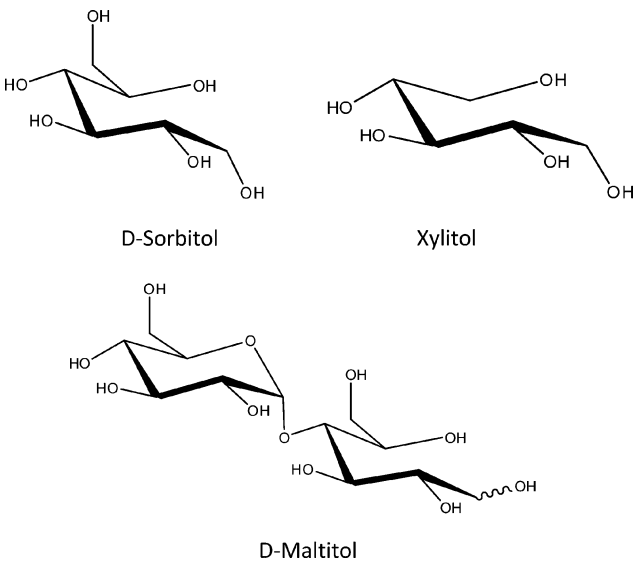


**Fig. 2** Chemical structures of the polyols studied.

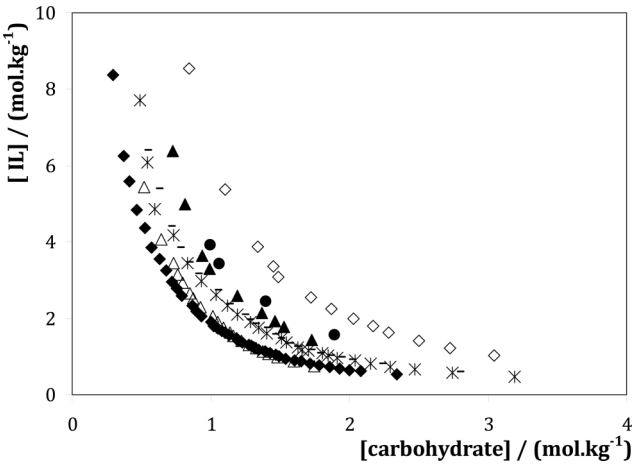


**Fig. 3** Phase diagrams for the ternary systems composed by $[C_4mim][CF_3SO_3]$ + carbohydrate + $H_2O$ at 298 K: (◆) D-(+)-glucose; (△) D-(+)-galactose; (✳) D-(−)-fructose; (–) D-(+)-mannose; (▲) D-(−)-arabinose; (●) L-(+)-arabinose; (◇) D-(+)-xylose.

An overview of all the carbohydrates potential in inducing ABS is presented in the ESI.† All binodal data are displayed in molality units, thus avoiding disparities in the carbohydrate-based ABS formation capability that could be a direct consequence of their distinct molecular weights (see ESI with experimental weight fraction data†).

The phase diagrams shown in Fig. 3 indicate that the monosaccharides capacity to induce IL-based ABS, follows the decreasing order: D-(+)-glucose ≈ D-(+)-galactose > D-(−)-fructose ≈ D-(+)-mannose > D-(−)-arabinose > L-(+)-arabinose > D-(+)-xylose. This trend suggests that hexoses are more effective in promoting ABS formation than pentoses. The number of hydroxyl groups results in a higher number of potential hydrogen bonds with water turning them into stronger salting-out agents. Furthermore, from the comparison between the structural isomers glucose and fructose, it is evident that pyranoses (6-sided ring) are more efficient salting-out agents than furanoses (5-sided ring). The slightly higher hydration

 

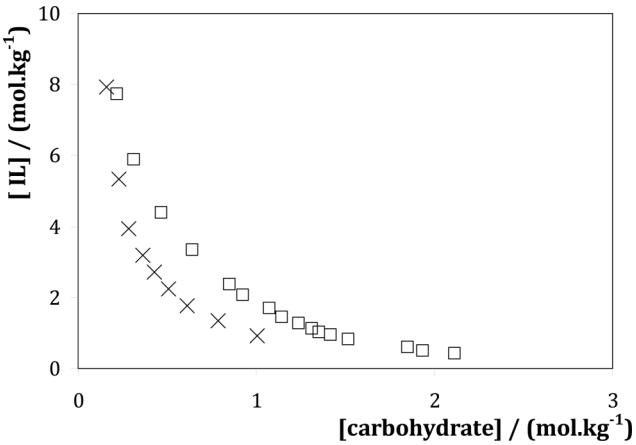


**Fig. 4** Phase diagrams for the ternary systems composed by $[C_4mim][CF_3SO_3]$ + carbohydrate + $H_2O$ at 298 K: (×) D-(+)-maltose; (□) sucrose.

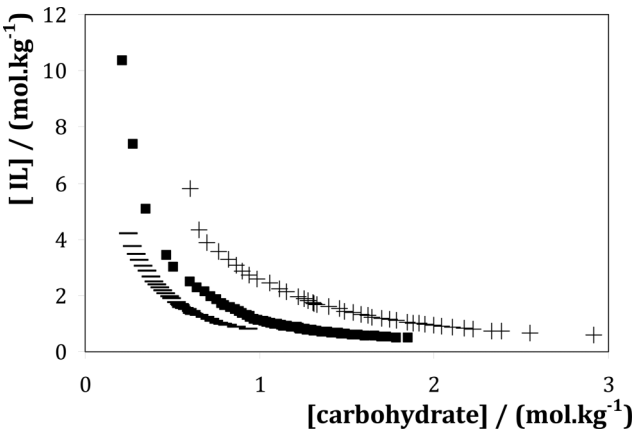


**Fig. 5** Phase diagrams for the ternary systems composed by $[C_4mim][CF_3SO_3]$ + carbohydrate + $H_2O$ at 298 K: (○) D-maltitol; (■) D-sorbitol; (+) xylitol.

of glucose over fructose is a direct consequence of the six-membered pyranose's more favourable conformation for hydrogen bonding with water. Several attempts have been reported in literature aiming at rationalizing carbohydrates hydration (salting-out inducing behaviour) in terms of hydration numbers, ratio of equatorial *versus* axial –OH groups, anomeric effect and hydrophobic/hydrophilic index.[53–56] From the results obtained, the preferential formation of hydrated complexes is the driving force behind the carbohydrates ability for the ionic liquid salting-out, and consequently for ABS formation.

Taking into account structures that have opposite configurations of hydroxyl groups at specific positions – the C(2) epimers D-glucose and D-mannose, and C(4) epimers D-glucose and D-galactose – there is a patent influence of the hydroxyl group orientation at C(2) and C(4). D-(+)-Glucose presents a higher ability to promote phase separation compared to D-(+)-mannose and D-(+)-galactose following the rank: glucose > galactose > mannose. This pattern is related with the carbohydrates hydration potential. It is well established that the hydration of carbohydrates depends on the ratio between axial and equatorial hydroxyl groups.[56,57] Hydration is favourable for carbohydrates presenting hydroxyl groups at equatorial positions. D-(+)-Mannose presents C(2) and C(4) hydroxyl groups at axial positions and is thus the less hydrated carbohydrate among the epimers. Consequently, it presents the lower ability to induce the ionic liquid salting-out from aqueous solutions. Analogous –OH groups in glucose occupy equatorial positions. Hence, D-(+)-glucose is the stronger salting-out species, among the three epimers considered, due to its preferential hydration. Sandwiched between these two appears D-(+)-galactose, with hydroxyl groups at both equatorial (C(2)) and axial (C(4)) positions, and thus occupying a middle position in the epimers salting-out ability. It should be emphasized that the difference between the C(2) epimers is particularly more pertinent in the carbohydrates ability to induce IL-based ABS.

For pentoses, two enantiomers, D-(−)-arabinose and L-(+)-arabinose, were additionally evaluated. From the results sketched in Fig. 3 the spatial configuration of the hydroxyl group at C(4) also influences the phase diagrams as discussed above for D-(+)-glucose and D-(+)-galactose. D-(−)-Arabinose is more capable of inducing ABS than L-(+)-arabinose. D-(−)-Arabinose presents the C(4) hydroxyl group at an equatorial position and has, therefore, a higher aptitude to be hydrated. From the results gathered, the relative positions of the hydroxyl groups at C(2) and C(4) are key factors determining the saccharides facility for creating IL-based ABS. Moreover, a deeper analysis of the results reveals that the IL-carbohydrate-based ABS pattern is more dependent on the C(4) axial/equatorial ratio of each carbohydrate than on the OH–C(2) group. Therefore, the carbohydrates hydrogen bonding ability with water depends on the spacing and orientation of their polar groups, regarding their –OH geometry in aqueous solutions.

Fig. 4 presents the experimental phase diagrams, at 298 K and atmospheric pressure, for each disaccharide + $[C_4mim][CF_3SO_3]$ + water system investigated. The influence of disaccharides in increasing the phase's immiscibility follows the trend: D-(+)-maltose > sucrose. Maltose is made up of two units of glucose whereas sucrose is composed of one molecule of glucose and one molecule of fructose. Six-membered pyronose rings in maltose are more favourable for interactions with water than the five-membered furanose ring in sucrose (as previously discussed with monosaccharides).

Fig. 5 shows that the sequence on forming ABS for polyols follows the rank: D-maltitol > D-sorbitol > xylitol. This pattern is directly related with the number of –OH groups present in each carbohydrate capable of hydrogen bonding with water. Among the reduced sugars, maltitol is the polyol that requires less ionic liquid to form ABS. A higher number of carbons, and consequently more hydroxyl groups, leads to higher efficiencies for the sugars hydration.

The salting-out ability of the overall carbohydrates studied (see ESI†) trails the following sequence: D-maltitol > D-(+)-maltose > D-sorbitol > sucrose > D-(+)-glucose ≈ D-(+)-galactose > xylitol ≈ D-(−)-fructose ≈ D-(+)-mannose > D-(−)-arabinose > L-(+)-arabinose > D-(+)-xylose. D-(+)-Maltose and D-maltitol are the stronger salting-out agents evaluated. It is quite remarkable the higher capacity of alditols, due to the higher number and solvability of their hydroxyl groups, to induce IL-based ABS. The reduction of aldoses and ketoses to an alcohol functionalized group leads to improved salting-out agents – polyols. In summary, it was observed that two main factors

are subjacent to the carbohydrates salting-out aptitude: (i) the number of –OH groups, and (ii) the related stereochemistry.

The salting-out potential of concentrated solutions of carbohydrates is usually assessed making use of the viscosity *B*- and *D*-coefficients accordingly to the extended Jones–Dole equation.[58,59] It should be remarked that the extended Jones–Dole equation[58] was selected to cover the carbohydrates concentrations applied in the IL-based ABS formation. The viscosity ratio of a carbohydrate containing mixture to that of the pure solvent, as a function of the carbohydrate concentration, provides the viscosity coefficients. Higher viscosity *B*-coefficient values indicate a stronger ability of the sugar to change the viscosity of the aqueous solution and to act as a salting-out agent: water–water and water–carbohydrate interactions are more significant than the interactions among carbohydrates. For carbohydrates to induce salting-out it is essential that the favourable dipole–dipole and H-bonding interactions between water and carbohydrates overwhelm the favourable interactions IL-water. On the other hand, the viscosity *D*-coefficients are related with the strength on both the carbohydrate-carbohydrate and carbohydrate-water interactions, coupled to the size and shape effects of the solute (entropic contributions). The trend gathered in this work on the carbohydrates potential to induce salting-out closely follows the viscosity *B*- and *D*-coefficients of aqueous solutions of carbohydrates taken from the literature,[59] as depicted in Fig. 6. Higher viscosity *B*- and *D*-coefficients point towards a stronger ability of each sugar to establish stronger intermolecular interactions. Thus, these values clearly correlate with the ability of each carbohydrate to induce the salting-out of [$C_4$mim][$CF_3SO_3$] from aqueous media.

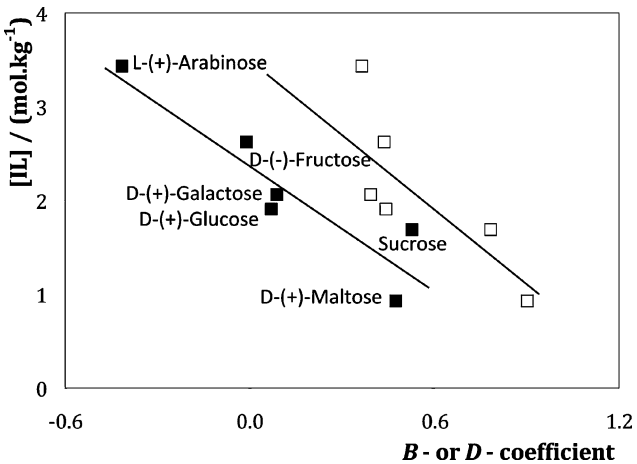


**Fig. 6** Ionic liquid molality, taken from each binodal curve, at which the carbohydrate molality is equal to 1.0 mol $kg^{-1}$ as a function of viscosity coefficients of aqueous solutions of carbohydrates at 308.15 K:[59] (□) *B*-coefficient; (■) *D*-coefficient.

The experimental binodal data were fitted using eqn (1),

$$[\mathrm{IL}] = A \exp[(B \times [\mathrm{CH}]^{0.5}) - (C \times [\mathrm{CH}]^3)] \quad (1)$$

where [IL] and [CH] are, respectively, the IL and carbohydrate weight percentages in the equilibrated phases, and *A*, *B* and *C* are constants obtained by the fitted regression of the experimental data.

**Table 1** Correlation parameters used in eqn (1) adjusted to the binodal experimental data (and standard error of estimate, $\sigma$, and correlation coefficients, $R^2$) at 298 K

| [$C_4$mim][$CF_3SO_3$] + carbohydrate + water | *A* | *B* | $10^5 \times C$ | $R^2$ | $\sigma$ |
|---|---|---|---|---|---|
| D-(+)-glucose | 169.6 | –0.3796 | 2.543 | 0.9971 | 0.8134 |
| D-(+)-galactose | 202.2 | –0.3971 | 4.041 | 0.9982 | 0.5282 |
| D-(–)-fructose | 213.9 | –0.3988 | 1.348 | 0.9959 | 1.0478 |
| D-(+)-mannose | 181.2 | –0.3428 | 2.186 | 0.9976 | 0.6939 |
| D-(–)-arabinose | 303.8 | –0.4949 | 1.000 | 0.9955 | 0.9297 |
| L-(+)-arabinose | 217.5 | –0.3863 | 1.120 | 0.9977 | 0.8064 |
| D-(+)-xylose | 259.8 | –0.3804 | 1.000 | 0.9991 | 0.4675 |
| D-(+)-maltose | 150.5 | –0.3311 | 1.292 | 0.9987 | 0.6528 |
| sucrose | 107.1 | –0.1636 | 1.821 | 0.9982 | 0.8253 |
| D-Maltitol | 170.4 | –0.3992 | 1.954 | 0.9981 | 0.4608 |
| D-sorbitol | 206.5 | –0.5096 | 2.643 | 0.9958 | 0.8920 |
| xylitol | 231.4 | –0.4624 | 1.100 | 0.9967 | 0.7043 |

The correlation coefficients *A*, *B* and *C*, the corresponding standard errors of the fitting ($\sigma$), and the correlation coefficients ($R^2$) for each individual system, are summarized in Table 1. Experimental tie-lines (TLs) measured for each IL–carbohydrate–water ternary system along with the respective tie-line lengths (TLLs) are reported in Table 2. An example of the TLs representation along with the correlation of the binodal data using eqn (1) for one monosaccharide, one disaccharide and one polyol is shown in Fig. 7.

With the exception of the sucrose-containing system (see below the density values and respective discussion for the corresponding phases), at the compositions evaluated, the IL-rich phase corresponds to the bottom phase while the carbohydrate-rich phase represents the top phase. This behaviour is quite interesting since the majority of typical (IL + inorganic salt)-based ABS present an upper phase mainly composed of ionic liquid and a bottom phase primarily constituted by the inorganic salt and water.[15–18] Even with [$C_4$mim][$BF_4$] + carbohydrates ABS[29–33] an upper IL-rich phase and a bottom carbohydrate-rich phase were found. The inversion in the phases composition observed in this work seems to be directly related with the [$C_4$mim][$CF_3SO_3$] high density (the density of [$C_4$mim][$BF_4$] is 1206.9 kg $m^{-3}$, and the density of [$C_4$mim][$CF_3SO_3$] is 1306.1 kg $m^{-3}$, at 293.15 K and 0.10 MPa).[60] It should be noted that an IL-rich bottom phase represents an additional advantage in extraction processes involving decantation.

The physical properties of ABS at various concentrations and temperatures are key issues for the design and scale up of a wide range of extraction processes and biorefinery approaches. High-molecular weight polymers are usually avoided due to their high viscosity that is a major hurdle in industrial scale operations. Therefore, polymer/salt ABS are usually preferred for large-scale applications since they provide relatively low cost and shorter separation times.[1–3] Aiming at further evaluate the enhanced potential of the ABS herein proposed, density and viscosity measurements were carried out in the temperature range between 298.15 K and 318.15 K, on both the top and bottom phases, for selected mass fraction compositions at the biphasic region. The exact mass fraction compositions for each ternary system are those represented in Table 2 at ≈ 40 wt % of IL and ≈ 25 wt % of each carbohydrate (and further used in the biomolecules partitioning studies – see results below).

 

**Table 2** Experimental weight fraction compositions (wt %) for the TLs and TLLs determination, and compositions of ionic liquid (IL) and each carbohydrate (CH) at the top (T) and bottom phases (B) at 298 K

| | wt % | | wt % | | wt % | | | |
|---|---|---|---|---|---|---|---|---|
| Carbohydrate | [IL] | [CH] | $[IL]_T$ | $[CH]_T$ | $[IL]_B$ | $[CH]_B$ | TLL | $\alpha$ |
| D-(+)-glucose | 37.90 | 16.67 | 15.23 | 26.31 | 59.59 | 7.441 | 48.21 | 0.5111 |
| | 29.29 | 21.09 | 14.08 | 27.17 | 67.54 | 5.819 | 57.57 | 0.7154 |
| | 40.11 | 24.94 | 1.469 | 44.36 | 82.63 | 3.577 | 90.83 | 0.5239 |
| D-(+)-galactose | 35.05 | 16.98 | 20.80 | 21.81 | 56.21 | 9.790 | 37.40 | 0.5976 |
| | 39.96 | 17.01 | 11.98 | 26.74 | 67.58 | 7.394 | 58.87 | 0.4967 |
| D-(−)-fructose | 29.99 | 24.96 | 13.83 | 32.53 | 64.17 | 8.964 | 55.59 | 0.6789 |
| | 39.95 | 24.99 | 6.169 | 41.63 | 77.65 | 77.65 | 79.69 | 0.5275 |
| D-(+)-mannose | 39.92 | 20.14 | 12.98 | 31.80 | 67.92 | 8.010 | 59.86 | 0.4901 |
| | 39.84 | 24.91 | 3.655 | 42.44 | 79.43 | 5.734 | 84.20 | 0.5225 |
| L-(+)-arabinose | 39.95 | 20.13 | 21.17 | 28.63 | 60.96 | 10.62 | 43.68 | 0.4719 |
| | 49.81 | 19.97 | 7.629 | 42.20 | 72.54 | 7.997 | 73.37 | 0.3502 |
| D-(+)-xylose | 52.11 | 21.18 | 11.06 | 41.64 | 72.38 | 11.07 | 68.52 | 0.3305 |
| | 40.08 | 24.93 | 16.84 | 35.82 | 66.88 | 12.37 | 55.27 | 0.4646 |
| | 39.62 | 24.71 | 18.02 | 34.84 | 64.69 | 12.95 | 51.55 | 0.4628 |
| D-(+)-maltose | 33.00 | 20.61 | 21.01 | 26.90 | 54.99 | 9.075 | 38.37 | 0.6472 |
| | 39.91 | 19.96 | 13.86 | 33.27 | 67.62 | 5.806 | 60.37 | 0.5157 |
| sucrose | 39.93 | 25.04 | 66.18 | 8.291 | 5.062 | 47.30 | 72.51 | 0.4295 |
| | 44.71 | 29.76 | 88.42 | 1.371 | 0.839 | 58.26 | 104.4 | 0.5009 |
| D-maltitol | 34.88 | 20.05 | 11.13 | 30.16 | 70.58 | 4.850 | 64.62 | 0.6005 |
| | 40.00 | 25.02 | 2.763 | 42.64 | 86.86 | 2.845 | 93.04 | 0.5572 |
| D-sorbitol | 41.61 | 12.89 | 14.40 | 21.91 | 65.13 | 5.096 | 53.44 | 0.4637 |
| | 39.77 | 14.93 | 10.17 | 25.49 | 68.56 | 4.658 | 62.00 | 0.5068 |
| | 40.01 | 24.97 | 0.582 | 45.22 | 82.27 | 3.255 | 91.84 | 0.5174 |
| xylitol | 49.80 | 14.88 | 14.083 | 29.57 | 69.71 | 6.696 | 60.14 | 0.3578 |
| | 39.82 | 24.89 | 5.585 | 41.06 | 81.84 | 5.039 | 84.33 | 0.5510 |

Experimental results for viscosities and densities of the co-existing phases are presented in the ESI.†

At 298.15 K, viscosities of IL-rich phases range between 9.8116 mPa s (for D-(+)-glucose) and 11.094 mPa s (for D-(−)-fructose) while carbohydrate-rich phases range between 8.2700 mPa s (xylitol) and 13.763 mPa s (sucrose). Generally, the IL-rich phase is shown to be slightly more viscous than the corresponding carbohydrate-rich phase. Two exceptions were observed for D-(+)-glucose- and sucrose-rich phases which are more viscous than the corresponding $[C_4mim][CF_3SO_3]$-rich phase. The systems here reported present viscosities similar to the $[C_4mim][CF_3SO_3]$ + $K_3PO_4$ ABS previously studied,[18] and are substantially lower than the viscosities of typical PEG-salt or PEG-polysaccharide ABS.[61,62] The low viscosity of these systems favours both the mass transfer on the extraction processes as well as the phases handling if a scale-up is needed.

Phase separation in ABS is usually attained by gravimetric separation, thus avoiding concomitant high-energy inputs. Differences in density values between the two phases enable faster and easier phase breakdown. The IL-rich phases are usually denser than the corresponding carbohydrate-rich phases. Only the sucrose system presents a carbohydrate-rich bottom phase. Curiously, an inversion of phases is observed within this system (at ≈ 40 wt % of IL and ≈ 25 wt % of sucrose) when the temperature is raised. Densities of carbohydrate-rich phases, at 298.15 K, range between 1.1824 g cm$^{-3}$ and 1.2247 g cm$^{-3}$ for the xylitol- and sucrose-rich phases, respectively. The densities of the IL-rich phase are higher and range from (1.2234 to 1.2363) g cm$^{-3}$ for sucrose and glucose composing systems. The carbohydrate-rich phases present lower densities than the $K_3PO_4$-rich phases previously reported by us.[18] The larger difference between the two phases densities was observed for xylitol which favours phase separation.

## Partitioning of biomolecules

In this work, three biomolecules were used as proof of principle to support the capability of the studied IL-based ABS in extraction routes and biorefinery conversion platforms. L-Tryptophan, caffeine and $\beta$-carotene were studied as representative examples of amino acids, alkaloids, and terpenoids, respectively. The molecular structures of the studied biomolecules are depicted in Fig. 8.

Partition coefficients, $K_X$, of the studied biomolecules ($K_{Trp}$ for L-tryptophan, $K_{\beta carot}$ for $\beta$-carotene and $K_{Caf}$ for caffeine) are defined as the ratio of the concentration of each biomolecule in the IL-rich phase to that in the carbohydrate-rich phase, as described by eqn (2),

$$K_X = \frac{[X]_{IL}}{[X]_{CH}} \quad (2)$$

where $[X]_{IL}$ and $[X]_{CH}$ are the concentration of each biomolecule in the ionic liquid and in the carbohydrate aqueous-rich phases, respectively. They are shown in Fig. 9 for a temperature of 298 K.

In order to isolate deviations that could arise from the ternary mixture compositions, the partitioning studies were performed at fixed overall compositions of IL, water, and each carbohydrate. The overall biphasic region mixture selected contains ≈40 wt % of $[C_4mim][CF_3SO_3]$ and ≈25 wt % of each carbohydrate. It should be pointed out that at such biphasic mixture composition one additional TL was further determined aiming at knowing the composition of all the components at

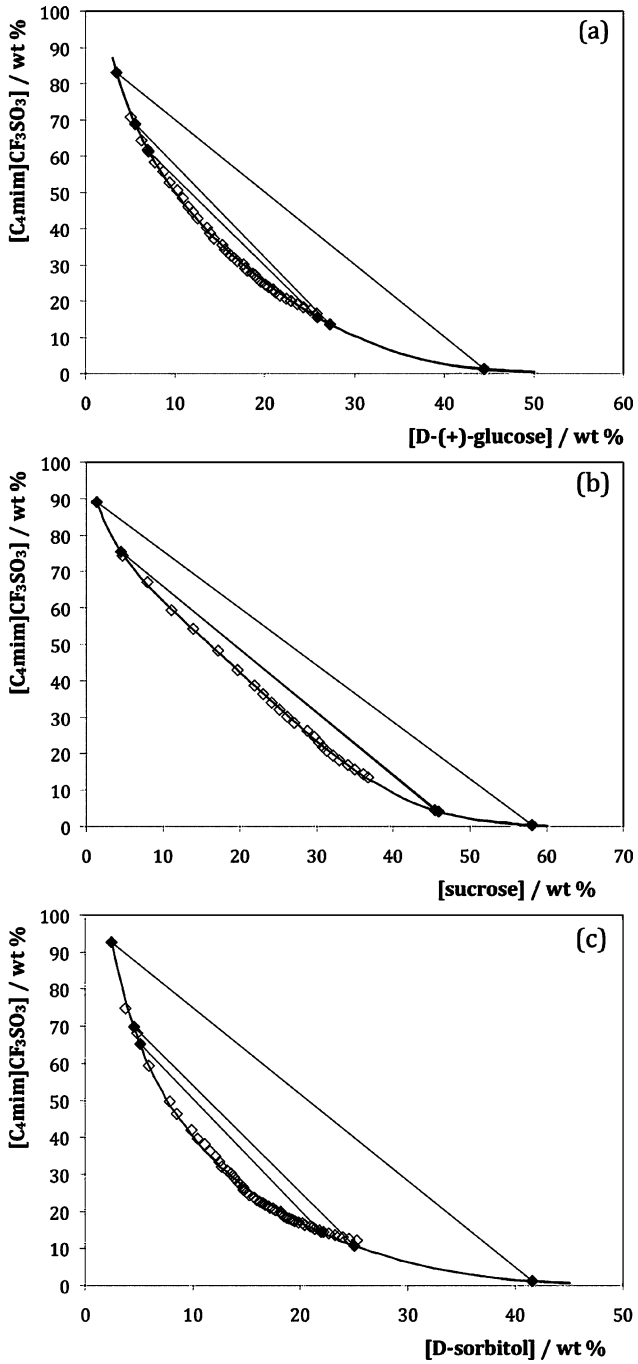


**Fig. 7** Phase diagrams for the ternary systems composed by $[C_4mim][CF_3SO_3]$ + D-(+)-glucose/sucrose/D-sorbitol + $H_2O$ at 298 K: (◇) binodal curve data; (◆) TL data; (—) binodal adjusted data through eqn (1).

both rich phases (Table 2). The partition coefficients, respective standard deviations, and exact mass fraction compositions of each component, are provided in the ESI.†

Partition coefficients of L-tryptophan ($K_{Trp}$) in IL-carbohydrates ABS are somewhat higher than those observed in PEG-polysaccharide systems.[63] This supports the idea that $[C_4mim][CF_3SO_3]$ has an improved extraction ability over polymers since weaker salting-out species are used in this work (monosaccharides, disaccharides and polyols instead of

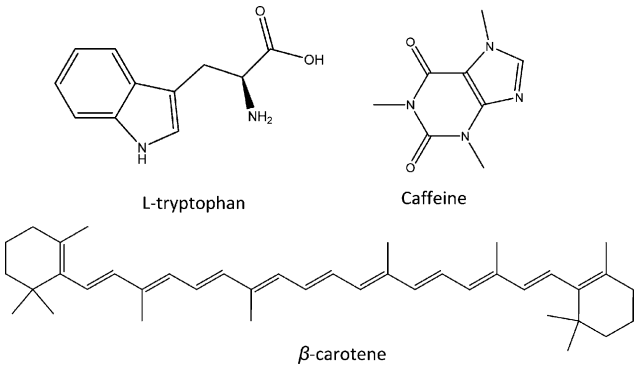


**Fig. 8** Chemical structures of the model biomolecules studied.

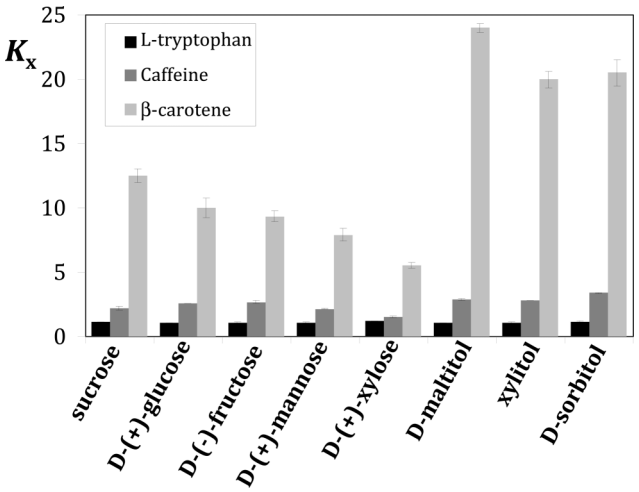


**Fig. 9** Partition coefficients of L-tryptophan, caffeine, and β-carotene, between the IL- and the carbohydrate-rich phases at 298 K.

polysaccharides). The partition coefficients, for L-tryptophan, indicate an extraction efficiency of approximately 50% to the IL-rich phase and showed to be almost independent of the carbohydrate used. This suggests that possible hydrophobic type interactions between carbohydrates and tryptophan are not significant and have no impact on the extraction ability of these molecules. As previously suggested, the enhanced ability for the extraction of L-tryptophan by hydrophilic imidazolium-based ionic liquids seems to result from the π···π and H-bonding interactions occurring between the ionic liquid cation and L-tryptophan.[15,17]

A slight improvement in the biomolecules hydrophobicity leads to higher extraction efficiencies. The partition coefficients of caffeine using the non-reduced sugars (monosaccharides and disaccharides) are higher than those of L-tryptophan and vary between 1.50 and 2.68. Among the non-reduced sugars the lower partition coefficient was observed with D-(+)-xylose – the weaker salting-out carbohydrate evaluated. Moreover, no major differences for the diverse non-reduced carbohydrates were observed. Again, and as observed before,[20] the favourable interactions between the ionic liquid cation and the caffeine purine ring seem to dominate the partitioning behaviour. Additionally, an increase in the partitioning of caffeine for the IL-rich phase was observed using alditols as salting-out species ($K_{Caf} \approx 3$). This trend is related with the stronger

 

salting-out ability of these sugars, due to their preferential hydration, enhancing thus the partitioning of the alkaloid for the IL-rich phase. In particular, the partitioning coefficients obtained for caffeine for IL-rich phases using carbohydrates are higher than those previously observed with IL-proline or polymer-polymer ABS.[14,64]

Remarkably high extraction efficiencies were observed with β-carotene. Partition coefficients range between 5.5 and 24.0 which correspond to β-carotene extraction efficiencies up to 95%. A macroscopic view of the extraction of β-carotene into the IL-rich phase is shown in the ESI.† The partition coefficients obtained for this hydrophobic solute largely depend on the carbohydrate employed. The trend on the partition coefficients of β-carotene follows the carbohydrates ability to form ABS, and therefore their hydration aptitude. The main driving force for the partitioning of β-carotene appears to relay on the inherent ability of carbohydrates to be hydrated (or not) and their consequent salting-out ability.

We have thus shown that IL-carbohydrate-based ABS extraction abilities depend on the hydrophobicity of the solute. Ranging from L-tryptophan, to caffeine, to β-carotene, a large range of solutes polarities was covered using distinct biomolecules with well known biological activities. The partitioning of hydrophilic and aromatic solutes, such as L-tryptophan, depends on the ionic liquid nature and on the IL-amino acid specific interactions. For the partitioning of caffeine, a middle rank polarity compound, a slight dependence on the carbohydrate hydration aptitude was verified. The results obtained for the extraction of an extreme hydrophobic solute, β-carotene, indicated a strong dependence on the carbohydrate hydration. As a result, the extraction of hydrophobic solutes can be controlled by a correct selection of the carbohydrate employed.

The systems here proposed showed to be highly efficient for the extraction of hydrophobic biomolecules and further applications using biomass samples or extractive fermentation media are directly envisaged. Compared with conventional extraction systems this new approach avoids the use of volatile organic compounds (usually used for the extraction of hydrophobic solutes) and corroborates the opportunity of using recyclable ionic liquid solvents.

## Conclusions

Aqueous solutions of a water-stable ionic liquid ([$C_4$mim][$CF_3SO_3$]), and a large range of distinct carbohydrates, have shown to undergo through phase separation. Phase diagrams, tie-lines, and tie-line lengths, at 298 K were determined and presented. Furthermore, densities and viscosities of the co-existing phases were determined to evaluate the adequacy of such systems for liquid–liquid extractions and their suitability in scale-up processes.

The ability of carbohydrates to induce ABS formation followed the order: D-maltitol > D-(+)-maltose > D-sorbitol > sucrose > D-(+)-glucose ≈ D-(+)-galactose > xylitol ≈ D-(−)-fructose ≈ D-(+)-mannose > D-(−)-arabinose > L-(+)-arabinose > D-(+)-xylose. It was demonstrated that the capability of sugars to induce phase separation closely follows their hydration capability. The aptitude of alditols to induce IL-based ABS was here demonstrated for the first time.

The proposed IL-carbohydrate-based ABS were additionally investigated and characterized accordingly to their extractive potential for three distinct biomolecules. L-Tryptophan, caffeine and β-carotene were studied as archetypal examples. The extraction efficiencies of the investigated IL-carbohydrate-based ABS – coupled to their low ionic strength, their low viscosity, and use of biomass – open new pathways towards their use as amenable candidates in extractive routes and biorefinery building blocks. Hence, the use of these novel systems in chemical derivatizations of carbohydrates is encouraged.

## Experimental

### Materials

The ABS studied in this work were established by using different aqueous solutions of carbohydrates and aqueous solutions of ionic liquid. The chemical structures of the studied carbohydrates are described in Fig. 2 and Fig. 3 and were as follows: D-sorbitol ≥99 wt% pure from Fluka, xylitol ≥99 wt% pure from Sigma, D-maltitol ≥98 wt% pure from Sigma, D-(+)-glucose >99.5 wt% pure from Scharlau, D-(+)-mannose >99 wt% pure from Aldrich, sucrose >99.5 wt% pure from Himedia, D-(+)-galactose ≥98.0 wt% pure from GPR Rectapur, D-(+)-xylose ≥99.0 wt% pure from Carlo Erba, L-(+)-arabinose ≥99.0 wt% pure from BHD Biochemicals, D-(−)-arabinose ≥99.5 wt% pure from Sigma, D-(+)-maltose ≥98 wt% pure from Sigma, and D-(−)-fructose >98.0 wt% pure from Panreac. The ionic liquids used in the ABS determination were 1-butyl-3-methylimidazolium tetrafluoroborate, [$C_4$mim][$BF_4$], and 1-butyl-3-methyl-imidazolium trifluoromethanesulfonate, [$C_4$mim][$CF_3SO_3$]. Both ionic fluids were acquired at Iolitec and are ≥99.0 wt% pure. The purity of each ionic liquid was additionally confirmed by $^1$H, $^{13}$C and $^{19}$F NMR spectra. The water used was ultra-pure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus. The biomolecules L-tryptophan, >99.0 wt% pure, and β-carotene, ≥97.0 wt% pure, were obtained from Fluka, and caffeine, ≥99.5 wt% pure, was acquired at José M. Vaz Pereira, SA.

### Methods

**Phase diagrams and tie-lines.** Ternary phase diagrams were determined through the cloud point titration method at 298 K (± 1 K).[15,16] Aqueous solutions of each carbohydrate at ≈(20–60) wt% (depending on the carbohydrate solubility saturation in water) and aqueous solutions of ionic liquid at ≈(40–90) wt% were prepared and used for the phase diagrams determination. Repetitive drop-wise addition of the aqueous carbohydrate solution to the aqueous solution of ionic liquid was carried out until the detection of a cloudy solution, followed by the drop-wise addition of ultra-pure water until the detection of a monophasic region and macroscopically limpid solution. Drop-wise additions were carried out under constant steering. Ternary mass fraction compositions were determined by the weight quantification of all components added within an uncertainty of $\pm 10^{-4}$ g. The experimental procedure adopted was validated with the [$C_4$mim][$BF_4$] + D-(+)-glucose + water ternary system

at 298 K against literature data.[30] The results obtained in this work show a good agreement with literature data (see ESI†).

It should be remarked that in all the ternary systems evaluated the top phase is the carbohydrate-rich phase while the bottom phase is the IL-rich phase, with the exception of sucrose where an inversion of phases was identified. The IL-rich phase was identified using an UV-Vis spectrophotometer, SHMADZU UV-1700 at a wavelength of 211 nm.

Measurements of viscosity and density were performed in the temperature range between (298.15 and 318.15) K, at atmospheric pressure, using an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter. The dynamic viscosity has a relative uncertainty of 0.35% while the absolute uncertainty of the density is within 0.0005 g $cm^{-3}$.[65] The thermophysical properties of each individual aqueous phase were determined at the ternary compositions used for the biomolecules partitioning (40 wt% of $[C_4mim][CF_3SO_3]$ + 25 wt% of carbohydrate + 35 wt% of water). The composition of each system is described in Table 2 for the systems evaluated.

Tie-lines (TLs) of each ternary system were determined by a gravimetric method originally described by Merchuck *et al.*[66] A ternary mixture at the biphasic region was prepared, vigorously stirred and allowed to reach phase separation for 12 h and at 298 K, using small ampoules (*ca.* 10 $cm^3$) designed previously for this purpose.[15,16] After equilibration, the top and bottom phases were carefully separated and individually weighed. Each individual TL was determined by application of the lever rule to the relationship between the top phase and the overall system composition.[66]

Each experimental binodal curve was fitted using eqn (1) previously described. The TLs determination was performed solving the following system of four equations (eqn (3) to (6)) to four unknown values ($[IL]_T$, $[IL]_B$, $[CH]_T$ and $[CH]_B$):[66]

$$[IL]_T = A\exp[(B\times[CH]_T^{0.5}) - (C\times[CH]_T^3)] \quad (3)$$

$$[IL]_B = A\exp[(B\times[CH]_B^{0.5}) - (C\times[CH]_B^3)] \quad (4)$$

$$[IL]_T = ([IL]_M/\alpha) - ((1-\alpha)/\alpha)\times[CH]_B \quad (5)$$

$$[CH]_T = ([CH]_M/\alpha) - ((1-\alpha)/\alpha)\times[CH]_B \quad (6)$$

where M, T, and B denote respectively the mixture, the top phase and the bottom phase, [CH] is the weight fraction of carbohydrate, [IL] the weight fraction of $[C_4mim][CF_3SO_3]$ and $\alpha$ is the ratio between the mass of the top phase and the total mass of the mixture.

Each tie-line length (TLL) was determined according to eqn (7):

$$TLL = \sqrt{([CH]_T - [CH]_B)^2 + ([IL]_T - [IL]_B)^2} \quad (7)$$

**Partitioning of biomolecules.** A mixture in the biphasic region was selected (≈40 wt% of $[C_4mim][CF_3SO_3]$ and ≈25 wt% of each carbohydrate) for evaluating the biomolecules partitioning and preferential affinity for each of the two aqueous phases. The mass fraction compositions of each component used in the partitioning studies are presented in Table 2. Aqueous solutions, with a concentration of approximately 0.78 g $dm^{-3}$ ($3.8\times10^{-3}$ mol $dm^{-3}$) for L-tryptophan, 0.15 g $dm^{-3}$ ($0.28\times10^{-3}$ mol $dm^{-3}$) for β-carotene and 5.0 g $dm^{-3}$ ($25\times10^{-3}$ mol $dm^{-3}$) for caffeine, were used in the water content composition at the ternary composition selected. All these aqueous solutions can be considered at infinite dilution and completely solvated in aqueous media avoiding thus specific interactions between biomolecules. After complete dissolution of all components in the mixture, by gentle stirring, the mixture was left to equilibrate for 12 h (a time period established in previous optimizing experiments), at 298.15 (±0.01) K, to achieve the complete partitioning of each biomolecule between the two aqueous phases. The temperature was maintained with a water bath, Julabo F34. Due care was taken with β-carotene, which suffers isomerisation on exposure to light, maintaining all the glass material covered by aluminium foil during all the partitioning process. The biomolecules quantification, in both phases, was carried out using an UV-Vis spectrophotometer, SHMADZU UV-1700, at wavelengths of 279 nm, 512 nm and 274 nm corresponding to L-tryptophan, β-carotene and caffeine, respectively. Calibration curves for each solute were established at the respective maximum absorption peaks. Possible interferences of both the saccharides and the ionic liquid with the analytical method were taken into account and found to be of no significance at the dilutions carried out for quantification. Three samples of each aqueous phase were quantified and the corresponding standard deviations determined.

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