CRITICAL REVIEW

Aqueous biphasic systems: a boost brought about by using ionic liquids†

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During the past decade, ionic-liquid-based Aqueous Biphasic Systems (ABS) have been the focus of a significant amount of research. Based on a compilation and analysis of the data hitherto reported, this *critical review* provides a judicious assessment of the available literature on the subject. We evaluate the quality of the data and establish the main drawbacks found in the literature. We discuss the main issues which govern the phase behaviour of ionic-liquid-based ABS, and we highlight future challenges to the field. In particular, the effect of the ionic liquid structure and the various types of salting-out agents (inorganic or organic salts, amino acids and carbohydrates) on the phase equilibria of ABS is discussed, as well as the influence of secondary parameters such as temperature and pH. More recent approaches using ionic liquids as additives or as replacements for common salts in polymer-based ABS are also presented and discussed to emphasize the expanding number of aqueous two-phase systems that can actually be obtained. Finally, we address two of the main applications of ionic liquid-based ABS: extraction of biomolecules and other added-value compounds, and their use as alternative approaches for removing and recovering ionic liquids from aqueous media.

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1. Introduction

In the mid-1950s, Albertsson proposed the use of aqueous biphasic systems (ABS) as an alternative to traditional liquid–liquid extraction techniques, which commonly made use of volatile organic solvents.[1] ABS consist of two immiscible aqueous-rich phases based on polymer–polymer, polymer–salt or salt–salt combinations. Although both solutes are water-soluble, they separate into two coexisting phases above a given concentration: one of the aqueous phases will be enriched in one of the solutes while in the other phase there is prevalence for the second polymer or salt.

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Since ABS are mainly composed of water they were immediately recognized as biocompatible media for cells, cell organelles and biologically active substances, and have been widely used for the recovery and purification of distinct (bio)molecules such as proteins and nucleic acids.[1,2] Conventional polymer-based ABS have been largely exploited since the 1980's and mainly consist of mixtures of two incompatible polymers or a polymer and a salting-out inducing salt. In 2003, Rogers and co-workers[3] reported the pioneering research pointing to the possible creation of ABS by the addition of inorganic salts to aqueous solutions of ionic liquids. These ABS were envisaged as new alternatives for the recycling and concentration of ionic liquids from aqueous solutions, for carrying out metathesis reactions in the formation of new ionic liquids, and as new separation techniques.[3] Since then, considerable effort has been directed towards the use of ionic liquids as feasible alternatives to polymer-rich phases,[3–59] and more recently, to the salt-rich phases.[60–64]

Ionic liquids (ILs) are salts that, in contrast to common electrolytes, are liquid at low temperatures. Due to their ionic nature, they present two outstanding properties: negligible volatility and non-flammability.[65–67] These two characteristics have contributed to their common epithet as "green solvents",

and as a result, they have been viewed as alternative replacements for the volatile and hazardous organic solvents presently used in a wide range of applications. Nevertheless, one of the main advantages of the applicability of ionic liquids in the formation of ABS is the ability to tailor their polarities and affinities by a proper manipulation of the cation/anion design and their combinations. This aspect is indeed a major benefit of ionic-liquid-based ABS given the difficulty of overcoming the limited polarity range of polymer-based ABS. Polymer-based systems usually display two hydrophobic phases and the difference in polarities depends essentially on the amount of water in each phase. On the other hand, polymer–salt ABS have a hydrophobic phase constituted by the polymer and a hydrophilic (and more ionic) phase, typically formed by high charge-density salts. The restricted difference in polarities between the two phases prevents a vast use of polymer-based ABS for extraction purposes. By virtue of their tunability, ionic liquids can "ideally" cover the whole hydrophilicity–hydrophobicity range.

The physical properties of the phase forming systems at various concentrations and temperatures are also indispensable requirements for the design and scale up of a wide range of separation processes. In this context, the replacement of polymers by ionic liquids also leads to other benefits since most of the polymer-based ABS are highly viscous and display slow phase separation – drawbacks generally avoided with ionic-liquid-based ABS. Fig. 1 illustrates the macroscopic appearance of the coexisting phases in a given ionic-liquid-based ABS.

Density and viscosity measurements of both phases for selected ionic-liquid-based ABS have been reported.[17,31,34,68] The systems evaluated are made up of inorganic salts and ionic liquids (phosphonium- and imidazolium-based)[31,34,68] and of carbohydrates and ionic liquids.[17] No significant differences in density values between ionic-liquid-based ABS and typical polymer-based systems have been observed. However, phosphonium-based phases are far less viscous (4–11 mPa s) than typical polymer-rich phases ($\approx$ 40 mPa s) at close mass fraction compositions.[31] In addition, imidazolium-based ABS present even lower viscosity values than their phosphonium-based counterparts.[68] Even for systems composed of carbohydrates and ionic liquids, the viscosities were found to be similar to those displayed by systems composed of inorganic salts

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Fig. 1 Macroscopic appearance of given ABS composed of an ionic liquid and an inorganic salt. Chloroanilic acid was added to colour (preferentially) the ionic-liquid-rich phase.

and ionic liquids, and substantially lower than those of typical polymer–salt or polymer–polysaccharide ABS.[17] The low viscosity of ionic-liquid-based ABS thus favours the mass transfer during extraction, as well as the handling of the phases in scaled-up processes.

Only ionic liquids miscible with water near room temperature can be considered for the formation of ionic-liquid-based ABS since only these are able to form two aqueous-rich phases. When dealing with hydrophobic ionic liquids, two phases already exist before the addition of any salt, and one of the phases is far from being aqueous-rich due to the low solubility of these ionic liquids in water. Moreover, the number of hydrophobic ionic liquids is limited to few cation–anion combinations, and most of them contain fluorinated ions, which can be expensive, unstable,[69] and less environmentally benign. In this context, there are a vast number of hydrophilic ionic liquids to choose from, while their environmental impact can be controlled in a more versatile manner. It is already well accepted that the toxicity of ionic liquids mainly depends on their hydrophobicity,[70,71] and it is useful to mention that most of the hydrophilic ionic liquids used to prepare ABS present low toxicities.[72] Still, one should be aware that the widespread application of ionic-liquid-based ABS will inevitably result in the loss of ionic liquids through aqueous effluents into water ecosystems; thus, more eco-friendly and biocompatible ionic liquids and salting-out species should be searched out in a near future. In fact, choline- and amino-acid-based ionic liquids have not yet been studied as ABS phase promoters and certainly suggest a path towards reducing the toxicity of the ionic liquid. On the other hand, the high charge density salts used as salting-out agents should also be replaced by more biocompatible species such as biodegradable organic salts, carbohydrates, polymers or amino acids.

2. Phase diagrams of ionic-liquid-based Aqueous Biphasic Systems

In general, ABS are ternary systems mainly composed of water and other two solute combinations. The triangular phase diagram in Fig. 2 conveniently represents the ternary composition of aqueous biphasic systems. The plotted graph represents only the top tip of the whole triangular diagram, stressing the fact that
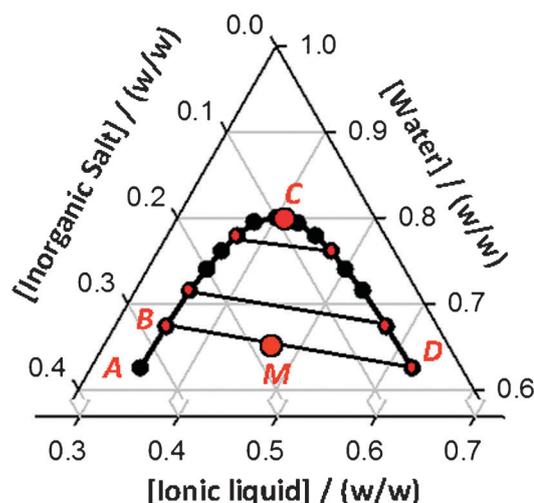

Fig. 2 Triangular phase diagram for a hypothetical system composed of ionic liquid + inorganic salt + water (weight fraction units).

ABS generally consist of two moderately diluted aqueous solutions.

All the mixtures with compositions below the coexistence binodal curve (A–B–C–D) undergo liquid–liquid demixing, while those above the line fit into the homogeneous and monophasic region. For a given total mixture (M) composition under the binodal solubility curve, the mixture suffers phase separation and forms two coexisting phases. For such mixtures of total content M, the compositions of each phase are represented by the points B and D, which are the end-points (nodes) of a specific tie-line (TL). The tie-line length (TLL) is a numerical indicator of the composition difference between the two phases and is generally used to correlate trends in the partitioning of solutes between both phases. Mixtures with total compositions along a specific tie-line have different mass or volume ratios from those of the two coexisting phases; yet, the composition of each phase remains the same. The critical point of the ternary system is Point C, where the two binodal nodes meet. Here, the compositions of the two would be coexisting phases become equal, and the biphasic system ceases to exist.

In the literature, most of the ABS ternary phase diagrams are depicted in an orthogonal representation, so that water concentration is omitted (pure water becomes the origin of the orthogonal axes). Fig. 3 illustrates such a representation. As in Fig. 2, the binodal curve, the total composition of the mixture, the composition of each phase and the critical point are defined as M, D, B and C, respectively. In this example, total mixture compositions above the binodal curve fall into the biphasic regime, whereas mixture compositions below the solubility curve are homogeneous. The vertical axis is commonly used for the solute that is enriched in the top (less dense) phase.

As in the case of the triangular phase diagrams, the orthogonal ternary phase diagrams depict concentration ranges corresponding to moderately diluted aqueous solutions. If the saturation limit of one of the solutes is reached, then other lines corresponding to the precipitation of that solute will have to be taken into account. These (less diluted) regions of the diagram may present three-phase regions that are outside of the scope of this work.
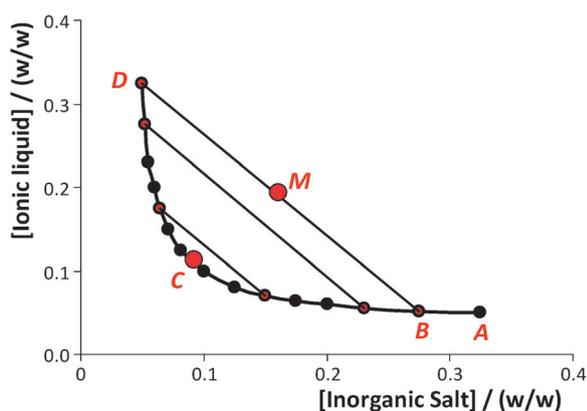
Fig. 3 Orthogonal ternary phase diagram for a hypothetical system composed of polymer + inorganic salt + water (weight fraction units).

Given all their potential features (highlighted before), several studies have focused on the determination of the phase diagrams of ionic-liquid-based ABS. A thorough survey of the literature from 2003 to September of 2011 yielded 62 articles in which the
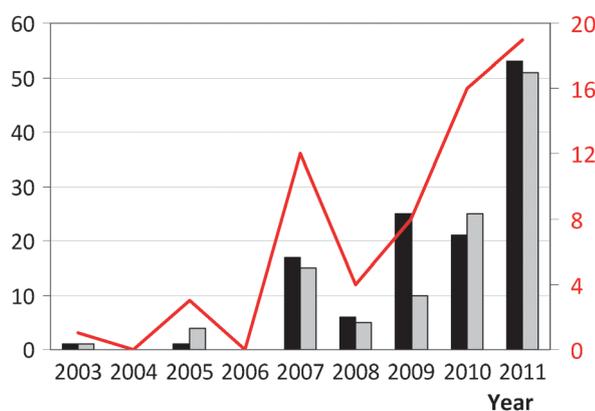

Fig. 4 Phase diagram data for ionic-liquid-based ABS from 2003 to 2011: number of articles *per* year (red line/scale); number of distinct ionic liquids studied *per* year (black bars); and number of distinct salting-out species evaluated *per* year (grey bars).

phase diagrams of ionic-liquid-based ABS were determined[3–64] – Fig. 4. It should be remarked that only manuscripts reporting phase diagrams are considered in this section. Those that only use ionic-liquid-based ABS for extraction purposes are discussed below.

After the initial work of Rogers and co-workers[3] in 2003, three articles were published in 2005; yet, only in 2007 did a greater number of manuscripts (12) appear in the literature. Since 2009 the number of manuscripts has increased rapidly and, more significantly, a large number of both ionic liquids and salting-out agents have been evaluated.

Most works studied ABS composed of ionic liquids and conventional salts (47 articles), whilst a few investigated the substitution of the high charge density salts by more benign species, such as amino acids (2 articles), carbohydrates (8 articles), and polymers (5 articles).[3–64]

The name and acronym of each ionic liquid cation and anion which were employed in the determination of the phase diagrams of ABS appear in Table 1.

Fig. 5 depicts the usage frequency of the distinct cation–anion combinations in the ABS formation *per* article.

Fig. 5 shows that, within the scope of this review, 1-alkyl-3-methylimidazolium-based ionic liquids have received by far the most scrutiny. In this class, $[C_4C_1im]Cl$, $[C_4C_1im]Br$ and $[C_4C_1im][BF_4]$ have been the most commonly investigated. Moreover, only for imidazolium-based ionic liquids was the introduction of functionalized groups evaluated, as was the cation side alkyl chain length. Regarding the ionic liquid anions, mostly halogen-, sulphate-, and tetrafluoroborate-based ionic liquids have been investigated. Although $[C_4C_1im][BF_4]$ was one of the most commonly studied fluids in the formation of ABS, it should be stressed that $[BF_4]$-based compounds are not water stable. $[BF_4]$-based ionic liquids suffer hydrolysis in contact with water releasing fluoridric acid, even at room temperature.[69] Although these phase diagrams can be accurately determined due to the short amount of time required to measure them, the user must take into account the fact that these systems are not good candidates for extraction or recycling purposes when longer periods of contact with water are required.

Aiming at defining general trends in the phase behaviour of ionic-liquid-based ABS, as well as to identify doubtful data,

Table 1 Name and respective acronym of the cation–anion combinations in ionic liquids

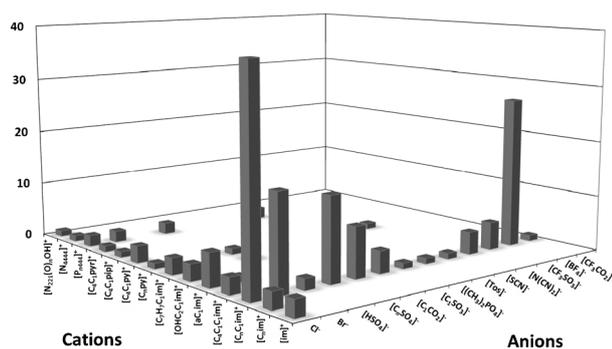| Cation		Anion	
Name	Acronym	Name	Acronym
Imidazolium	$[im]^+$	Chloride	Cl^-
1-Alkylimidazolium	$[C_nim]^+$	Bromide	Br^-
1-Alkyl-3-methylimidazolium	$[C_nC_1im]^+$	Hydrogen sulfate	$[HSO_4]^-$
1-Butyl-2,3-dimethylimidazolium	$[C_4C_1C_1im]^+$	Alkylsulfate	$[C_nSO_4]^-$
1-Allyl-3-methylimidazolium	$[aC_1im]^+$	Acetate	$[C_1CO_2]^-$
1-Hydroxyethyl-3-methylimidazolium	$[OHC_2C_1im]^+$	Methylsulfonate	$[C_1SO_3]^-$
1-Benzyl-3-methylimidazolium	$[C_7H_7C_1im]^+$	Dimethylphosphate	$[(CH_3)_2PO_4]^-$
1-Alkylpyridinium	$[C_npy]^+$	Tosylate	$[tos]^-$
1-Butyl-2-methylpyridinium	$[C_4\text{-}2\text{-}C_1py]^+$	Thiocyanate	$[SCN]^-$
1-Butyl-3-methylpyridinium	$[C_4\text{-}3\text{-}C_1py]^+$	Dicyanamide	$[N(CN)_2]^-$
1-Butyl-4-methylpyridinium	$[C_4\text{-}4\text{-}C_1py]^+$	Triflate	$[CF_3SO_3]^-$
1-Butyl-1-methylpiperidinium	$[C_4C_1pip]^+$	Tetrafluoroborate	$[BF_4]^-$
1-Butyl-1-methylpyrrolidinium	$[C_4C_1pyr]^+$	Trifluoroacetate	$[CF_3CO_2]^-$
Alkyl(tributyl)phosphonium	$[P_{n444}]^+$		
Alkyl(triisobutyl)phosphonium	$[P_{ni(444)}]^+$		
(Tetrabutyl)ammonium	$[N_{4444}]^+$		
Ammoeng110™	$[N_{221(O)nOH}]^+$		

Fig. 5 Graphical representation of the available data on ionic-liquid-based ABS as a function of different combinations of cations and anions in ionic liquids.

we have collected all liquid–liquid phase equilibrium results available in the literature[3–64] and converted the usual reported weight fraction units to molality units. All the phase diagrams are shown in molality units to avoid inconsistencies that may result from the varying molecular weights exhibited by the ionic liquids and/or the salting-out species used. In fact, this type of representation allows for a broader analysis of the effect of individual solutes in promoting liquid–liquid demixing. In the material below, we discuss the determination of the phase diagrams and respective tie-lines as well as common correlations applied to rationalize the experimental data. Subsequent sections are grouped into major classes of salting-out agents. They present and discuss the influence of the ionic liquid chemical structure, the nature of the salting-out species, pH effects, and the influence of temperature on the liquid–liquid demixing phenomenon. All phase diagrams were determined at atmospheric pressure and studies of the pressure effect were not found in the literature.[3–64]

2.1. Determination of the phase diagrams and correlation

Concerning ionic-liquid-based ABS, the visual determination of cloud points has been used in most reports to determine the experimental phase diagrams.[3–25,27–64] One exception is that of Jiang et al.,[26] who examined the turbidity of the systems making use of a spectrophotometer at 600 nm. Briefly, the experimental procedure consisted of two major steps: (i) drop-wise addition of an aqueous solution of the salting-out agent to an ionic liquid aqueous solution (or pure ionic liquid) until the detection of a cloudy and biphasic solution, or *vice versa*; (ii) drop-wise addition of ultra-pure water until the formation of a clear and limpid solution corresponding to the monophasic regime. The systems' compositions were always determined by weight quantification.

Rogers and co-workers[3] were the first to apply a mathematical description to correlate the experimental coexisting curve which was initially proposed by Merchuk et al.[73] to describe polymer-based systems. The binodal curves were fitted to a three-parameter equation according to,

$$Y = A \exp[(B \times X^{0.5}) - (C \times X^3)] \qquad (1)$$

where Y and X are the mass fraction percentages of the ionic liquid and the major salting-out agent, respectively. A, B and C are fitting parameters obtained by least squares regression.

Most researchers applied eqn (1) to describe the experimental binodal data since, in general, it provides a good description of the coexistence curves of ionic-liquid-based ABS.[3–5,9–14,17,18,21,22,27,31,34,35,40,43,45,46,48,50–55,60,61,64] On the basis of the correlation coefficients obtained from the fitting, eqn (1) was found to satisfactorily reproduce the experimental binodal boundaries. An example of the application of eqn (1) to correlate the experimental data points is depicted in Fig. 6.

Zafarani-Moattar and Hamzehzadeh[55,56] and Wang et al.[48] further expressed the three fitting parameters of eqn (1) as a function of temperature in a linear form with $(T - T_0)$ K as a variable, and as follows,

$$A = A^0 + A^1(T - T_0) \qquad (2)$$

$$B = B^0 + B^1(T - T_0) \qquad (3)$$

$$C = C^0 + C^1(T - T_0) \qquad (4)$$

where T_0 was assumed as the reference temperature, 273.15 K, and A^0, A^1, B^0, B^1, C^0 and C^1 are independent temperature-adjustable parameters.

Subsequently, Zafarani-Moattar and Hamzehzadeh[57] expressed the dependence of the coefficients A, B and C of eqn (1) as a function of the pH of the medium as follows,

$$A = A^0 + \frac{A^1}{\text{pH}} \qquad (5)$$

$$B = B^0 + \frac{B^1}{\text{pH}} \qquad (6)$$

$$C = C^0 + \frac{C^1}{\text{pH}} \qquad (7)$$

where A^0, A^1, B^0, B^1, C^0 and C^1 are pH-independent adjustable parameters.

Zafarani-Moattar and Hamzehzadeh[55–57] found that eqn (1)–(7) satisfactorily correlate the binodal experimental curves and their dependence on temperature and pH.

Deive et al.[10] applied eqn (1) to correlate their experimental data points and also varied the exponents 0.5 and 3 to optimise the fittings. In general, the five-parameter fitting yielded better correlations with smaller associated standard deviations.
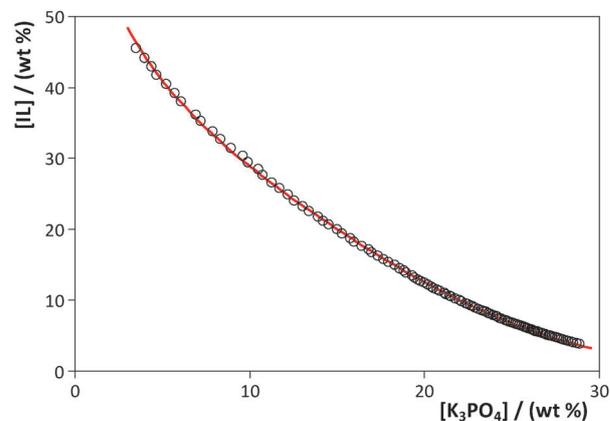

Fig. 6 Ternary phase diagram composed of [C$_7$C$_1$im]Cl + K$_3$PO$_4$ + water at 298 K.[18] The symbols represent the experimental data and the line corresponds to the fitting by eqn (1).

The binodal experimental curves have also been fitted by a logarithmic expression[20,37,63] described by,

$$Y = A\ln(X + C) + B \qquad (8)$$

where Y and X are the mass fraction percentages of the ionic liquid and of the salting-out agent, respectively. A, B and C are fitting parameters obtained by non-linear regression. A previous estimation of these values is usually necessary, and in general, a plot of $\partial Y/\partial X$ against X gives a straight line and the initial values of A and C are calculated. Once the parameters A and C are determined, B is further estimated from eqn (8).

In addition, Li et al.[30] applied the following equation (9), while Yu et al.[53] fitted their experimental phase diagrams based on eqn (10), both of which presenting four adjustable constants,

$$Y = A + BX^{0.5} + CX + DX^2 \qquad (9)$$

$$Y = \exp(A + BX^{0.5} + CX + DX^2) \qquad (10)$$

where, again, Y and X are the weight fraction percentages of the ionic liquid and of the salting-out species, and A, B, C and D are fitted and constant parameters.

It should be stressed that more complex equations have even been used, such as those proposed by Chen and co-workers[6,8,59] with 5 fitted coefficients.

In summary, all the proposed equations closely correlate the experimental data on ionic-liquid-based ABS and their selection largely depended on the aim of the piece of research.

2.2.   Determination of tie-lines and their correlation

Merchuk et al.[73] initially proposed a gravimetric method to determine the TLs of polymer-based systems using the fitting of the binodal data by eqn (1) coupled to a mass balance relationship. The compositions of the top and bottom phases and the overall system compositions are determined by the lever-arm rule. Rogers and co-workers[3] successfully applied this approach to ionic-liquid-based ABS. Each TL is determined according to the solution of the following system of four unknown constants,

$$Y_T = A \exp[(B \times X_T^{0.5}) - (C \times X_T^3)] \qquad (11)$$

$$Y_B = A \exp[(B \times X_B^{0.5}) - (C \times X_B^3)] \qquad (12)$$

$$Y_T = \frac{Y_M}{\alpha} - \frac{1 - \alpha}{\alpha} \times Y_B \qquad (13)$$

$$X_T = \frac{X_M}{\alpha} - \frac{1 - \alpha}{\alpha} \times X_B \qquad (14)$$

In eqn (11)–(14), T, B, and M, designate the top phase, the bottom phase and the mixture, respectively; X and Y represent the weight fraction percentage of the salting-out agent and of the ionic liquid, respectively; and α is the ratio between the mass of the top phase and the total mass of the mixture. A, B, and C are the fitted constants obtained by the application of eqn (1). In fact, most authors[4,5,9–12,14,17,18,20,31,34,35,40,43,45,46,61] used this mathematical approach to determine the TLs corresponding to each phase diagram. A general example appears in Fig. 7.

Other researchers[6–8,13,21–23,27,37,48,53–57,59,63,64] have determined the TLs, i.e., the composition of each component at the coexisting phases by analytical methods. After a careful separation of the

equilibrated phases, Zafarani-Moattar and Hamzehzadeh[54–57] determined the concentration of potassium or sodium salts by flame photometry, the ionic liquid concentration by refractive index measurements (or by nitrogen elemental analysis to validate the proposed technique), and the water content by Karl–Fischer titration. In the particular case of polymer–ionic-liquid-based ABS, Zafarani-Moattar et al.[64] and Wu et al.[63] quantified the ionic liquid at the coexisting phases by UV-Vis spectrometry and/or the polymer and water contents by refractive index measurements and Karl–Fischer titration, respectively. For the determination of TLs in carbohydrate-based ABS, Chen et al.[6–8] and Zhang et al.[59] applied high performance liquid chromatography (HPLC) to determine the ionic liquid composition and Karl–Fischer titration to quantify the water content, whilst the amount of carbohydrates was determined by calculation and based on a complete mass balance. On the other hand, Han et al.[21] identified the concentration of conventional salts at each phase by refractive index measurements whereas the amount of ionic liquid was directly taken from the fitting using eqn (1). In addition, the same research group[22,23] determined the amount of salt in the top and bottom phases by density measurements and the amount of ionic liquid was also taken from the fitting by eqn (1). Pei et al.[37] quantified the ionic liquids by UV-Vis spectrometry and the water by a drying procedure under vacuum until a constant weight was obtained. Finally, three works[27,48,53] have determined the mass fraction of ionic liquid by UV-Vis spectrometry and the amount of salts by acid–base titration. In all cases the water content was determined by the mass balance equation.[27,48,53]

The reliability of the tie-line compositions has also been ascertained by several authors,[6,8,10,12,13,20,22,27,34,37,53,57,59] with the Othmer–Tobias and Bancroft equations,[75,76] and as described below,

$$\frac{1 - Y_T}{Y_T} = k_1 \left(\frac{1 - X_B}{X_B}\right)^n \qquad (15)$$

$$\frac{Z_B}{X_{BT}} = k_2 \left(\frac{Z_T}{Y_T}\right)^r \qquad (16)$$

where X, Y and Z represent the weight fraction percentage of the salting-out agent, the ionic liquid, and water, respectively,
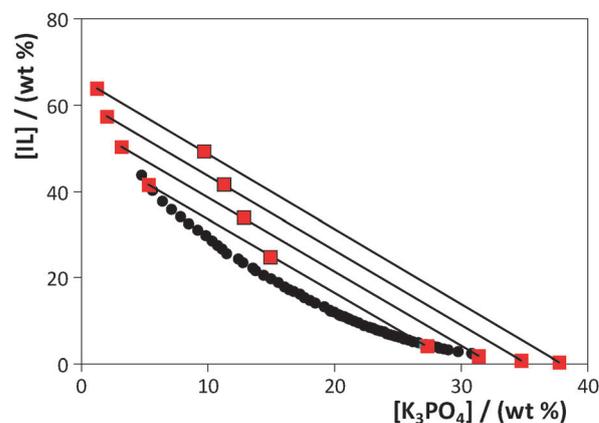

Fig. 7   Ternary phase diagram composed of $[C_7H_7C_1im]Cl + K_3PO_4 +$ water at 298 K: ●, experimental binodal data; ■, tie-line data.[35,74]
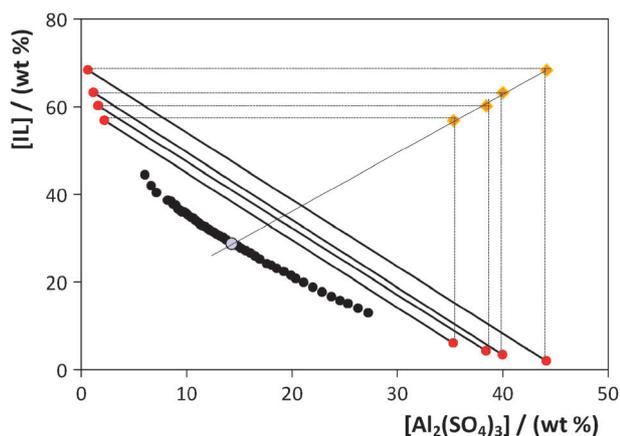
Fig. 8 The binodal curve of the ternary system composed of $[C_2C_1im][CF_3SO_3]$ + $Al_2(SO_4)_3$ + water at 298 K with the identification of the critical point.[34]

in the top (T) and bottom (B) phases. k_1, k_2, n and r are adjustable parameters.

Eqn (15) and (16) are linearized by taking the logarithm on both sides of the equations to determine the fitting parameters. A proper fitting by linearization indicates an acceptable consistency in the TL results. Based on the correlation coefficients and standard deviations obtained, these equations were found to satisfactorily describe the tie-line data.[6,8,10,12,13,20,22,27,34,37,53,57,59]

The tie-line length (TLL) and slope of the TLs (S_{TL}) have also been frequently addressed[6–14,17,18,21,22,27,30,31,34,35,37,40,43,45,46,48,53,55,61,63,64] and are described by,

$$TLL = \sqrt{(X_T - X_B)^2 + (Y_T - Y_B)^2} \qquad (17)$$

$$S_{TL} = \frac{Y_T - Y_B}{X_T - X_B} \qquad (18)$$

where T, B, and M designate the top phase, the bottom phase and the mixture, respectively, and X and Y represent the equilibrium compositions of the salting-out agent and of the ionic liquid. These single values were found useful for verifying trends in the partition coefficients or recovery efficiencies discussed below.

The location of the critical point has, nevertheless, been addressed by few authors.[34,53,59] The critical point of the ternary systems was estimated by extrapolation from the TLs compositions applying the following linear equation,

$$Y = f + gX \qquad (19)$$

where Y and X are the ionic liquid and salting-out agent compositions, and f and g are fitting parameters. As an example, the locus of the estimated critical point (C) for the $[C_2mim][CF_3SO_3]$ + $Al_2(SO_4)_3$ + water system, along with the described procedure, appears in Fig. 8.

2.3. Ionic liquids + conventional salts + water

Rogers and co-workers[3] were the first to show that a mixture of an aqueous solution of $[C_4C_1im]Cl$ (a hydrophilic ionic liquid) and a concentrated aqueous solution of K_3PO_4 can produce an aqueous biphasic system formed by an upper ionic-liquid-rich phase and a lower inorganic-salt-rich phase.

Since all the phase-forming components are ionic, in contrast to the typical polymer-based ABS where the ion exchange cannot occur, the different ions in ionic-liquid-based ABS are expected to distribute themselves between the coexisting phases. In this context, Bridges *et al.*[4] have shown that although there is migration of ions in ionic-liquid-based ABS, the electroneutrality of the system and of each phase is always maintained. Moreover, the authors[4] have concluded that the interpretation of the results based on whole salt concentrations rather than on individual ion concentrations yields, from an operational perspective, a sufficiently precise speciation of the ions present along any given tie-line.

The cornerstone of the formation of ABS comprising ionic liquids and conventional salts is the salting-out effect which is mainly a result of the creation of water–ion complexes that cause the dehydration of the solute and the increase in the surface tension of the cavity in the aqueous media.[77–79] Freire *et al.*[77–79] recently demonstrated the role of ion-specific effects in the solvation phenomenon associated to the mixing of ionic liquids in aqueous media. Salting-out inducing ions act mainly through entropic effects resulting from the formation of water–ion complexes whereas salting-in inducing ions directly interact with the hydrophobic moieties of the ionic liquid.[77–79] The salting-in/-out effects are thus dominated by the ion–ion *versus* ion–water interactions and not by underlying water-structure modifications, as classically accepted.[77–79] The addition of high charge-density salts to aqueous solutions of ionic liquids leads to liquid–liquid demixing due to a preferential hydration of the high charge density salt over the ionic liquid, leading therefore to the salting-out (exclusion) of the ionic liquid to the ionic-liquid-rich phase. Ionic liquids are generally made up of low-symmetry charge-delocalized ions only capable of weak directional intermolecular interactions (and hence weakly hydrated as compared to the common salting-out inducing salts).

Most (47) of the published articles on ionic liquid-based ABS included conventional salts.[3–5,9–13,15,16,18–49,53–57] Table 2 reports the combinations of ionic liquids and distinct salts for the formation of ABS studied hitherto.

2.3.1. Influence of the ionic liquid. Twenty-two of the 47 articles concerning ABS composed of ionic liquids and salts have addressed several ionic liquids and compared the phase diagrams in order to infer on the ionic liquid effect.[4,5,9–12,18,20,30–35,37,38,41,43,45,46,53,56]

Despite the immense versatility inherent to the cation–anion permutations in ionic liquids, most of the phase diagrams of ionic-liquid-based ABS have made use of imidazolium-based compounds,[4,5,9–12,18,20,30–35,37,38,41,43,45,46,53,56] whereas relatively few studies have used phosphonium-, ammonium-, pyridinium-, piperidinium-, or pyrrolidinium-based ionic liquids.[4,9,10,31,34,44,45] The first investigation of the effect of the cation core on the formation of ABS was conducted by Bridges *et al.*[4] in 2007. The authors[4] evaluated the phase behaviour of imidazolium-, pyridinium-, and quaternary ammonium-, and phosphonium-based chloride salts salted-out by K_3PO_4, K_2HPO_4, K_2CO_3, KOH and $(NH_4)_2SO_4$. Fig. 9 compares the phase behaviour of four ionic liquids with K_3PO_4 (the strongest salting-out agent evaluated).

Table 2 ABS composed of ionic liquids and inorganic/organic salts reported in the literature

Ionic liquid	Conventional salt
[im]Cl	K_3PO_4 [35]
[$C_n$im]Cl	K_3PO_4 [35]
[$C_nC_1$im]Cl	$K_3C_6H_5O_7$ [56], K_3PO_4 [3,4,12,18,24,28,33,35,37,43] K_2HPO_4 [4,5,16,24,28,29,37,45] K_2HPO_4–KH_2PO_4 [45,46] K_2CO_3 [4,12,24,28,29,37] KOH [4,24,28,29,37] Na_2CO_3 [10] Na_2SO_4 [9]
[$C_4C_1C_1$im]Cl	K_3PO_4 [4,18] K_2HPO_4 [4] K_2CO_3 [4] KOH [4]
[aC_1im]Cl	K_3PO_4 [13,35] K_2HPO_4 [13] K_2CO_3 [13]
[OHC_2C_1im]Cl	K_3PO_4 [35]
[$C_7H_7C_1$im]Cl	K_3PO_4 [35] K_2HPO_4–KH_2PO_4 [45] Na_2CO_3 [10] Na_2SO_4 [9]
[C_4py]Cl	K_3PO_4 [4]
[C_4-2-C_1py]Cl	K_2HPO_4–KH_2PO_4 [45]
[C_4-3-C_1py]Cl	K_2HPO_4–KH_2PO_4 [45]
[C_4-4-C_1py]Cl	K_2HPO_4–KH_2PO_4 [45]
[C_4C_1pip]Cl	K_2HPO_4–KH_2PO_4 [45]
[C_4C_1pyr]Cl	K_2HPO_4–KH_2PO_4 [45]
[P_{4444}]Cl	$Al_2(SO_4)_3$ [34]
[$C_nC_1$im]Br	$K_3C_6H_5O_7$ [32,55] $K_3C_6H_5O_7$–$C_6H_8O_7$ [57] K_3PO_4 [37,38,43,54] K_2HPO_4 [37,38,54] K_2CO_3 [37,38] KOH [37,38] $Na_3C_6H_5O_7$ [39,56] Na_2HPO_4 [25] Na_2SO_4 [9]
[C_4C_1im][HSO_4]	K_3PO_4 [43]
[$C_nC_1$im][C_1SO_4]	K_3PO_4 [33,43] K_2HPO_4–KH_2PO_4 [46] Na_2CO_3 [10] Na_2SO_4 [9]
[$C_7H_7C_1$im][C_1SO_4]	Na_2SO_4 [10]
[C_1py][C_1SO_4]	Na_2CO_3 [10]
[$C_nC_1$im][C_2SO_4]	K_3PO_4 [11,33,43] K_2HPO_4–KH_2PO_4 [46] K_2CO_3 [11] Na_2CO_3 [11] Na_2SO_4 [9] $(NH_4)_2SO_4$ [11]
[$C_7H_7C_1$im][C_2SO_4]	Na_2SO_4 [9] $Al_2(SO_4)_3$ [34]
[$C_nC_1$im][C_4SO_4]	K_3PO_4 [11] K_2CO_3 [11] Na_2CO_3 [11] $(NH_4)_2SO_4$ [11]
[$C_nC_1$im][C_6SO_4]	K_3PO_4 [11] K_2CO_3 [11] Na_2CO_3 [11] $(NH_4)_2SO_4$ [11]
[$C_nC_1$im][C_8SO_4]	K_3PO_4 [11] K_2CO_3 [11] Na_2CO_3 [11] Na_2SO_4 [9] $(NH_4)_2SO_4$ [11]
[$C_nC_1$im][C_1CO_2]	K_3PO_4 [30,41,43] K_2HPO_4 [30,41] K_2HPO_4–KH_2PO_4 [46] K_2CO_3 [30]
[$C_nC_1$im][C_1SO_3]	K_3PO_4 [43] K_2HPO_4–KH_2PO_4 [46]
[C_4C_1im][tos]	K_2HPO_4–KH_2PO_4 [46] Na_2SO_4 [9] $Al_2(SO_4)_3$ [34]
[C_4C_1im][SCN]	K_2HPO_4–KH_2PO_4 [46] Na_2SO_4 [9] $Al_2(SO_4)_3$ [34]
[C_4C_1im][$N(CN)_2$]	K_3PO_4 [36,43] K_2HPO_4 [36] K_2HPO_4–KH_2PO_4 [46] Na_2SO_4 [9] $Al_2(SO_4)_3$ [34]
[C_8py][$N(CN)_2$]	K_2HPO_4–KH_2PO_4 [44] Na_2SO_4 [9] $Al_2(SO_4)_3$ [34] $AlK(SO_4)_2$ [34]
[$C_nC_1$im][CF_3SO_3]	K_3PO_4 [43] K_2HPO_4–KH_2PO_4 [46] $Na_3C_6H_5O_7$ [40] Na_3PO_4 [40] Na_2HPO_4 [40] NaH_2PO_4 [40] NaK-$C_4H_4O_6$ [40] Na_2CO_3 [40] Na_2SO_4 [9,40] Na_2SO_3 [40] $NaCH_3CO_2$ [40] $NaHCO_3$ [40] $NaHSO_4$ [40] NaCl [40] NaOH [40] KCH_3CO_2 [40] KCl [40] $Ca(CH_3CO_2)_2$ [40] $CaCl_2$ [40] $Mg(CH_3CO_2)_2$ [40] $MgCl_2$ [40] $Al_2(SO_4)_3$ [34]
[$C_nC_1$im][BF_4]	$Na_3C_6H_5O_7$ [21–23] Na_3PO_4 [27,42,53] Na_2HPO_4 [19] NaH_2PO_4 [19,26,27,47,48,53] Na_2CO_3 [19,27,47,48] Na_2SO_4 [19,27,42,47] Na_2SO_3 [48,53] $Na_2CH_3CO_2$ [21,23] $Na_2C_4H_4O_4$ [23] $Na_2C_4H_4O_6$ [27,55] NaCl [4,19,42] $(NH_4)_2C_4H_4O_6$ [20] $(NH_4)_2SO_4$ [48] $(NH_4)_3C_6H_5O_7$ [22,23] SDBS [49]
[C_4C_1im][CF_3CO_2]	K_3PO_4 [43] Na_2SO_4 [9]
[P_{4444}]Br	K_3PO_4 [31] $Al_2(SO_4)_3$ [34] $AlK(SO_4)_2$ [34]
[P_{1444}][C_1SO_4]	K_3PO_4 [31] $Al_2(SO_4)_3$ [34]
[P_{1444}][tos]	K_3PO_4 [31] $Al_2(SO_4)_3$ [34]
[N_{4444}]Cl	$(NH_4)_2SO_4$ [4]
[$N_{221(O)n OH}$]Cl	K_2HPO_4–KH_2PO_4 [15]

The closer to the axis origin a binodal curve is, the higher the ionic liquid hydrophobicity (or salting-in behaviour), i.e., the lower the density charge of the ionic liquid ions and the higher their ability to phase split. The overall order of the ionic liquids' ability to undergo liquid–liquid demixing in the presence of potassium phosphate concentrated aqueous solutions is as follows: [P_{4444}Cl] > [N_{4444}]Cl > [C_4py]Cl > [C_4C_1im]Cl. The two quaternary salts ([P_{4444}]Cl and [N_{4444}]Cl) have highly shielded charges, located mostly on the heteroatom surrounded by four alkyl chains, thus leading to a higher tendency toward salting-out from aqueous media.[4] The pyridinium-based ionic liquid has less shielding compared to that of the quaternary ammonium salt, although the charge is also mostly located on the nitrogen atom. Finally, the imidazolium-based ionic liquid has a cation with a charge more evenly dispersed along the entire heterocycle, and a greater ability to interact with water via hydrogen-bonding. Similar results for other salting-out agents like K_2HPO_4 or K_2CO_3 have also been reported.[4] Subsequently, Louros et al.[31] published the ternary phase diagrams of distinct phosphonium-based ionic liquids, also using K_3PO_4 as salting-out agent, at 298 K. Again, the phosphonium-based ionic liquids are more effective in promoting ABS when compared to their imidazolium-based counterparts with similar anions. Similar results have recently been published by Neves et al.[34] Independently of the salt employed and the pH of the aqueous media, the phosphonium-based ionic liquids are always more efficacious in creating ABS.[4,31,34]

Ventura et al.[45] have assessed the influence of nitrogen-based cyclic ionic liquids (imidazolium-, pyridinium-, pyrrolidinium-, and piperidinium-based) on the formation of ABS. The inorganic salt used consists of a mixture of K_2HPO_4–KH_2PO_4 to control the pH values of the coexisting phases. The formation of ABS with aqueous solutions of a phosphate buffer for the various families of cations occurs in the following sequence: [C_4C_1pyr]Cl < [C_4C_1im]Cl < [C_4C_1pip]Cl < [C_4-3-C_1py]Cl. The graphical representation of these data is given in ESI.†

The authors[45] made use of liquid–liquid equilibria data for binary systems composed of ionic liquids and water to support their results and showed that, albeit the solubility of water in ionic liquids largely depends on the availability of electrons at the aromatic cores for privileged hydrogen-bonding, in contrast, the solubility of ionic liquids in water is mainly ruled by the molar volume of the ionic fluids. Larger cations such as pyridinium and piperidinium (6-atom heterocyclic compounds) are better at inducing ABS than smaller cations such as imidazolium and pyrrolidinium (containing 5-atom rings).
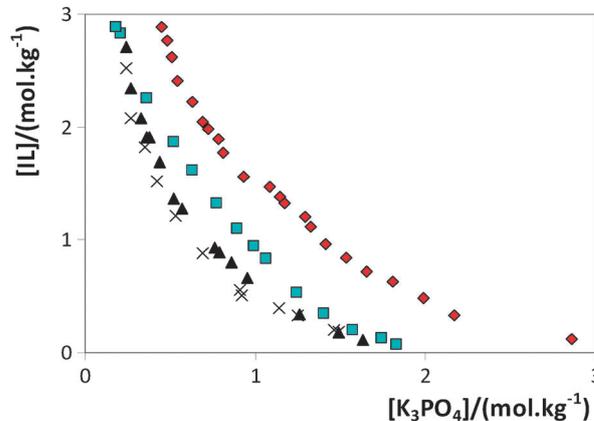

Fig. 9 Ternary phase diagrams for ABS composed of chloride-based ionic liquids + K_3PO_4 at room temperature: ♦, [C_4C_1im]Cl; ■, [C_4py]Cl; ▲, [N_{4444}]Cl; ×, [P_{4444}]Cl.[4]

In summary, considering the liquid–liquid phase behaviour of ionic liquids and water,[80,81] and supported by the results regarding the influence of the ionic liquid cation core, it seems that the formation of ionic-liquid-based ABS is dominated by steric and entropic contributions (for ionic liquids with a fixed anion and different cations). As a matter of fact, the results reported by Bridges et al.[4] for the phase diagrams of imidazolium-, pyridinium-, and quaternary ammonium- and phosphonium-based chloride salts can be interpreted using the same argument. The trend shown in Fig. 9 also closely correlates with the ionic liquid molar volume.

Few authors have investigated the influence of the nature of the ionic liquid anion towards the formation of ABS.[9,10,33,37,43,46] Najdanovic-Visak et al.[33] used a distinct approach from the remaining works[9,10,33,37,43,46] and investigated the salting-out effect produced by the addition of K_3PO_4 to aqueous solutions of sulphate- and chloride-based ionic liquids through the determination of the corresponding temperature-composition pseudo-binary phase diagrams.

In 2007, Pei et al.[37] highlighted the differences among chloride- and bromide-based ionic liquids by analysing their capacity to undergo phase splitting. However, only in 2009 was a more comprehensive analysis of the anion effect provided by Coutinho and co-workers.[43] Their results appear in Fig. 10.

The experimental phase diagrams shown in Fig. 10 reveal that the ability of the ionic liquids to form ABS increases in the order: $[C_4C_1im]Cl \approx [C_4C_1im][C_1SO_3] < [C_4C_1im]Br < [C_4C_1im][CF_3CO_2] < [C_4C_1im][N(CN)_2] < [C_4C_1im][HSO_4] < [C_4C_1im][CF_3SO_3]$. The authors[43] also determined the ternary phase diagrams for $[C_2C_1im]$-based ionic liquids, K_3PO_4, and water at 298 K, and reported the following ABS formation rank: $[C_2C_1im][C_1SO_3] < [C_2C_1im][C_1CO_2] \approx [C_2C_1im]Cl < [C_2C_1im]Br < [C_2C_1im][C_1SO_4] < [C_2C_1im][C_2SO_4] < [C_2C_1im][CF_3SO_3]$. In general, bromide-based ionic liquids present higher aptitude to form ABS than their chloride-based counterparts, and in good agreement with the data previously reported by Pei et al.[37]

The ABS studied reflect the competition between the salting-out ions and the ionic liquid ions for the formation of hydration
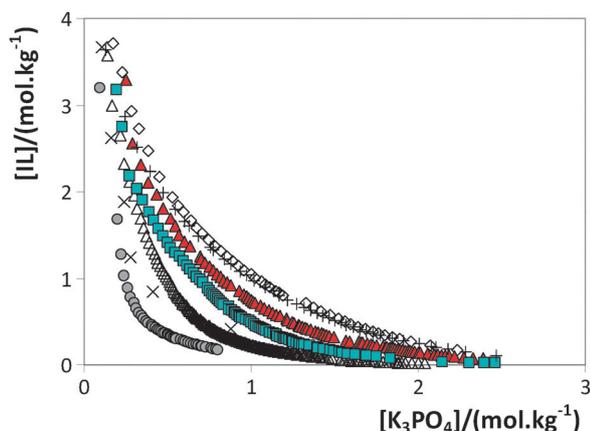
complexes, or in other words, their relative hydrogen-bond acceptor capacities. Taking this idea into account, the authors[43] showed, for the first time, that the ability of an ionic liquid anion to produce ABS closely follows the decrease in their hydrogen-bond accepting strength or electron pair donation ability. Anions with lower hydrogen bond basicity values (usually quantified using polarity scales obtained with different solvatochromic probes) present lower abilities to form coordinative bonds and to create hydration complexes, and therefore are more easily salted-out by conventional salts.[43] Nevertheless, it should be stressed that this phenomenon has not been previously observed when evaluating the effect of the ionic liquid cation (where steric contributions play the major role). Anions have a higher aptitude for hydration since they are more polarizable and present a more diffuse valence electronic configuration. Furthermore, most ionic liquid anions are characterized by the absence of long alkyl side chains which mainly contribute to steric hindrance (some recent exceptions are alkylsulfonate, alkylsulfate and carboxylate anions).

Additional works[9,46] have provided new information on the ionic liquid anions' ability to form ABS, either by studying new ionic liquid anions or by using different salts. With Na_2SO_4, the ability of the ionic liquid for phase splitting at 298 K follows the rank: $[C_4C_1im][CF_3SO_3] > [C_4C_1im][C_8SO_4] > [C_4C_1im][Tos] \approx [C_4C_1im][SCN] > [C_4C_1im][N(CN)_2] > [C_4C_1im][C_2SO_4] > [C_4C_1im][CF_3CO_2] \approx [C_4C_1im][C_1SO_4] > [C_4C_1im]Br.[9]$ Also with a phosphate buffer solution, the ability of the ionic liquid to induce liquid–liquid demixing at 298 K follows a similar order.[46] Therefore, for the ionic liquids investigated, the anions rank is mainly universal and independent of the salt used, and closely follows their decreased ability to hydrogen-bond with water (hydrogen bond basicity values).[9,43,46]

Several authors[5,9,10,12,18,20,30,32,33,35,37,38,41,45,53] have addressed the influence of the ionic liquid cation alkyl chain length on the formation of ABS. However, this trend is limited to imidazolium-based ionic liquids combined with the anions $[C_1CO_2]^-$, $[BF_4]^-$, Cl^- and Br^-. Fig. 11 shows the binodal boundaries for the ternary systems composed of $[C_{1-6}C_1im]Cl$, K_3PO_4, and water published by Neves et al.[35]

The inspection of Fig. 11 indicates that the longer the cation alkyl side chain (and thus the ionic liquid molar volume) the

Fig. 10 Ternary phase diagrams for ABS composed of $[C_4C_1im]$-based ionic liquids + K_3PO_4 at 298 K: ◇, $[C_4C_1im]Cl$; +, $[C_4C_1im][C_1SO_3]$; ▲, $[C_4C_1im]Br$; ■, $[C_4C_1im][CF_3CO_2]$; Δ, $[C_4C_1im][N(CN)_2]$; ×, $[C_4C_1im][HSO_4]$; ●, $[C_4C_1im][CF_3SO_3]$.[43]

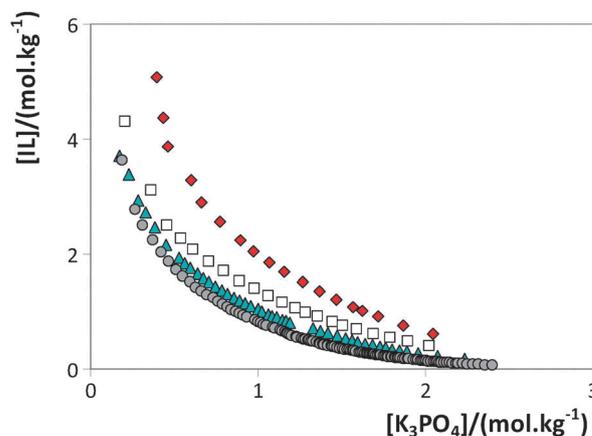**Fig. 11** Ternary phase diagrams for ABS composed of $[C_nC_1im]Cl$ ionic liquids + K_3PO_4 at 298 K: ◆, $[C_1C_1im]Cl$; □, $[C_2C_1im]Cl$; ▲, $[C_4C_1im]Cl$; ●, $[C_6C_1im]Cl$.[35]

greater is the ability of the ionic liquid to phase separate. An increase in the cation alkyl chain length leads to an increase in the fluid hydrophobicity (an intrinsic result of the aliphatic part extension), and thus to a poorer solubility of the ionic liquid in water. In fact, it has been previously shown with liquid–liquid equilibria data for binary systems constituted by ionic liquids and water[82,83] that the decrease in the ionic liquids' solubility in water with their cation size increase is driven by a decrease in the entropy of solution. For this reason, ionic liquids with longer aliphatic chains require less salt for salting-out and are more easily excluded from the salt-rich phase to the ionic-liquid-rich phase. Other authors have observed the same trend in the ABS formation aptitude.[9,10,20,32,37,41,53] Hence, independently of the ionic liquid used or salt employed, the increased ability of ionic liquids with longer aliphatic chains to form ABS is always verified; yet, only if alkyl chains up to hexyl are considered.

Najdanovic-Visak et al.,[33] who reported the temperature-composition pseudo-binary systems for K_3PO_4, water and chloride ionic liquids combined with the cations 1-butyl-3-methylimidazolium, 1-methyl-3-octylimidazolium and 1-decyl-3-methylimidazolium verified that the locus of the homogeneous region increased as the alkyl side chain of the cation increased. In other words, opposite patterns began to appear for ionic liquids with alkyl side chains longer than 6 carbons. Within this context, other authors[12,30,37,38,45] have also reported phase diagrams where this anomalous behaviour has been detected. Nevertheless, no major conclusions concerning this pattern have been provided. Taking this issue into account, the liquid–liquid phase behaviour of ternary systems composed of 1-alkyl-3-methylimidazolium chloride ionic liquids (from methyl to tetradecyl), water, and K_3PO_4 has recently received an extensive analysis by Freire et al.[18] The gathered results indicate that the ionic liquid abilities to form ABS, for instance at 1.0 mol kg^{-1} of K_3PO_4, follow the order: $[C_1C_1im]Cl < [C_2C_1im]Cl < [C_4C_1im]Cl < [C_{10}C_1im]Cl \approx [C_8C_1im]Cl < [C_7C_1im]Cl \approx [C_6C_1im]Cl < [C_{12}C_1im]Cl < [C_{14}C_1im]Cl – cf.$ ESI.† Therefore, an increase in the cation side alkyl chain length leads to an improved ability to form aqueous biphasic systems if alkyl chain lengths until hexyl are considered (and as previously observed by several authors).[9,10,20,32,37,41,53] For ionic liquids with longer aliphatic chains, the locus of the homogeneous region increases as the alkyl side chain length increases, and agrees well with the initial work of Najdanovic-Visak et al.[33] Based on the extended analysis of the cation side alkyl chain length, these results showed that the ABS formation phenomenon is more complex than initially admitted and that the capacity of the ionic liquid to self-aggregate also interferes with phase separation. Self-aggregation does not exist in systems containing ionic liquids with alkyl lengths below hexyl but can occur in aqueous solutions of $[C_{8-14}C_1im]Cl$ (after the corresponding critical micelle concentration values are attained).[84] Still, the authors argued that, if the new trend found for ABS formation above hexyl is dominated by micelle formation, such a trend should continue uninterruptedly up to $[C_{14}C_1im]Cl$ since critical micelle concentrations progressively decrease along the homologous series.[84] Hence, the authors evaluated the ability of each ionic liquid to be hydrated making use of a salting-out Stechnow-type coefficient.[18] In summary, these authors[18] concluded

that the complex behaviour of the alkylmethylimidazolium-series is a result of a delicate balance between two opposite effects: (i) an entropic contribution that decreases the solubility of ionic liquids in water with an increase in the cation alkyl chain length, and (ii) the inherent aptitude of ionic liquids with longer aliphatic chains to self-aggregate in aqueous media.

Although the effect of the cation alkyl chain length has been largely explored by various research groups, the effect of the alkyl chain length of the anion has received less attention.[9,11,33,43] The most complete study was reported recently by Deive et al.[11] The authors[11] studied the influence of the side chain length of the sulphate anion on ABS formation and their phase behaviour at 298.15 K – a graphical representation is given in ESI.† In the same line as observed before with the ionic liquid cations, ionic liquids with longer aliphatic chains at the anion also require less inorganic salt to undergo phase splitting. This trend was found to be general and mostly independent of the salt employed (K_3PO_4, K_2CO_3, Na_2CO_3 or $(NH_4)_2SO_4$).[11] Remarkably, the influence of the self-aggregation of ionic liquids with long alkyl chains at the anion is less significant than that observed and discussed before for the ionic liquid cation. Nevertheless, it should be pointed out that only anions with alkyl chains up to octyl were studied. Indeed, no extended analysis of this effect has yet been published and deserves further investigation.

Few authors investigated the effect of the number of alkyl substitutions at the imidazolium core.[4,18,35] Neves et al.[35] investigated ABS composed of the ionic liquids [im]Cl, $[C_1im]Cl$, $[C_2im]Cl$, and $[C_1C_1im]Cl$, and the salt K_3PO_4. A general overview of the results is shown in Fig. 12.

Fig. 12 shows the binodal data for imidazolium-based ionic liquids, namely one unsubstituted ([im]Cl), two monosubstituted ($[C_1im]Cl$ and $[C_2im]Cl$) and one disubstituted ($[C_1C_1im]Cl$) ionic liquid. The unsubstituted and monosubstituted ionic liquids containing systems reveal "atypical" behaviour, verified for the first time for ionic-liquid-based ABS, and which is similar to that observed for systems composed of polymers and low molecular weight polysaccharides. The authors[35] further pointed out the potential of these novel systems as interesting approaches for product separations since, at a fixed K_3PO_4 concentration, two
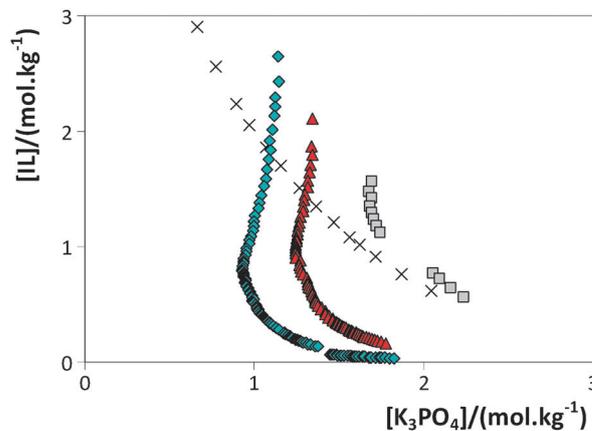

Fig. 12 Ternary phase diagrams for ABS composed of imidazolium-based ionic liquids + K_3PO_4 at 298 K: ■, [im]Cl; ▲, $[C_1im]Cl$; ♦, $[C_2im]Cl$; ×, $[C_1C_1im]Cl$.[35]

monophasic and one biphasic regions are present. The system can be moved between the various phases by the simple variation of the ionic liquid content.[35] Moreover, there are specific ionic liquid ranges where [im]Cl, [C$_1$im]Cl and [C$_2$im]Cl are more effective in promoting phase separation than the disubstituted imidazolium-based ionic liquids (for instance, [C$_1$C$_1$im]Cl).

Additional data on the number of alkyl substitutions were reported by Bridges *et al.*[4] and Freire *et al.*[18] for systems composed of [C$_4$C$_1$C$_1$im]Cl and the salts K$_3$PO$_4$, K$_2$HPO$_4$, K$_2$CO$_3$, and KOH. The binodal boundaries for systems constituted by K$_3$PO$_4$, and the ionic liquids [C$_4$C$_1$C$_1$im]Cl, [C$_4$C$_1$im]Cl, and [C$_6$C$_1$im]Cl, at 298 K, are depicted in ESI.† It is well accepted that the substitution of the most acidic hydrogen at C2 by an alkyl group largely reduces the ability of the ionic liquid to hydrogen-bond. Nonetheless, the aptitude of this ionic liquid to undergo liquid–liquid demixing falls between [C$_4$C$_1$im]Cl and [C$_6$C$_1$im]Cl, behaving like a structural isomer of [C$_5$C$_1$im]Cl. The authors[18] concluded that these results support the idea that the hydrogen bonding of the ionic liquid cation with water is not significant in regards to the phase behaviour of ABS. The researchers proposed that the hydrogen bonding with water is more relevant at the ionic-liquid-rich phase, whilst the ionic fluid solvation in water is more dependent on the cation size, and, thus, on steric contributions.[18] Similar results were published by Bridges *et al.*[4] Nonetheless, a significant deviation was reported in the phase diagrams of both ionic liquids when combined with the salt KOH.[4] The authors[4] pointed out that such behaviour could be derived from the decomposition of both ionic liquids in the presence of such a strong base. [C$_4$C$_1$im]Cl decomposed within minutes whilst noticeable decomposition of [C$_4$C$_1$C$_1$im]Cl took over 12 h (colour change from clear to yellow, and finally to a black liquid with pungent smell).[4]

One particular work[45] was found in the literature regarding the positional isomerism at the ionic liquid cation and its effect on ABS formation. Ventura *et al.*[45] focused on ABS composed of butylmethylpyridinium chloride ionic liquids with the methyl group at the positions C2, C3, and C4, and the mixture of salts K$_2$HPO$_4$–KH$_2$PO$_4$. A summary of the results is given in ESI.† The sequence for the increasing ability of phase splitting follows the rank: [C$_4$-2-C$_1$py]Cl < [C$_4$-3-C$_1$py]Cl ≈ [C$_4$-4-C$_1$py]Cl. The *ortho* isomer presents a slightly lower ability to undergo liquid–liquid demixing whilst no major differences between the fluid phase behaviour of *meta* and *para* isomers were found. Based on previous results on the mutual solubilities between pyridinium isomers-based ionic liquids and water,[80] the authors called attention to the weak dependence of the cation to hydrogen-bond with water (at the water-rich phase) and their impact on the ABS formation.[45]

The influence of functionalized alkyl side chains at the ionic liquid cation has also been addressed by the Coutinho group[9,34,35,45] and more recently by Deive *et al.*[10] The presence of double bonds, additional aromatic rings, and hydroxyl groups at the side chains of the cation was evaluated. Some results for functionalized imidazolium-based ionic liquids, K$_3$PO$_4$, and water systems are displayed in Fig. 13. Data for the corresponding non-functionalized analogues are also included for comparison purposes.[18,35]

The presence of a hydroxyl group at the longer alkyl side chain of the imidazolium-based ionic liquid, [OHC$_2$C$_1$im]Cl,
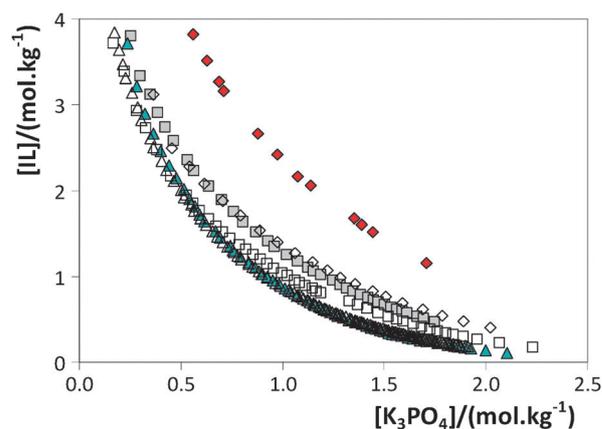

Fig. 13 Ternary phase diagrams for ABS composed of chloride-based ionic liquids + K$_3$PO$_4$ at 298 K: ♦, [OHC$_2$C$_1$im]Cl; ■, [aC$_1$im]Cl; ▲, [C$_7$H$_7$C$_1$im]Cl; ◇,[C$_2$C$_1$im]Cl; □, [C$_4$C$_1$im]Cl; Δ, [C$_7$C$_1$im]Cl.[18,35]

leads to an evident decrease in the ABS-promoting ability when compared with [C$_2$C$_1$im]Cl. The presence of a double bound (allyl group) at [aC$_1$im]Cl also decreases the ability of ABS to form (taking into account that the phase diagram for the analogue [C$_3$C$_1$im]Cl should be within [C$_2$C$_1$im]Cl and [C$_4$C$_1$im]Cl). These patterns reflect the introduction of functionalized groups with higher affinity for water and a higher tendency to hydrogen-bond. Therefore, ionic liquids with functionalized groups enhancing their hydrophilic character are more easily hydrated and are more difficult to separate from aqueous media by the addition of a high charge density salt. Finally, the effect of a benzyl group at [C$_7$H$_7$C$_1$im]Cl does not bring significant changes in the phase diagram behaviour as compared to [C$_7$C$_1$im]Cl.[18,35] Similar trends in the weak effect of the benzyl group towards the phase diagrams behaviour were found in additional research for ABS formed by Na$_2$SO$_4$,[9] Na$_2$CO$_3$[10] or the phosphate buffer solution composed of K$_2$HPO$_4$–KH$_2$PO$_4$.[45]

2.3.2. Influence of the salt. Twenty-five out of the 47 articles concerning ABS composed of ionic liquids and common salting-out agents (salts) addressed the effect of the nature of the salt with a given ionic liquid.[4,11–13,19,21–24,27–30,34,36–38,40–42,45,47,48,53,54] Most works discussed the use of inorganic salts,[4,11–13,19,24,27–30,34,36–38,40–42,45,47,48,53,54] whereas fewer have considered different organic salts.[21–23] Some additional studies[20,32,39,55–57] have also used organic salts in the preparation of ionic-liquid-based ABS but their scope is rather limited in this context since they only focused on one salt and/or one ionic liquid.

Amongst the organic salts, the most comprehensive study has recently been reported by Han *et al.*[21,23] The binodal curves for the phase diagrams constituted by [C$_4$C$_1$im][BF$_4$] + sodium citrate, + sodium tartarate, and + sodium acetate, at 298.15 K, are shown in ESI.† From the binodal curves trend, the salting-out ability of the sodium-based organic salts follows the order: Na$_3$C$_6$H$_5$O$_7$ > Na$_2$C$_4$H$_4$O$_6$ > NaC$_2$H$_3$O$_2$. A similar rank has been reported by Shahriari *et al.*[40] with the same organic salts and one distinct ionic liquid ([C$_4$C$_1$im][CF$_3$SO$_3$]). The increase in the salting-out strength of the salt is represented by the decrease in

the amount of salt required for the formation of two phases under the same composition of ionic liquid. Anions with higher valence are better salting-out agents than those with lower one because the latter are more hydrated.

Several authors have addressed the inorganic salts[4,11–13,19,24,27–30,34,36–38,40–42,45,47,48,53,54] effect toward the formation of ionic-liquid-based ABS. Even so, and among all the available literature, Shahriari et al.[40] published the most complete study on the effect of salts through the liquid–liquid demixing of [C$_4$C$_1$im][CF$_3$SO$_3$] aqueous mixtures. They[40] evaluated both the cation and anion influence of the salt on ABS formation. Aiming at exploring a wide range of salts, the authors[40] used the common water-stable ionic liquid [C$_4$C$_1$im][CF$_3$SO$_3$] which has low hydrogen bond basicity and is easily salted-out from the aqueous solution (even by NaCl, KCl or CsCl). The corresponding ABS data[40] are depicted in Fig. 14 and 15.

The results[40] indicate that the salting-out strength of sodium-based salts to induce the formation of a second aqueous phase
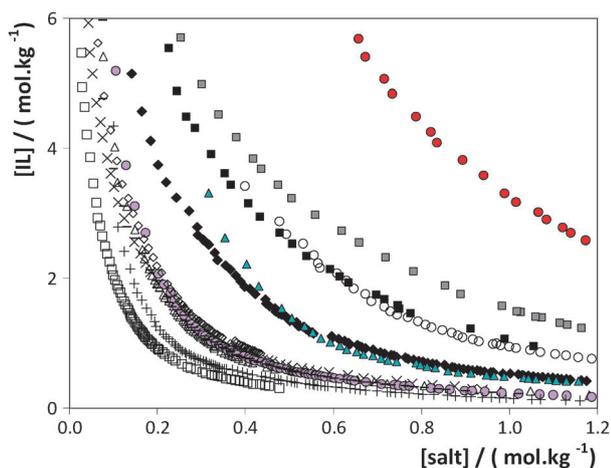

Fig. 14 Ternary phase diagrams for ABS composed of [C$_4$C$_1$im]-[CF$_3$SO$_3$] + sodium-based salts at 298 K: ●, NaCl; ▦, NaHCO$_3$; ■, NaHSO$_4$; ○, NaCH$_3$CO$_2$; ▲, NaOH; ◆, NaH$_2$PO$_4$; ◇, KNaC$_4$H$_4$O$_6$; •, Na$_2$SO$_3$; −, Na$_2$SO$_4$; Δ, Na$_2$SO$_3$; ×, Na$_2$HPO$_4$; +, Na$_3$C$_6$H$_5$O$_7$; □, Na$_3$PO$_4$.[40]
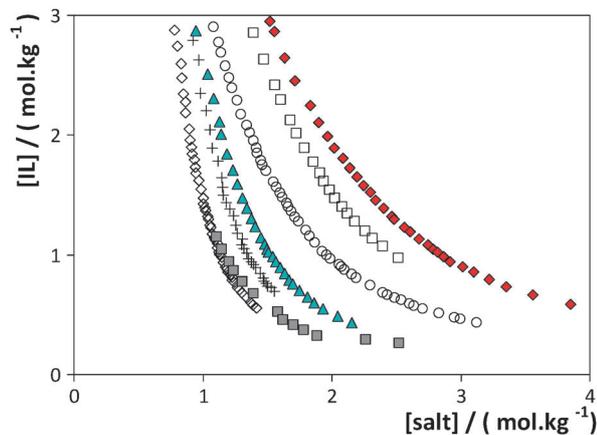

Fig. 15 Ternary phase diagrams for ABS composed of [C$_4$C$_1$im][CF$_3$SO$_3$] + chloride-based salts at 298 K: ◆, CsCl; □, KCl; ○, NaCl; ▲, NiCl$_2$; +, CaCl$_2$; ▦, SrCl$_2$; ◇, MgCl$_2$.[40]

follows the rank: PO$_4$$^{3-}$ > C$_6$H$_5$O$_7$$^{3-}$ > HPO$_4$$^{2-}$ ≈ CO$_3$$^{2-}$ > SO$_4$$^{2-}$ ≈ SO$_3$$^{2-}$ > C$_4$H$_4$O$_6$$^{2-}$ ≫ H$_2$PO$_4$$^-$ > OH$^-$ > CH$_3$CO$_2$$^-$ ≈ HSO$_4$$^-$ ≈ HCO$_3$$^-$ > Cl$^-$. The series of the anion salting-out strength shown in Fig. 14 correlates well with that proposed by other authors.[4,27] On the other hand, the effect of the salt cation was performed with chloride- and acetate-based salts. From the collected data[40] it is observed that the salting-out ability in the two series of salts was identical and follows the order: Mg^{2+} ≈ Ni^{2+} ≈ Sr^{2+} > Ca^{2+} ≫ Na$^+$ > K$^+$ > Cs$^+$.

All published salt effect trends[4,11–13,19,24,27–30,34,36–38,40–42,45,47,48,53,54] follow the well-known Hofmeister series. This series was initially explained based on the ordering of bulk water and on the ability of the ions to increase or decrease the water structure by a simple hydration phenomenon. Therefore, most authors[4,11–13,19,24,27–30,34,36–38,40–42,45,47,48,53,54] explained their ranks based on the Gibbs free energy of hydration of the ions. Nevertheless, novel mechanisms to explain the salting-in-/-out phenomena of ionic liquids have recently been proposed.[77–79] These studies have shown that the salting-out of ionic liquids from aqueous media is driven by an entropic process resulting from the formation of water–ion complexes (causing the dehydration of the solute and the increase in the surface tension of the cavity). Making use of this information, Deive et al.[11] explained their trends in the binodal boundaries with the entropy of hydration of the salt ions. Remarkably, Shahriari et al.[40] used the large compilation of data shown in Fig. 14 and 15 and demonstrated that there is a close correlation between the ionic liquid molality necessary for the formation of a given ABS and the molar entropy of hydration of the ions. In addition, the correlation was found to yield a poorer description if the Gibbs free energy of hydration is used.[40] In conclusion, these results support the idea that the salting-out in ionic-liquid-based ABS is an entropically driven process, as previously proposed for hydrophobic ionic liquids and a wide range of salts.[77–79]

One isolated work has recently been published by Wei et al.[49] and falls into the concept of ionic-liquid-based ABS formed by the addition of ionic species and, in this particular case, of an ionic surfactant. Wei et al.[49] have shown, for the first time, that aqueous mixtures of sodium dodecyl benzene sulfonate (SDBS) and [C$_4$C$_1$im][BF$_4$], within a certain range of compositions, can spontaneously separate and form ABS. The authors[49] also used transmission electron microscopy to characterize the biphasic system and depicted the existence of aggregates in both phases: large micelles are present in the upper phase (surfactant-rich phase) whilst relatively smaller micelles appear in the bottom phase (ionic-liquid-rich phase).

2.3.3. Influence of the pH. The use of distinct salts generates different pH values at the coexisting phases of ionic-liquid-based ABS. Most works have dealt with alkaline aqueous solutions since, in general, the formation of biphasic systems is easily achieved under such conditions. The use of neutral or acidic inorganic salt solutions to form ionic-liquid-based ABS has been either contested or poorly understood. Nonetheless, the use of ABS with specific pH values (or a limited pH range) can be of crucial importance for extracting vital biomolecules in the biotechnological field, such as proteins.

Although the effect of inorganic salts on the formation of ionic-liquid-based ABS has been widely studied and discussed, no major attention has been devoted to the effect of pH.

Zafarani-Moattar and Hamzehzadeh[57] illustrated the pH effect on the binodal boundaries of systems composed of [C$_4$C$_1$im]Br, water and potassium citrate–citric acid mixtures. Different ratios of potassium citrate–citric acid lead to different pH values of the aqueous media and, in general, a decrease in the pH of the aqueous solution (increase in the citric acid content) leads to an increase in the area of the monophasic region.[57] On the other hand, He et al.[24] have shown that systems with [C$_4$C$_1$im]Cl can undergo phase separation by the addition of appropriate amounts of alkaline aqueous salt solutions, namely K$_2$HPO$_4$, K$_3$PO$_4$, K$_2$CO$_3$, KOH, Na$_2$HPO$_4$ and NaOH. However, when acidic to neutral salt solutions, such as KH$_2$PO$_4$, K$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, KCl or NaCl are used, two-phases have not been formed.[24] In the same line of investigation, Li et al.[28] reported ternary phase diagrams of water, [C$_4$C$_1$im]Cl, and distinct inorganic salts, and reported that ionic-liquid-based ABS can be readily formed in alkaline aqueous solutions. When using acidic or neutral salts, no phase separation has been observed.[28] Thus, both reports[24,28] confirm that besides the salting-in/-out aptitude of the conventional salts, the creation of ionic-liquid-based ABS largely depends on the pH of the aqueous solutions. Both works[24,28] have demonstrated the inability of acidic and neutral aqueous solutions to form ionic-liquid-based ABS with [C$_4$C$_1$im]Cl. Yet, later on, Cláudio et al.[9] provided a critical assessment regarding the formation of ionic-liquid-based ABS under acidic media. The authors[9] published novel ternary phase diagrams composed of different water-stable ionic liquids, water, and Na$_2$SO$_4$, and at 298 K. A large array of ionic liquids was explored,[9] and in contrast to the results of He et al.[24] and Li et al.,[28] it was verified that a proper selection of the ionic liquid structural features was able to lead to liquid–liquid demixing using aqueous solutions of Na$_2$SO$_4$. Moreover, most of the ionic liquids investigated were shown to produce acidic aqueous systems. The authors[9] concluded that, although the Hofmeister series is obeyed, the pH values of the aqueous solutions are important in determining the number of successful ionic liquids that are capable of undergoing liquid–liquid demixing. When the pH decreases, the number of ionic liquids that are able to suffer phase splitting is largely reduced.

2.3.4. Influence of the temperature. The influence of temperature on the phase diagram behaviour of ionic-liquid-based ABS has been assessed by several authors[16,19,20,22,27,37,39,48,55,56] for imidazolium-based ionic liquids combined with distinct salts, namely NaCl, NaH$_2$PO$_4$, Na$_2$CO$_3$, Na$_2$SO$_3$, Na$_2$SO$_4$, Na$_3$PO$_4$, Na$_3$C$_6$H$_5$O$_7$, KOH, K$_2$HPO$_4$, K$_2$CO$_3$, K$_3$PO$_4$, K$_3$C$_6$H$_5$O$_7$, (NH$_4$)$_2$SO$_4$, (NH$_4$)$_2$C$_4$H$_4$O$_6$, and (NH$_4$)$_3$C$_6$H$_5$O$_7$. Half of the manuscripts deal with [BF$_4$]-based ionic liquids[19,20,22,27,48] while the remaining are devoted to bromide and chloride-based fluids.[55,56,16,37,39] Two works from the Rebelo research group have determined the cloud-point temperature shifts of aqueous systems composed of [C$_4$C$_1$im][BF$_4$] and NaCl, Na$_2$SO$_4$, and Na$_3$PO$_4$,[42] and distinct chloride- and sulphate-based ionic liquids with the common salt K$_3$PO$_4$.[33] All the studied salts triggered a salting-out

phenomenon leading to significant upward shifts of the demixing temperatures.[33,42]

From all the available literature, Sadeghi et al.[39] published the most extensive temperature study for the [C$_4$C$_1$im]Br + Na$_3$C$_6$H$_5$O$_7$ + water system. The influence of temperature on the phase diagrams of ionic-liquid-based ABS is shown in Fig. 16.

The locus for the experimental binodal curves of the ABS composed of [C$_4$mim]Br and tri-sodium citrate demonstrates that the two-phase region decreases with the increase in temperature.[39] The higher the temperature, the higher are the salt and ionic liquid concentrations required for phase separation. Hence, lower temperatures are favourable for the creation of ionic-liquid-salt-based ABS. The favourable interactions of hydrophilic ionic liquids with water increase with temperature, enhancing thus their mutual solubilities. The same pattern on the temperature effect has been observed in all the remaining studies using distinct ionic liquids or salts.[15,16,19,20,22,27,37,39,48,55,56] Nevertheless, it should be remarked that the intensity of the temperature effect on the phase diagrams depends on the inorganic salt employed.

Besides the general agreement on the favorable formation of ionic-liquid-salt-based ABS at lower temperatures, some contradictions among authors have been found. Han et al.[20,22] and Wang et al.[48] suggested that the slope of the tie-lines decreases as temperature increases, meaning that a decrease in temperature drives water from the ionic-liquid-rich phase to the salt-rich phase, so that the concentration of the ionic liquid at the ionic-liquid-rich phase increases, whereas the salt-rich phase becomes more diluted. On the contrary, Zafarani-Moattar and Hamzehzadeh[55,56] stated that temperature has no major influence on the tie-line slopes. As a result, additional data on this matter are required to obtain a more comprehensive picture of the effect of temperature on the tie-line slopes.

In typical polymer + inorganic salt systems, an increase in temperature leads to larger immiscibility regions, whilst in polymer–polymer ABS the opposite effect is observed. Therefore, the dependence on temperature of the phase diagrams behaviour for ionic liquid + inorganic salt systems resembles that of polymer–polymer ABS. Although ionic liquids tend to
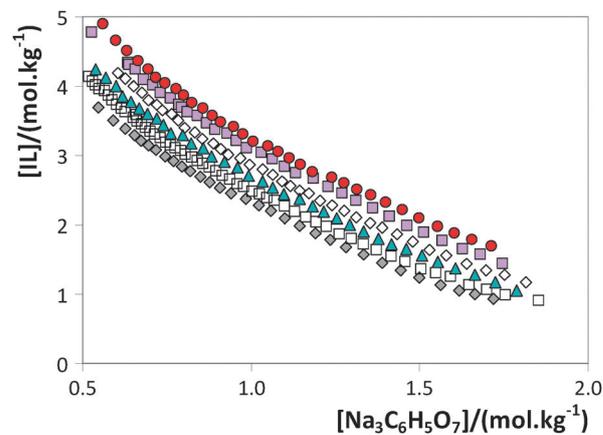

Fig. 16 Ternary phase diagrams for ABS composed of [C$_4$C$_1$im]Br + Na$_3$C$_6$H$_5$O$_7$ at: ♦, 288.15 K; □, 298.15 K; ▲, 308.15 K; ◇, 318.15 K; ■, 328.15 K; ●, 338.15 K.[39]

fall into the salting-in regime and can be salted-out by common salts, Sadeghi *et al.*[39] have observed that water soluble polymers are more easily salted-out by inorganic salts than ionic liquids due to their higher hydrophobic nature, and, thus, the behaviour of their phase diagrams is more dependent on temperature variations. Finally, the authors[39] calculated the free energy of phase separation taking into account the clouding concentration of the ionic liquid (or salt) as a function of temperature. They[39] concluded that the thermodynamic functions of clouding for ionic-liquid-salt systems are smaller than those observed with polymer-based aqueous two-phase systems, and that in contrast to polymer-based ABS where entropy plays a dominant role, in ionic-liquid-based ABS both entropy and enthalpy are driving the formation of the two coexisting phases.

2.4. Ionic liquids + amino acids + water

Besides the large exploitation of organic salts in the composition of ionic-liquid-based ABS, amino acids have also been used as phase separation promoters in aqueous systems involving hydrophilic ionic liquids.[14,58] Indeed, the substitution of high charge density salts by amino acids (inner charged molecules) can lead to more benign extraction procedures, or to less aggressive steps in the ionic liquid recovery from aqueous effluents. Amino acids decrease the ionic strength of the overall solution and may prevent or minimize the ion exchange between the two phases. Despite the growing literature involving ionic-liquid-based ABS, only two works have been found regarding systems composed of amino acids + ionic liquids + water.[14,58] This is a direct consequence of the weaker salting-out ability of amino acids which leads to a reduced number of ionic liquids that are able to undergo liquid–liquid demixing in aqueous media. A summary of the studied systems is presented in Table 3.

Zhang *et al.*[58] reported in 2007 the existence of ABS constituted by $[C_4C_1im][BF_4]$ + glycine/L-serine/L-proline at different temperatures. Later on, Domínguez-Pérez *et al.*[14] published the ternary phase diagrams at 298 K for 1-butyl-3-methylimidazolium-based ionic liquids combined with the anions $[BF_4]^-$, $[CF_3SO_3]^-$, and $[N(CN)_2]^-$, and L-lysine, D,L-lysine·HCl and L-proline.

2.4.1. Influence of the ionic liquid. Only 3 ionic liquids ($[C_4C_1im][BF_4]$, $[C_4C_1im][CF_3SO_3]$ and $[C_4C_1im][N(CN)_2]$) have been found to be able to form ABS when amino acids are used as phase separation promoters. Zhang *et al.*[58] claimed that although $[C_4C_1im][BF_4]$ was able to form ABS with amino acids, it was not possible to obtain ionic-liquid-based ABS with $[C_2C_1im][BF_4]$ or $[C_4C_1im]Cl$. The later results from Domínguez-Pérez *et al.*[14] show that there are indeed more ionic liquids able to form ABS with amino acids besides

$[C_4C_1im][BF_4]$. Nonetheless, all these ionic liquids must present weak hydrogen-bond acceptor abilities.

Fig. 17 depicts the ability of the three studied ionic liquids to form ABS in L-lysine + water solutions.

Since amino acids are weaker salting-out species than the commonly used inorganic salts, the ionic liquids capable of undergoing liquid–liquid separation have to be carefully selected. From Fig. 17, the ionic liquids' propensity for salting-out follows the rank: $[C_4C_1im][BF_4]$ > $[C_4C_1im][CF_3SO_3]$ > $[C_4C_1im][N(CN)_2]$.[14] With the other amino acids studied, D,L-lysine·HCl and L-proline, the $[C_4C_1im][N(CN)_2]$ could not form ABS, whereas the same order for the remaining ionic liquids was observed.[14] This sequence of ionic liquids is identical to that observed for ionic liquid + inorganic salt systems.[43] Since the ABS formation reflects the competition between all the ions for the formation of hydration complexes, it can be expected that ionic liquid anions with lower hydrogen bond basicity values will have a higher tendency to separate from aqueous media, and as discussed by Domínguez-Pérez *et al.*[14]

2.4.2. Influence of the amino acid. Data collected from the two available sources[14,58] allowed us to provide a more comprehensive analysis of the effect of the amino acid on the formation of ABS – *cf.* ESI.† The amino acids' tendency to form ABS follows the order: L-lysine ≈ D,L-lysine·HCl ≈ L-serine > glycine > L-proline. This sequence indicates that a lower molality of L-lysine is needed to form an ionic-liquid-based ABS. Domínguez-Pérez *et al.*[14] explained the above series of amino acids based on their hydrophobicities expressed by their solubilities in water. The higher the solubility of an amino acid in water, the greater is its capacity to form hydration complexes, and, hence, to salt-out the ionic liquid from aqueous environments. For instance, the additional –NH₂ group at the side chain of lysine (when compared to L-proline) enhances its solubility in water and consequently its ability to form ABS. In contrast, the addition of a hydrochloride group to lysine does not lead to significant variations in the salting-out aptitude of the amino acid. In summary, the amino acids seem to behave like weak salting-out agents, acting mainly by forming water–amino acid complexes which further dominate the ABS formation.
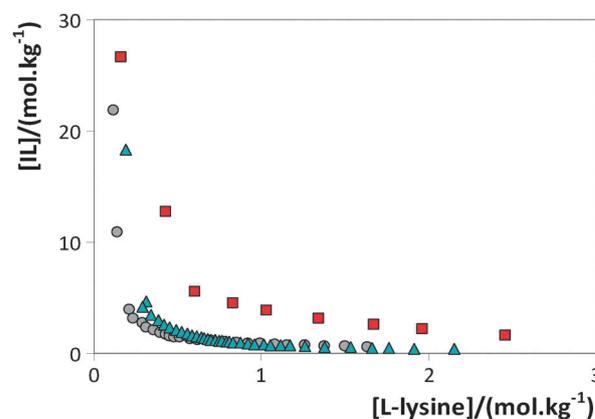
Fig. 17 Ternary phase diagrams for ABS composed of $[C_4C_1im]$-based ionic liquids + L-lysine at 298 K: ■, $[C_4C_1im][N(CN)_2]$; ▲, $[C_4C_1im][CF_3SO_3]$; ●, $[C_4C_1im][BF_4]$.[14]

Table 3 ABS composed of ionic liquids and amino acids reported in the literature

Ionic liquid	Amino acid
$[C_4C_1im][N(CN)_2]$	L-Lysine[14]
$[C_4C_1im][CF_3SO_3]$	L-Proline,[14] L-lysine,[14] D,L-lysine·HCl[14]
$[C_4C_1im][BF_4]$	Glycine[58], L-serine,[58] L-proline,[14,58] L-lysine,[14] D,L-lysine·HCl[14]

2.4.3. Influence of the temperature. Domínguez-Pérez et al.[14] determined the ionic-liquid-based ternary phase diagrams only at 298 K, while Zhang et al.[58] provided a deeper study of the effect of temperature on the formation of ionic liquid + amino acid + water systems. The influence of temperature on the demixing regime is shown in ESI.† In general, an increase in temperature leads to smaller immiscibility regions among amino acid–ionic-liquid-based aqueous systems. As temperature increases, the mutual solubility of the coexisting phases also increases, and a larger amount of at least one of the phase-forming components is needed for phase splitting. Ionic-liquid–amino acid-based ABS are favourably formed at lower temperatures since a decrease in temperature leads to a fall in the solubility of both the ionic liquid and of the amino acid in aqueous solutions. This trend is similar to that observed in common ABS composed of ionic liquids and inorganic salts.

2.5. Ionic liquids + carbohydrates + water

Some researchers have addressed the finding of more benign ABS promoters and shifted to ternary phase systems formed by ionic liquids + carbohydrates + water. Indeed, the ionic exchange among phases can be fully avoided using this type of systems whilst falling under the biorefinery concept. The use of carbohydrates to induce ionic-liquid-based ABS can be visualized as a step towards the usage of a broad spectrum of valuable biocompounds available from biomass. Since carbohydrates are non-toxic and renewable resources, greener processes involving ionic-liquid-based ABS can be immediately envisaged. Eight reports regarding this special kind of ionic-liquid-based ABS have been published.[6–8,17,50–52,59] Nevertheless, in the presence of these weaker salting-out agents, the number of ionic liquids that are able to form ABS is limited, and that is the main reason why few ionic liquids have been studied in this context.[6–8,17,50–52,59] The carbohydrate systems are summarized in Table 4.

The proof of principle which demonstrated the ability of carbohydrates to salt-out ionic liquids dates from 2007 and was proposed by Zhang et al.[59] Subsequently, three works appeared in 2008,[50–52] one in 2009,[7] two in 2010,[6,8] and one in 2011.[17] The carbohydrates employed range from monosaccharides,[7,8,17,50,59] to disaccharides,[6,8,17,50–52] and, lastly, polyols.[17]

2.5.1. Influence of the ionic liquid. Wu et al.[51] reported the liquid–liquid behaviour of systems composed of sucrose and distinct ionic liquids: [aC$_1$im]Cl, [aC$_1$im]Br, and [C$_4$C$_1$im][BF$_4$]. Chen et al.[7,8] investigated the influence of the increase in the cation alkyl side chain length with tetrafluoroborate-based ionic

liquids and claimed that [C$_n$C$_1$im]Cl and [C$_n$C$_1$im]Br (with n = 2 to 10) do not undergo phase separation in the presence of aqueous solutions containing carbohydrates. More recently, Freire et al.[17] reported phase diagrams for systems composed of [C$_4$C$_1$im][CF$_3$SO$_3$] + carbohydrates + water as suitable and water-stable alternatives to the largely studied, yet water-unstable, [BF$_4$]-based ionic liquids.

In general, [BF$_4$]-based ionic liquids are more able to form ABS than [CF$_3$SO$_3$]-based ones[7,17,50] – trend displayed in ESI.† In the same line, and as previously discussed for ionic-liquid-based ABS formed by the addition of inorganic salts or amino acids, this ionic liquid sequence is inverse to the ability of the anion to hydrogen-bond with water.

More ubiquitous differences have been observed when the ionic liquids [aC$_1$im]Cl and [aC$_1$im]Br are included. For instance, at 1.0 mol kg^{-1} of carbohydrate, the ionic liquids' ABS formation follows the order: [C$_4$C$_1$im][CF$_3$SO$_3$] < [aC$_1$im]Cl < [aC$_1$im]Br < [C$_4$C$_1$im][BF$_4$]. Generally, the higher salting-in capacity of the bromide-based ionic liquid relative to their chloride-based counterpart agrees well with previous trends found in inorganic-salt-based ABS. However, no clear explanation can be provided for the higher propensity of the halogen-based ionic liquids towards phase separation as compared to [C$_4$C$_1$im][CF$_3$SO$_3$]. Indeed, contradictory results have been found by Freire et al.,[17] who reported the inability of [C$_4$C$_1$im]Cl, [C$_6$C$_1$im]Cl, [C$_4$mim][C$_1$SO$_4$], [C$_4$C$_1$im][HSO$_4$], and [C$_4$C$_1$im][N(CN)$_2$] towards macroscopic liquid–liquid demixing in the presence of concentrated carbohydrate solutions. Chen et al.[7] have also indicated that [C$_n$C$_1$im]Cl and [C$_n$C$_1$im]Br (with n = 2 to 10) do not suffer phase separation with carbohydrates. Neither the increase in the length of the cation alkyl side chain nor ionic liquid anions with lower ability to hydrogen-bond with water are capable of forming carbohydrate–ionic-liquid–water biphasic systems. Only [C$_4$C$_1$im][BF$_4$] and [C$_4$C$_1$im][CF$_3$SO$_3$] have shown to undergo phase separation in the presence of aqueous solutions of carbohydrates.[17] To resolve these discrepancies, the determination of the ternary phase diagram of [aC$_1$im]Cl + sucrose + water at room temperature was attempted by us (during the preparation of this review); however, no phase separation was observed at any composition ratio. Moreover, a mixture composed of 40 wt% of [aC$_1$im]Cl + 30 wt% of sucrose + 30 wt% of water (biphasic region according to Wu et al.[51]) was prepared and no liquid–liquid demixing was observed within a period of 48 h.

Regarding the influence of the cation side alkyl chain length of the ionic liquid on the phase equilibria behaviour when glucose is used as a fixed carbohydrate, the phase area expands in the order: [C$_4$C$_1$im][BF$_4$] > [C$_3$C$_1$im][BF$_4$] (phase diagrams shown in ESI†). Again, the formation of ionic-liquid–carbohydrate-based systems is a direct reflection of the competition between the two solutes to be hydrated. Since the ability of a specific ionic liquid ion to salt-out from aqueous media depends on its affinity for water, an increase in the size of the aliphatic chain at the cation increases its hydrophobicity and, therefore, the ability of the ionic liquid to be separated from the aqueous media. This trend closely correlates with similar systems constituted by ionic liquids combined with inorganic salts and amino acids, as discussed above.

Table 4 ABS composed of ionic liquids and carbohydrates reported in the literature

Ionic liquid	Carbohydrate
[aC$_1$im]Cl	Sucrose[51]
[aC$_1$im]Br	Sucrose[51]
[C$_n$C$_1$im][BF$_4$]	Glucose,[7,8,50] fructose,[50,59] xylose,[7,50] sucrose,[50–52,6,7] maltose[6]
[C$_4$C$_1$im][CF$_3$SO$_3$]	D-(+)-Glucose,[17] D-(+)-galactose,[17] D-(−)-fructose,[17] D-(+)-mannose,[17] D-(−)-arabinose,[17] L-(+)-arabinose,[17] D-(+)-xylose,[17] sucrose,[17] D-(+)-maltose,[17] D-maltitol,[17] D-sorbitol,[17] xylitol[17]

2.5.2.   Influence of the carbohydrate. Wu *et al.*[50] have published phase diagrams for ABS composed of $[C_4C_1im][BF_4]$ and sucrose, glucose, xylose or fructose. Subsequently, Chen *et al.*[6] demonstrated the formation of ABS with $[C_4C_1im][BF_4]$ and disaccharides (sucrose and maltose), and later on reported the phase diagrams of the same ionic liquid with monosaccharides (glucose and xylose) and disaccharides (sucrose and maltose).[7] The most complete work on carbohydrate–ionic-liquid-based ABS was published by Freire *et al.*[17] for the water-stable ionic liquid $[C_4C_1im][CF_3SO_3]$ and a large array of structurally iterated sugars: glucose, mannose, galactose, xylose, arabinose, fructose, maltose, sucrose, sorbitol, xylitol, and maltitol.

Mono- and disaccharides are polyhydroxy aldehydes or ketones, whereas the hydrogenated forms of such aldoses or ketoses are known as polyols. All the structures have diverse –OH groups with dual donor–acceptor character, and can establish hydrogen bonds with water and act as salting-out species. Fig. 18 displays the results of the different monosaccharides in promoting ionic-liquid-based ABS. Additional figures illustrating the phase diagrams behaviour for disaccharide and polyols are given in ESI.†

Liquid–liquid equilibrium data for all the $[C_4C_1im][CF_3SO_3]$-based systems investigated by Freire *et al.*[17] indicate that ABS can be formed with a wide range of saccharides. Therefore, phase tuneable systems can be obtained based on a series of distinct carbohydrates.

The phase diagrams depicted in Fig. 18 reveal that the monosaccharides' aptitude to induce ionic-liquid-based ABS formation follows the decreasing order: D-(+)-glucose ≈ D-(+)-galactose > D-(−)-fructose ≈ D-(+)-mannose > D-(−)-arabinose > L-(+)-arabinose > D-(+)-xylose.[17] From this rank, it can be concluded that hexoses are more effective in promoting ABS than pentoses. This fact is directly related to the number of hydroxyl groups at hexoses, which results in a higher number of potential hydrogen bonds with water turning them into stronger salting-out agents. On the other hand, the comparison between two structural isomers – glucose and fructose – indicates that pyranoses (6-sided rings) are more efficient salting-out agents than furanoses (5-sided rings). The higher hydration of glucose over fructose is a direct
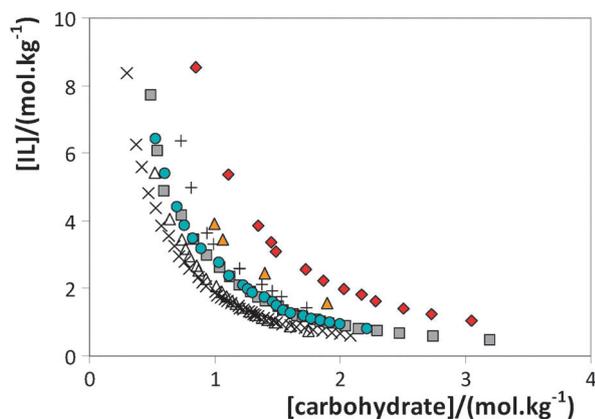
outcome of the six-membered pyranose which is a more favourable conformation for hydrogen-bonding with water.[17]

When analysing the influence of epimers (structures that have opposite configurations of hydroxyl groups at specific positions), for instance the C(2) epimers D-glucose and D-mannose, and C(4) epimers D-glucose and D-galactose, it is evident that there is an influence of the hydroxyl group orientation at C(2) or C(4) on the ABS formation capacity. The ability of epimers to liquid–liquid demixing follows the order: glucose > galactose ≫ mannose. Freire *et al.*[17] have demonstrated that this pattern is related to the carbohydrates hydration potential which depends on the ratio between axial and equatorial hydroxyl groups. Indeed, hydration is more favourable for carbohydrates presenting hydroxyl groups at equatorial positions, which agrees well with the verified trend. Moreover, when considering two enantiomers, D-(−)-arabinose and L-(+)-arabinose, it can be concluded that the spatial configuration of the hydroxyl group at C(4) also influences the phase diagram behaviour. D-(−)-Arabinose is more able to induce ABS than L-(+)-arabinose, since it presents the C(4) hydroxyl group at an equatorial position and presents, consequently, a higher tendency toward hydration. Thus, the relative positions of the hydroxyl groups at C(2) and C(4) are crucial factors in the saccharides facility to be hydrated and, as a consequence, in creating ionic-liquid-based ABS.

The influence of disaccharides on enhancing the phase's immiscibility follows the trend: D-(+)-maltose > sucrose.[17] In the same line, and as observed for the monosaccharides, the six-membered pyronose rings in maltose interact more favourably with water than the five-membered furanose ring in sucrose, and, therefore, present a higher ability to salt-out the ionic liquid. Furthermore, the polyols tendency to form ionic-liquid-based ABS follows the rank: D-maltitol > D-sorbitol > xylitol.[17] This trend is also related to the number of –OH groups present in each carbohydrate and their hydrogen-bonding capabilities. A higher number of carbons, and consequently more hydroxyl groups, lead to higher efficiencies in the interactions of sugars with water.

In summary, the data gathered by Freire *et al.*[17] show that the overall salting-out ability of the carbohydrates investigated follows the sequence: D-maltitol > D-(+)-maltose > D-sorbitol > sucrose > D-(+)-glucose ≈ D-(+)-galactose > xylitol ≈ D-(−)-fructose ≈ D-(+)-mannose > D-(−)-arabinose > L-(+)-arabinose > D-(+)-xylose. From the studied bio-based products, D-(+)-maltose and D-maltitol are the strongest salting-out agents.[17] Particularly, the higher capacity of alditols to induce ionic-liquid-based ABS is quite remarkable – a phenomenon derived from the higher number and solvability of their hydroxyl groups. Freire *et al.*[17] concluded that in addition to the preferential formation of hydrated complexes, which was directly evaluated using the viscosity B- and D-coefficients of aqueous solutions of carbohydrates, two main factors are subjacent to the carbohydrates' salting-out aptitude: (i) the number of –OH groups, and (ii) the corresponding intrinsic stereochemistry.

Fig. 18 Ternary phase diagrams for ABS composed of $[C_4C_1im][CF_3SO_3]$ + monosaccharides at 298 K: ×, D-(+)-glucose; Δ, D-(+)-galactose; ■, D-(−)-fructose; ●, D-(+)-mannose; +, D-(−)-arabinose; ▲, L-(+)-arabinose; ◆, D-(+)-xylose.[17]

2.5.3.   Influence of the temperature. Fig. 19 depicts the influence of temperature on the $[C_4C_1im][BF_4]$ + glucose + water system from 278.15 to 308.15 K.

A decrease in temperature leads to an expansion of the two-phase region of the phase diagram of the system constituted by
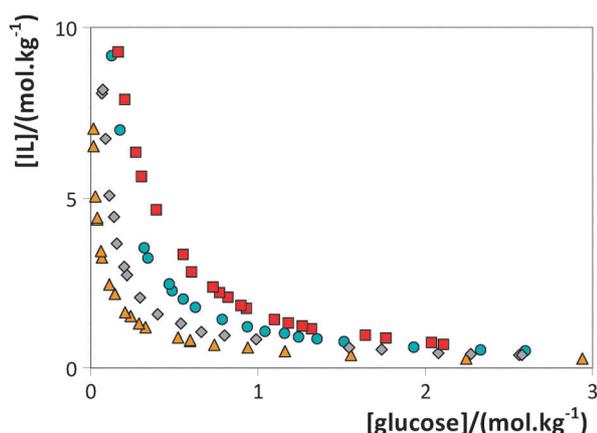
Fig. 19 Ternary phase diagrams for ABS composed of $[C_4C_1im][BF_4]$ + glucose at: ▲, 278.15 K; ◆, 288.15 K; ●, 298.15 K; ■, 308.15 K.[8]

$[C_4C_1im][BF_4]$ + glucose + water.[8] This behaviour has also been observed for similar ternary systems composed of aqueous solutions of $[C_4C_1im][BF_4]$ and fructose,[59] sucrose,[6,52] and maltose,[6,7] and it is analogous to that previously observed for ionic-liquid-based ABS formed by the addition of inorganic salts or amino acids.

Wu *et al.*[52] suggested that the ABS formation between carbohydrates and ionic liquids is an end result of the binding of the ions to the saccharides, which further induces the transition of the sugars from cyclic to chain structures. Hence, according to the authors,[52] the trend depicted in Fig. 19 is a direct outcome of the decrease of the $[C_4C_1im]$-induced decyclization process of the sugar with the increase in temperature. Furthermore, the authors[52] affirmed that the interaction between the ionic liquid and carbohydrates is exothermic. This statement contradicts the findings of Freire *et al.*,[17] who concluded, based on a large series of carbohydrates, that the preferential formation of hydrated complexes is the driving force behind the carbohydrates' salting-out ability. In this context, a broader range of ionic-liquid–carbohydrate-based systems should be studied in order to gather a definite conclusion.

2.6. Ionic liquids + polymers + water

Four main works have been published for aqueous systems involving polymers, ionic liquids and water.[60,62–64] Polymer–polymer ABS usually display two main hydrophobic phases and the difference in polarities mainly depends on the amount of water at each phase. On the other hand, polymer–salt ABS present a hydrophobic phase composed majorly of the polymer and a highly charged and hydrophilic aqueous phase. Therefore, the substitution of a high charge density salt and/or a polymer by an ionic liquid will allow for tighter control of the phases' polarities. Further advantages can be associated with the use of ionic liquids in ABS instead of conventional high-melting inorganic salts. Salt crystallization problems can be avoided when employing an ionic liquid which melts below room temperature. Even in the case of an ionic liquid melting above room temperature, the presence of water will largely prevent its solidification since the saturation levels of ionic liquids in aqueous solutions are usually higher than those observed with common salts. In addition, ionic liquids can optionally be designed to have a low corrosive character.[60]

Table 5 ABS composed of ionic liquids and polymers reported in the literature

Ionic liquid	Polymer
[im]Cl	PEG 2000[60]
$[C_1im]Cl$	PEG 2000[60]
$[C_nC_1im]Cl$	PPG 400,[63] PPG 1000,[63] PEG 2000,[60] PEG 4000,[60] PEG 35 000[62]
$[aC_1im]Cl$	PPG 400,[63] PPG 1000,[63] PEG 2000[60]
$[OHC_2C_1im]Cl$	PEG 2000[60]
$[C_4-3-C_1py]Cl$	PEG 2000[60]
$[C_4C_1pyr]Cl$	PEG 2000[60]
$[C_4C_1pip]Cl$	PEG 2000[60]
$[P_{4444}]Cl$	PEG 2000[60]
$[C_nC_1im]Br$	PPG 400,[64] PEG 2000[60]
$[C_2C_1im][HSO_4]$	PEG 2000[60]
$[C_2C_1im][C_2SO_4]$	PEG 35 000[62]
$[C_4C_1im][C_1CO_2]$	PPG 400,[63] PEG 2000[60]
$[C_2C_1im][(CH_3)_2PO_4]$	PEG 2000[60]
$[C_nC_1im][C_1SO_3]$	PEG 2000[60]

Rebelo and co-workers[62] reported the pioneer work on the salting-in and salting-out effects of ionic liquids through polymers dissolved in aqueous media. Subsequently, two works concerning ABS composed of poly(propylene glycol) (PPG),[63,64] and one work with poly(ethylene glycol) (PEG),[60] and ionic liquids have been published. Table 5 summarizes the aqueous two-phase systems composed of polymers and ionic liquids available in the literature.

2.6.1. Influence of the ionic liquid. Rebelo and co-workers[62] evaluated the changes in the fluid phase behaviour of PEG aqueous solutions by the addition of ionic liquids through the determination of the critical solution temperature shifts at atmospheric pressure. Both the effect of the ionic liquids' anion nature and the cation alkyl side chain length were addressed with ionic liquids of the type: $[C_2C_1im][C_2SO_4]$ and $[C_nC_1im]Cl$ (n = 2, 4, 6, 8). Both salting-in and salting-out effects of the ionic liquid towards the PEG solubility were identified: ionic liquids with long aliphatic chains improve the solubility of PEG 35 000, whereas ionic liquids with short alkyl chains induce its salting-out.[62]

Wu *et al.*[63] published salting-out effects of ionic liquids towards PPG 400 and PPG 1000 dissolved in aqueous media. Since PPG is a thermo-sensitive polymer, the recycling of hydrophilic ionic liquids from aqueous solutions making use of heat was additionally proposed.[63] The researchers[63] reported phase diagrams for $[aC_1im]Cl$, $[C_4C_1im][C_1CO_2]$, and $[C_4C_1im]Cl$, combined with PPG 400 or PPG 1000. Moreover, they indicated that no phase separation was observed with $[C_4C_1im][BF_4]$ and any of the polymers.[63] More recently, Zafarani-Moattar *et al.*[64] reported ternary phase diagrams composed of PPG 400 + water + $[C_nC_1im]Br$ (n = 2, 4). Combining the data from both reports with PPG 400,[63,64] the amount of ionic liquid required for phase separation increases in the order: $[aC_1im]Cl \approx [C_4C_1im]Cl < [C_4C_1im][C_1CO_2] < [C_2C_1im]Br < [C_4C_1im]Br$ (phase diagrams shown in ESI†). Comparing the results for $[C_2C_1im]Br$ and $[C_4C_1im]Br$, it can be concluded that the formation of PPG-based systems is promoted by ionic liquids with shorter aliphatic chains at the cation. Similar results were obtained by Freire *et al.*[60] for PEG-based systems. Longer aliphatic chains enhance the
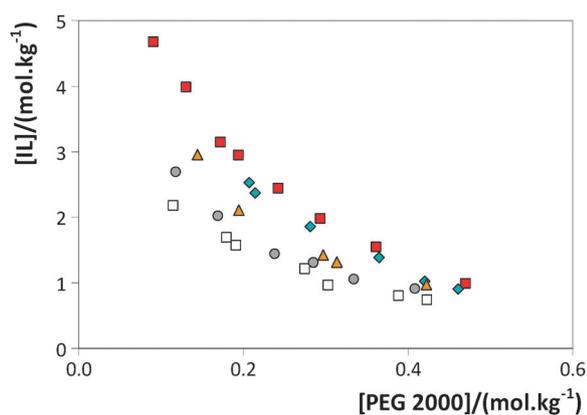
Fig. 20 Ternary phase diagrams for ABS composed of ionic liquid + PEG 2000 at 298 K: ■, [C$_2$C$_1$im]Cl; ♦, [C$_2$C$_1$im][HSO$_4$]; ▲, [C$_2$C$_1$im][C$_1$SO$_3$]; ●, [C$_2$C$_1$im][C$_1$CO$_2$]; □, [C$_2$C$_1$im][(CH$_3$)$_2$PO$_4$].[60]

hydrophobic character of the ionic liquid while increasing the ABS promoting ability in common systems of the type ionic liquid + inorganic salt + water. In contrast, in polymer-based ABS the opposite trend is identified, indicating that in these examples the ionic liquid acts as the salting-out species. Therefore, ionic liquids with shorter alkyl chains present a higher affinity for water and thus hydrate more easily and induce the formation of a second liquid phase.

Regarding the effect of the ionic liquid anion, the ternary phase diagrams formed by PEG 2000 and distinct ionic liquids were selected as major indicatives of a general trend.[60] The ability of [C$_2$mim]-based ionic liquids to induce PEG-based ABS, *i.e.*, the minimum required combined concentrations, follows the trend: [C$_2$C$_1$im][(CH$_3$)$_2$PO$_4$] > [C$_2$C$_1$im][C$_1$CO$_2$] > [C$_2$C$_1$im][C$_1$SO$_3$] > [C$_2$C$_1$im][HSO$_4$] > [C$_2$C$_1$im]Cl – Fig. 20.

Freire *et al.*[60] also reported experimental binodal curves for [C$_4$C$_1$im]-based ABS where a similar rank was observed: [C$_4$C$_1$im][C$_1$CO$_2$] ≈ [C$_4$C$_1$im][C$_1$SO$_3$] > [C$_4$C$_1$im]Cl > [C$_4$C$_1$im]Br > [C$_4$C$_1$im][CF$_3$SO$_3$]. The relative ability of an ionic liquid anion to induce the formation of a second liquid phase in PEG-based ABS follows an opposite order to that observed for those composed of inorganic salts and ionic liquids discussed above. Indeed, when dealing with high charge density salts, the ability to induce ABS increases with the decrease in the ionic liquid affinity for water – the ionic liquid is being salted-out by the inorganic ions. Inversely, when dealing with aqueous systems of polymers and ionic liquids, the ABS promoting agents are the hydrophilic ionic liquids. As verified by Wu *et al.*[63] and Zafarani-Moattar *et al.*[64] in PPG-based systems, the anions rank in PEG-based systems also follows upon their aptitude for hydration and therefore for inducing salting-out.[60]

The effect of the ionic liquid cation core on PEG-2000-based ABS was also assessed by Freire *et al.*[60] with five ionic liquids containing the common chloride anion. The results are summarized in ESI.† The ability of ionic liquids to induce PEG-based ABS follows the trend: [P$_{4444}$]Cl > [C$_4$C$_1$pip]Cl ≈ [C$_4$C$_1$pyr]Cl > [C$_4$C$_1$py]Cl ≈ [C$_4$C$_1$im]Cl. This ability correlates with the relative hydrophobic character of each fluid. The most hydrophobic ionic liquids display cloud points at lower concentrations of ionic

liquid. Ubiquitously, this cationic pattern is in good agreement with the above-mentioned results obtained with ABS of imidazolium-, phosphonium- and ammonium-based ionic liquids in the presence of inorganic salts. Hence, the cation trend here observed is in stark contradiction to that obtained for the anions discussed above. In this context, the authors[60] stated that the ABS formation of PEG-based systems is driven by a different phenomenon than initially expected. When dealing with the more hydrophobic ionic liquids (quaternary ammonium and phosphonium-based compounds), the ABS formation is, as for the salt + ionic liquid systems, a direct result of the PEG salting-out aptitude over the moderate hydrophobic ionic liquids considered. PEG seems to be preferentially hydrated, and, thus, tends to salt-out the ionic liquids. The differences between the cation- and anion-series were explained[60] as a direct result of the stronger hydration displayed by the anions (when compared with the cations) due to their higher polarizability.

Freire *et al.*[60] additionally studied the functionalization of the alkyl substituents at the imidazolium cation on the formation of PEG-based systems. The influence of a double bond or a hydroxyl group at the alkyl chain leads to the following sequence: [HOC$_2$C$_1$im]Cl > [C$_2$C$_1$im]Cl ≈ [aC$_1$im]Cl > [C$_4$C$_1$im]Cl – *cf.* ESI.† The presence of a hydroxyl group or double bond at the aliphatic chain of the cation shifts the binodal curves to the left when compared with [C$_2$C$_1$im]Cl and [C$_4$C$_1$im]Cl, respectively. The functionalized ionic liquids present thus a higher aptitude to promote PEG-based ABS. In this example, the trend for the ionic liquids to form ABS is the opposite of that in those composed of ionic liquids and inorganic salts. Functionalized ionic liquid ions present a higher ability to form hydration complexes, and, hence, to act as the major salting-out species.

Based on the opposite behaviours gathered with the large number of phase diagrams determined by the authors,[60] and where both the ionic liquid and the PEG were found to act as salting-out agents, they concluded that the formation of PEG–IL-based ABS is not only a direct consequence of the ions' ability to form hydration complexes. Instead, the authors[60] stated that the hydrogen-bonding interactions that occur in ionic-liquid–PEG pairs of solutes are additional driving forces in the formation of the respective ABS. In all examples the ABS tendency correlates well with the trend verified in the binary systems of the type PEG + ionic liquid.[60] In summary, the phase behaviour of complex systems of PEG-based ABS is not only dominated by the skill of the solutes to interact with water; it is also a direct consequence of the favourable (or non-favourable) interactions occurring between the PEG and the ionic fluid. The larger the immiscibility between the ionic liquid and PEG, the greater is the ability of the ionic liquid to induce the polymer separation from aqueous media.[60]

2.6.2. Influence of the polymer. The tuneable character of the phase behaviour of polymer-based ABS is not exclusively derived from the manipulation of the ionic liquid structure. Polymers also offer an additional degree of design, for instance by varying the length of the polymeric chains, *i.e.*, by changing their average molecular weight, or by altering the structure of the monomer unit. Two works have assessed the influence of

the polymer on ABS formation.[60,63] Wu et al.[63] demonstrated that several ionic liquids are able to salt-out PPG 400 and PPG 1000 from aqueous media, and Freire et al.[60] have more recently studied the phase diagrams composed of PEG 1000, PEG 2000, PEG 3400, and PEG 4000 combined with the common ionic liquid [C_4C_1im]Cl. The effect of increasing the molecular weight of the polymer, as well as the difference in the phase diagrams between PPG and PEG with a similar molecular weight, is depicted in ESI.† In general, PPG and PEG are able to lead to liquid–liquid demixing in the following order: PPG 1000 > PPG 400 and PEG 4000 > PEG 3400 > PEG 2000 > PEG 1000. For a common ionic liquid, an increase in the molecular weight of the polymer facilitates the separation into two coexisting phases. The analogous trend is well defined in conventional polymer–salt ABS. Polymers with higher molecular weight present lower affinity for water and are preferentially salted-out by the ionic liquid. Moreover, when comparing the data between PPG- and PEG-based systems, it is clear that PPG 1000 is much more likely to undergo phase separation in aqueous media than PEG 1000. PPG is more hydrophobic than PEG due to the additional methyl group at the side chain. Hence, more hydrophobic substances are more easily separated due to their lower affinity for aqueous phases as discussed before.

2.6.3. Influence of the temperature. The effect of temperature on phase diagrams was firstly addressed by Rebelo and co-workers,[62] who determined the critical solution temperatures for fixed compositions of solutes. This work[62] analysed the change of the lower critical solution temperature (LCST) locus of the binary mixture composed of PEG and water as a function of the ionic liquid concentration. Freire et al.[60] reported the experimental solubility data for PEG 2000–[C_2C_1im]Cl and PEG 2000–[C_2C_1im][C_1CO_2] ABS at three temperatures. Zafarani-Moattar et al.[64] presented the ternary phase diagrams for [C_4C_1im]Br + PPG 400 + water at 298.15 K and 318.15 K, and [C_2C_1im]Br + PPG 400 + water at 298.15 K, 308.15 K and 318.15 K. The temperature effect on the phase diagrams for systems composed of PEG or PPG is shown in ESI.†

An increase in temperature decreases the immiscibility region of the aqueous system containing PEG 2000 and [C_2C_1im]Cl or [C_2C_1im][C_1CO_2].[60] In addition, the temperature effect was found to be more pronounced with the acetate-based ionic liquid, which was further explained based on the higher salting-out aptitude of the acetate anion.[60] Overall, the reduction of the immiscibility regime with an increase in temperature within these systems is in close agreement with that observed in ABS composed of ionic liquids and inorganic salts, carbohydrates or amino acids, and as previously presented and discussed. In contrast, the trend of the system composed of PPG 400 + [C_2C_1im]Br + water displays an opposite behaviour. The published results[60,64] seem thus to indicate that a complex phenomenon governs the phase behaviour of ABS containing polymers and ionic liquids, and despite their similarities, largely depends if the polymer is PEG or PPG. This fact may arise from the difference in polarities and hydrogen bond ability between both polymers. When dealing with polymer-based ABS an increase in temperature leads to larger immiscibility regimes in PEG + inorganic salt systems, whilst in polymer–polymer ABS

the opposite pattern is verified. Therefore, the dependence on temperature of the phase diagrams of the ionic-liquid–polymer-based ABS resembles that of polymer + polymer or polymer + inorganic salt aqueous systems[60,64] depending on the polymer used. Hence, new data concerning these particular systems are obviously needed to gather a broader picture of the mechanisms involved in the formation of such ABS.

2.7. Ionic liquids + inorganic salts + polymers + water

One particular example of quaternary systems composed of distinct ionic liquids, one inorganic salt, one polymer, and water was reported in 2010 by Pereira et al.[61] The goal of this work was to use ionic liquids as additives to control the polarities of the phases of common polymer–inorganic-salt-based ABS. These systems were shown to constitute interesting advances in biotechnological separation processes aiming at replacing the approach of PEG functionalization.[61] Diverse ionic liquids at 5 wt% were added to the systems and their phase behaviours were compared to the ternary system composed of PEG 600 + Na_2SO_4 + water.[61]

Within the system PEG 600 + Na_2SO_4 + water, only the effect of adding different ionic liquids was addressed through the phase diagrams behaviour.[61] Several structural features of the ionic liquids were evaluated, namely the effect of the ionic liquid cation side alkyl chain length, the number of alkyl substitutions at the cation, the addition of functionalized groups at the aliphatic chain of the cation, and the anion nature. The results for the influence of the ionic liquid anion are illustrated in Fig. 21, while the remaining effects are shown in ESI.† The binodal curve for the control system without ionic liquid is also represented for comparison purposes.

The influence of 5 wt% of ionic liquids on ABS composed of PEG 600 and Na_2SO_4 is not very pronounced, although general trends can be identified. Nevertheless, it should be remarked that the influence of such small amounts of ionic liquid was found to be highly significant in the tailoring of extraction of an archetypal amino acid.[61] An increase in the cation side alkyl chain length, as well as the presence of more aliphatic chains, reduces the phases' miscibility when compared with the system with no ionic liquid. In addition, the presence of
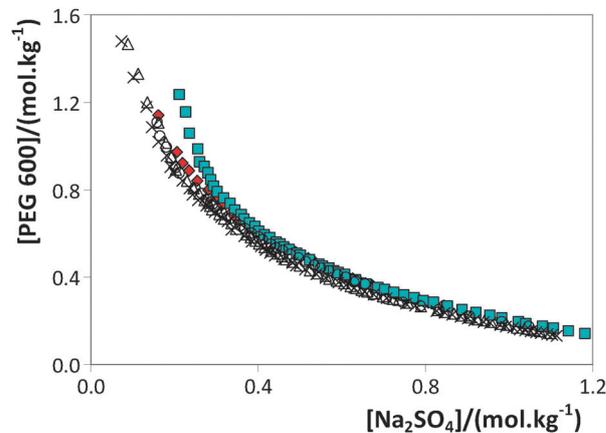

Fig. 21 Phase diagrams for ABS composed of PEG 600 + Na_2SO_4 + 5 wt% ionic liquid at 298 K: ◆, no ionic liquid; ■, [C_4C_1im][HSO_4]; Δ, [C_4C_1im]Cl; ○, [C_4C_1im][C_1CO_2]; ×, [C_4C_1im][C_1SO_4].[61]

a hydroxyl group or a double bond at one of the alkyl chains of the imidazolium cation reduces the ability for phase separation, following hence their hydrophobicity. Regarding the cation influence, only $[C_4C_1im]Cl$ and $[C_4C_1C_1im]Cl$ provide a larger immiscibility region when compared with the system with no ionic liquid. In fact, these results agree well with the work of Rebelo and co-workers[62] for the changes of the fluid phase behaviour of PEG aqueous solutions by the addition of ionic liquids. The authors[62] identified both salting-in and salting-out effects of the ionic liquid towards the PEG solubility, while the salting-out was only achieved with ionic liquids with shorter alkyl chains. Concerning the influence of the ionic liquid anion nature, depicted in Fig. 21, only $[C_4C_1im][HSO_4]$ decreases the demixing locus, whilst $[C_4C_1im]Cl$, $[C_4C_1im][C_1CO_2]$ and $[C_4C_1im][C_1SO_4]$ increase the ability for phase separation. The tendency of ionic liquids to induce liquid–liquid demixing closely correlates with that previously shown for ABS of the type ionic liquid + inorganic salt + water, and depends on the hydrogen-bonding ability of the anion. In order to deepen the understanding of the phase behaviour mechanism, the authors also determined the partition coefficients of the ionic liquid in the PEG–Na_2SO_4-based system. With the exception of the high charge density [im]Cl, the remaining ionic liquids preferentially partition into the PEG-rich phase. Moreover, the intensity of the partitioning of each ionic liquid for the polymeric phase is directly proportional to the salting-in/-out effect they induce regarding their ability to increase (or decrease) the two-phase region.[61] Despite the interesting results on this type of quaternary phase diagrams, especially in what concerns their tailoring ability for partitioning of added-value compounds, no further results were found in the literature. This fact deserves further attention of the research community working with ABS.

3. Applications of ionic-liquid-based ABS

Two main applications were found in the literature for ionic-liquid-based ABS: the extraction of a wide range of solutes, ranging from simple alcohols to complex enzymes; and the recovery of ionic liquids from aqueous media. These two applications are discussed separately in the next sub-sections presented below.

3.1. Extractive approaches

Due to their favourable properties, ABS have been regarded as an economical and efficient downstream processing method, which can be widely used for the recovery and purification of many different types of solutes.[80–82] The efficient application of ABS for preparative purposes, e.g. for separation, isolation, or purification, requires the knowledge of the specific mechanism behind the product partitioning between the two aqueous phases. Generally, such mechanisms are complex, commanded mainly by solute–solvent interactions, such as van der Waals, hydrogen-bonding, and electrostatic forces, and steric and conformational effects. Different net effects of these contributions in each phase allow for the selective partition of the solute into one of the phases.[1] The solute partitioning depends on the properties of the two aqueous phases as well as those of the solute. Achieving successful ABS separation requires the ability to manipulate such phase properties to obtain suitable partition coefficients

(and selectivity) for the target solute molecule. Several procedures can be used to manipulate the partition of a target solute, namely: (i) controlling the solute affinity for one of the two aqueous phases by using different salts and/or ionic liquids; (ii) changing the system composition by manipulating the concentration of salt and/or ionic liquid; (iii) inserting additional co-solvents, anti-solvents, or amphiphilic structures into the system.

In the following divisions, the extraction capacity of ionic-liquid-based ABS for several solutes, with the exception of enzymes where activity is preferred to concentration, is evaluated through the partition coefficient (K) values defined as,

$$K = \frac{[\text{solute}]_{\text{ionic liquid}}}{[\text{solute}]_{\text{salting-out agent}}} \quad (20)$$

which is defined by the ratio of concentrations of a solute in each phase, and where the "ionic liquid" and "salting-out agent" stand for the ionic-liquid-rich and the inorganic-salt-rich phases, respectively.

For enzymes, the extraction efficiency (W_u) is defined according to,

$$W_u = \frac{W_{\text{ionic liquid}}}{W_0} \times 100 \quad (21)$$

where $W_{\text{ionic liquid}}$ is the activity of the enzyme in the ionic-liquid-rich phase, in U mg^{-1}, and W_0 is the initial activity of the enzyme in water, also in U mg^{-1} units.

Until now, we have preferred to use the concentration units in mol kg^{-1} to avoid potential discrepancies from differing molecular weights of the solutes involved in ABS formation. However, for extraction purposes, most literature data are presented in terms of weight fraction units, namely wt%. Most authors compared the efficiency of the extraction steps amongst different ABS at the same mass fraction compositions of the ternary mixture. Therefore, these units will be used below.

The results gathered from the litera-ture[5,7,14–17,19,23–26,28–31,35,36,38,43,44,47,49,61,68,74,83–96] are divided and discussed by four main classes of solutes: alkaloids, pharmaceuticals, amino acids and proteins. The remaining articles were grouped in a section entitled other solutes where the extraction of steroid hormones, short chain alcohol homologues, aromatic and phenolic compounds and metals is addressed.

3.1.1. Alkaloids. Despite legal (e.g. doping agents and drugs control) and academic interest in the analysis of alkaloids, the development of methods for their recovery and quantitative determination remains a challenge. No rapid and effective procedure for preparing samples for quantitative analysis of trace-level alkaloids has yet been developed. Nowadays, the two most common methods for sample pre-treatment are liquid–liquid extraction (LLE)[97] and solid-phase extraction (SPE).[98] Although SPE presents good purification and concentration effects, it requires a relatively time-consuming solvent desorption step using traditional volatile organic solvents and pre-treatment processes. LLE also requires the use of toxic volatile organic compounds and sample recovery is not always adequate. Accordingly, the development of simple and environmentally friendly pre-treatment methods is of great interest.

Table 6 Ionic-liquid-based ABS reported in the literature for the extraction of alkaloids

Alkaloid	System
Caffeine	$[C_4C_1im][CF_3SO_3]$ + proline/lysine,[14] ($[C_4C_1im][BF_4]$, $[C_4C_1im][N(CN)_2]$) + lysine,[14] ($[C_nC_1im]Cl$ (n = 2, 4, 6–8, 10, 12), $[C_4C_1C_1im]Cl$, $[aC_1im]Cl$, $[OHC_2C_1im]Cl$, $[C_7H_7C_1im]Cl$, $[C_nC_1im][CF_3SO_3]$ (n = 2, 4)) + K_3PO_4,[74] $[P_{1444}][C_1SO_4]$ + K_3PO_4,[31] $[C_4C_1im][CF_3SO_3]$ + (sucrose, D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, D-(+)-xylose, D-maltitol, xylitol, D-sorbitol)[17]
Nicotine	($[C_nC_1im]Cl$ (n = 2, 4, 6–8, 10, 12), $[C_4C_1C_1im]Cl$, $[aC_1im]Cl$, $[OHC_2C_1im]Cl$, $[C_7H_7C_1im]Cl$, $[C_nC_1im][CF_3SO_3]$ (n = 2, 4)) + K_3PO_4[74]
Codeine	$[C_4C_1im]Cl$ + K_2HPO_4[28]
Papaverine	$[C_4C_1im]Cl$ + K_2HPO_4[28]

The results found in the literature for the extraction of alkaloids using ionic-liquid-based ABS are presented in Table 6.

In 2005, Li et al.[28] suggested a pioneering ionic-liquid-based ABS ($[C_4C_1im]Cl$ + K_2HPO_4) as a new pre-treatment strategy in the analysis of opium alkaloids from *P. papaveris*. Both phases of the ionic-liquid-based system are clear and mostly composed of water, facilitating its use for coupling with HPLC (High Performance Liquid Chromatography) techniques. The obtained results[28] clearly opened up new possibilities in the enrichment and separation of other drugs from biological samples and the development of new sample pre-treatment procedures.

Based on the evidence that no interference with the ionic liquid had been observed during the quantification of opium alkaloids using HPLC,[28] Freire and co-workers[74] later showed that the complete extraction of archetypal alkaloids, such as caffeine and nicotine, is possible and achieved in a single-step procedure by a proper tailoring of the ionic liquid employed in the ABS formulation. The study focused on seventeen different imidazolium-based ionic liquids which yield ABS in the presence of inorganic salt aqueous solutions (water or urine-type samples). The partition coefficients of caffeine and nicotine in the ABS containing imidazolium-based ionic liquids, K_3PO_4, and water are depicted in Fig. 22(a) – ionic liquid cation effect – and in Fig. 22(b) – ionic liquid anion effect.

The results showed that complete extraction of each alkaloid (no detection of alkaloid in the inorganic salt-rich phase) is attained at a partition coefficient greater than *circa* 120. After fine-tuning the ABS composition, the direct quantitative extraction of the studied alkaloids into the ionic-liquid-rich phase from a synthetic biological sample – artificial human urine – was also evaluated.[74] The corresponding partition coefficients are depicted in ESI.† The results show that the extraction effectiveness for both alkaloids from human urine samples is significantly improved; the presence of a more complex matrix, which includes NaCl and urea, favours the alkaloids partitioning to the ionic-liquid-rich phase. These results[74] – which can be extended to other bioactive drugs – clearly indicate that new applications of ionic-liquid-based ABS for analytical purposes can be immediately conceptualized.

Compared to conventional LLE and SPE, this new approach of ABS avoids the use of volatile organic solvents, replacing them with relatively small amounts of recyclable ionic liquid solvents in a second aqueous phase. Moreover, the difficulty of
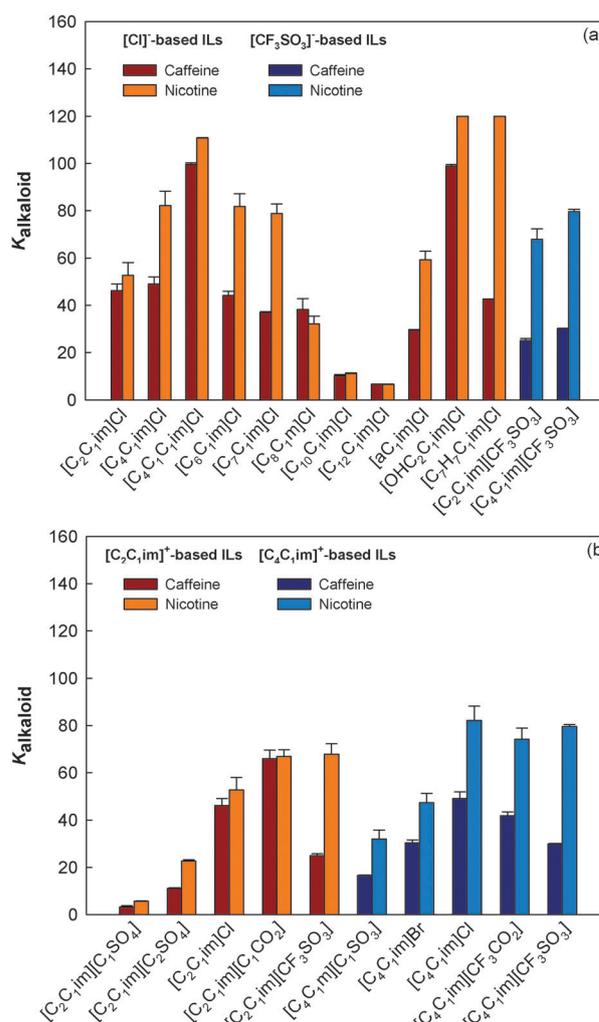

Fig. 22 Influence of the ionic liquid (a) cation and (b) anion on the partitioning coefficients of caffeine and nicotine between ionic-liquid- and K_3PO_4-rich aqueous phases at 298 K.[74]

recycling, namely the recovery of the ionic liquid from the ABS, depends on the nature of the salting-out agent: "greener" salting-out inducing agents *versus* traditional inorganic salts. Indeed, some authors have successfully used non-traditional salting-out agents, namely amino acids[14,31] and a large range of distinct carbohydrates[17] for the extraction of caffeine.

3.1.2. Pharmaceuticals. Pharmaceuticals are designed to stimulate a physiological response in humans, animals, bacteria or other organisms. During the past decade, concern has grown about the potentially adverse effects of the intensive use and disposal of pharmaceuticals on human and ecological health. Research has shown that after passing through wastewater treatment, pharmaceuticals, amongst other compounds, are released directly into the environment. As a result of their persistence and relatively high mobility in the environment, antibiotics can also enter groundwater and be transported in aquifers, surface waters and soil. In addition, some of these compounds are resistant to degradation. However, the relatively low concentration of most antibiotics and the inherent complexity of the environmental samples means that a pre-concentration step

Table 7 Ionic-liquid-based ABS reported in the literature for the extraction of pharmaceuticals

Pharmaceutical	System
Penicillin	$[C_4C_1im]Cl + NaH_2PO_4$,[90]
	$[C_4C_1im][BF_4] + NaH_2PO_4$,[26]
	$[C_4C_1im]Br + Na_2HPO_4$[25]
Chloroamphenicol	$[C_4C_1im]Cl + K_2HPO_4$,[88]
	$[C_4C_1im][BF_4] + Na_3C_6H_5O_7$[23]
Roxithromycin	$[C_4C_1im][BF_4] + Na_2CO_3$[89]
Tetracycline	$[C_4C_1im][BF_4] + NaH_2PO_4$[91]
Sulfadimidine	$[C_4C_1im][BF_4] + (NH_4)_2SO_4$[94]
Acetylspiramycin	$[C_4C_1im][BF_4] + NaH_2PO_4$[47]
Azithromycin	$[C_4C_1im][BF_4] + (Na_2CO_3, NaH_2PO_4, Na_2SO_4, (NH_4)_2SO_4)$[19]
Mydecamycin	$[C_4C_1im][BF_4] + (Na_2CO_3, NaH_2PO_4, Na_2SO_4, (NH_4)_2SO_4)$[19]
Ciprofloxacin	$[C_4C_1im][CF_3SO_3] + lysine$[14]
Papaverine	$[C_4C_1im]Cl + K_2HPO_4$[29]
Morphine	$[C_4C_1im]Cl + K_2HPO_4$[29]

is needed for the reliable quantitative determination of these compounds.[99] In recent years, various separation methods such as liquid–liquid extraction, solid-phase extraction, ion-exchange, chromatography, crystallization or a combination of two or more of these methods have been used for the pre-concentration of antibiotics.[100] The use of these techniques is time-consuming and poses environmental issues regarding the greenness of the solvents used. On the other hand, antibiotics that are produced *via* fermentative processes, such as penicillin and tetracycline, need to be extracted in order to be further processed. The traditional way to obtain penicillin includes its extraction with organic solvents at low pH, back extraction into aqueous solutions at alkaline pH and crystallization of the product.[101]

A summary of ionic-liquid-based ABS found in the literature for the extraction of pharmaceuticals is presented in Table 7.

Liu and co-workers have published 3 articles[25,26,90] studying the experimental factors governing the extraction of penicillin using ionic-liquid-based ABS. They used $[C_4C_1im][BF_4]$, $[C_4C_1C_1im][BF_4]$, $[C_4C_1im]Cl$, $[C_4C_1im]Br$ and $[C_8C_1im]Br$ combined with Na_2HPO_4, NaH_2PO_4, or Na_2SO_4 as salting-out agents and obtained extraction efficiencies higher than 90% in all the systems investigated. The partition coefficients were further plotted against the corresponding tie-line lengths for an optimal extraction evaluation.[25,26,90] The authors[26] have also shown that the critical tie-line length is related to the hydrophobicity of the ionic liquid, *i.e.*, ln K (ln of the partition coefficients of penicillin) decreases in the following order: $[C_4C_1im]Br$ < $[C_4C_1im][BF_4]$ < $[C_4C_1C_1im][BF_4]$ < $[C_8C_1im]Br$. The same authors[26] also compared the efficiency of the extraction of ABS containing $Na_2HPO_4 \cdot 2H_2O$ and $[C_4C_1im]Br$, PEG 4000, a PEG function-alized with a imidazole terminal, and a PEG functionalized with a carboxylic terminal. The partition coefficients using ionic liquids and PEGs are graphically compared in ESI.† In general, ionic-liquid-based ABS provide higher partition coefficients than typical polymer-based systems.

Han *et al.*[88] used ABS composed of $[C_4C_1im]Cl$ and K_2HPO_4 combined with solvent sublation to analyse chloroamphenicol in lake water, feed water, milk and honey samples, using HPLC as the analytical method. They[88] have shown that the extraction efficiency of the proposed combined method (98.5%) is much higher than that of solvent sublation (53.40%) and liquid–liquid

extraction (2.9%), whilst being slightly higher than that of conventional ABS (95.7%). Still worthwhile mentioning is the work of Domínguez-Pérez *et al.*,[14] who presented the extraction of ciprofloxacin using $[C_4C_1im][CF_3SO_3]$ and an amino acid – lysine – to promote phase separation. The extraction was carried out at just one single concentration, ionic liquid : amino acid ratio of 40 : 20, and a partition coefficient of 2.7 was obtained.[14] This value is much lower than those obtained for the above-mentioned antibiotics using ionic-liquid-based ABS with inorganic salts. However, it should be pointed out that amino acids have a lower salting-out capacity than inorganic salts, thus leading to less efficient extractions to the ionic-liquid-rich phase.

All the remaining relevant literature is summarized in Table 7. All these studies[19,23,47,89,91,94] addressed the extraction of pharmaceutical compounds, mainly antibiotics, while using $[C_4C_1im][BF_4]$-based ABS combined with different salts. These data further confirmed that ionic-liquid-based ABS are real and improved alternatives to the traditional extraction methods, offering simpler, greener, quicker and more efficient procedures. Indeed, when coupled with a suitable analytical method, such as HPLC, ionic-liquid-based ABS constitute viable pre-treatment and clean-up techniques, able to operate in the region of very low solute concentration

3.1.3. Amino acids. The separation of bio-products from bio-reaction media is an important step in biotechnology. Extraction can be applied to first-stage separation or purification processes. Amino acids are a class of useful bio-products for various applications. The conventional separation of amino acids includes ionic exchange, reversed micelle methods,[102] and liquid-membrane extraction processes.[103] Organic solvents are used in the majority of these processes with all their inherent problems, such as high flammability and toxicity to humans and micro-organisms. In this context, the partition behaviour of amino acids in ABS is both of academic and practical importance; since amino acid residues determine the surface properties of proteins, further understanding of the driving forces for the partitioning of proteins in given ABS can be obtained from the study of single amino acids. Furthermore, amino acids are very important bio-products, so their recovery *via* aqueous two-phase extraction from bio-reaction media may represent realistic alternatives to more traditional methods.[104]

Ionic-liquid-based ABS have been successfully applied to the extraction of amino acids.[17,30,31,35,43,64,95] Some of these studies confirmed the potential to control the ABS physico-chemical properties by judiciously selecting the ionic liquid cations and/or anions, thus enabling the manipulation of the properties of the extraction phases for enhanced product recovery.[30,31,35,43] The results found in the literature for the extraction of amino acids are presented in Table 8.

A systematic study of the extractive potential for amino acids of ABS formed by nineteen hydrophilic imidazolium-based ionic liquids and the inorganic salt K_3PO_4 has been reported in three studies.[30,35,43] The influence of the number of alkyl groups present in the cation, the cation side alkyl chain length, the presence of double bonds, aromatic rings, and hydroxyl groups on the alkyl side chain, and the influence of a set of different anions were evaluated. The obtained partition

Table 8 Ionic-liquid-based ABS reported in the literature for the extraction of amino acids

Amino acid	System
L-Tryptophan	$[P_{1444}][C_1SO_4]$ + K_3PO_4,[31] ([im]Cl, [C_1im]Cl, [C_2C_1im]Cl, [C_4C_1im]Cl, [aC_1im]Cl, [OHC_2C_1im]Cl, [C_7H_7C1im]Cl) + K_3PO_4,[35] ([C_2C_1im]Cl, [C_2C_1im][C_1CO_2], [C_2C_1im][C_1SO_4], [C_2C_1im][C_2SO_4], [C_2C_1im][CF_3SO_3]) + K_3PO_4,[43] ([C_4C_1im]Cl, [C_4C_1im]Br, [C_4C_1im][C_1SO_4], [C_4C_1im][N(CN)_2], [C_4C_1im][CF_3CO_2], [C_4C_1im][CF_3SO_3]) + K_3PO_4,[43] $[C_nC_1im][C_1CO_2]$ (n = 4, 6, 8) + K_3PO_4,[30] $[C_4C_1im][CF_3SO_3]$ + (sucrose, D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, D-(+)-xylose, D-maltitol, xylitol, D-sorbitol),[17] $[C_4C_1im]Br$ + $K_3C_6H_5O_7$,[95] $[C_2C_1im]Br$ + PPG 400[64]
L-Phenylalanine	$[C_4C_1im]Br$ + $K_3C_6H_5O_7$,[95]
L-Tyrosine	$[C_4C_1im]Br$ + $K_3C_6H_5O_7$,[95] $[C_2C_1im]Br$ + PPG 400[64]
L-Leucine	$[C_4C_1im]Br$ + $K_3C_6H_5O_7$[95]
L-Valine	$[C_4C_1im]Br$ + $K_3C_6H_5O_7$[95]

coefficients of L-tryptophan (a model amino acid) between the ionic-liquid- and the K_3PO_4-aqueous rich phases are depicted in Fig. 23.

The results demonstrate that the partition coefficient of L-tryptophan (K_{Trp}) in ionic-liquid-based ABS is considerably higher than those obtained with conventional PEG-inorganic salt systems ($K_{Trp} \approx 1–7$),[105] PEG–polysaccharide systems ($K_{Trp} \approx 1$)[106] or with water-immiscible ionic liquids two-phase extractions.[107] Generally, the influence of the imidazolium-based cation on the extraction capacity of ABS is more relevant than the influence of the ionic liquid anion.[35,43] The presence of benzyl groups or double bonds in the imidazolium side alkyl chain has been observed to increase the partition coefficients.[35] Besides the $\pi \cdots \pi$ stacking, the possibility of solute–ionic-liquid hydrogen-bonding seems to rule the partition behaviour. Regarding the effect of the ionic liquid anion on the partition coefficients, it was further found that they closely follow the Hofmeister series.[43]

The work of Louros et al.[31] is the only one reporting the evaluation of the extraction capability for amino acids of ionic-liquid-based ABS with ionic liquids not based on imidazolium cations. This is an unfortunate limitation given the vast number of ionic liquids that can be formed by cation–anion permutations. In this study,[31] the extraction aptitude for amino acids of the ABS system composed of $[P_{1444}][C_1SO_4]$ and K_3PO_4 was tested using the amino acid L-tryptophan. The values of the partition coefficients obtained (K_{Trp} = 9.00) are larger than those observed with the imidazolium-based ionic liquid system with the same anion (K_{Trp} = 4.47),[43] $[C_2C_1im][C_1SO_4]$, for a mass fraction composition of ≈ 25 wt% of ionic liquid and ≈ 15 wt% of K_3PO_4 at 298 K. In addition, the alkylphosphonium-based ionic liquids are, in general, less dense than water, whereas the imidazolium-based compounds are usually denser than water.[108] This fact can be a plus in product work-up steps for decanting aqueous systems. Furthermore, phosphonium-based fluids have higher thermal stability and no acidic protons, making them more stable under nucleophilic and basic conditions than imidazolium-based ionic liquids.[109] Consequently, some of these intrinsic characteristics of
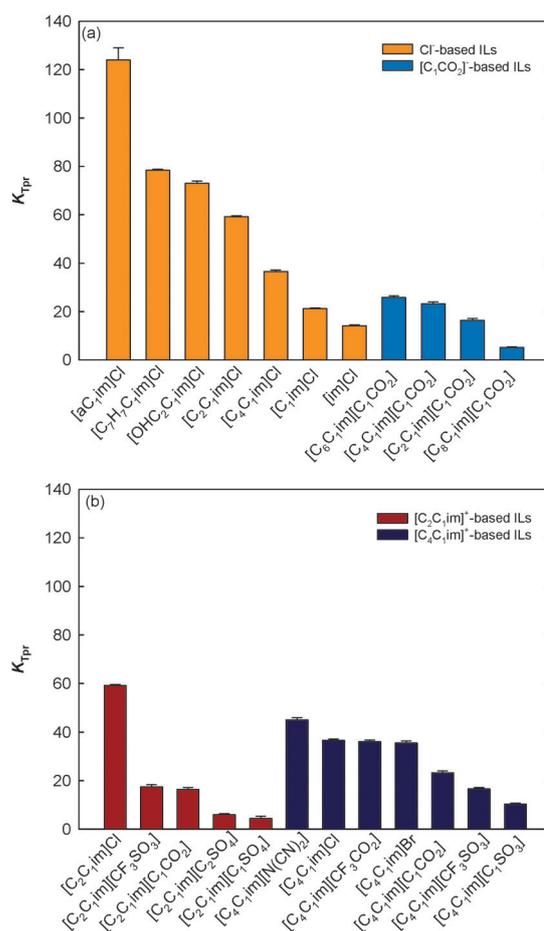
Fig. 23 Influence of the ionic liquid (a) cation and (b) anion on the partitioning coefficients of L-tryptophan between ionic-liquid- and K_3PO_4-rich aqueous phases at 298 K.[30,35,43]

phosphonium-based ionic liquids can be valuable for specific applications.

In the search for greener salting-out inducing agents, Zafarani-Moattar and Hamzehzadeh[95] have used a citrate-based salt with aqueous solutions of 1-butyl-3-methylimidazolium bromide to form ABS. Citrates are biodegradable and can be discharged into biological wastewater treatment plants. These authors also studied the extraction capability of ABS composed of 1-butyl-3-methylimidazolium bromide and potassium citrate for five model amino acids: L-tryptophan (Trp), L-phenylalanine (Phe), L-tyrosine (Tyr), L-leucine (Leu), and L-valine (Val).[95]

Zafarani-Moattar and Hamzehzadeh[95] studied the partition behaviour of amino acids at their isoelectric points, providing an interesting approach to disclosing the effect of their charge and structural/physicochemical properties on the partition coefficient. The results reported by the authors[95] are illustrated in ESI.† In general, the partition coefficients of the amino acids at pH = 6 decrease in the following order: $K_{Trp} > K_{Tyr} > K_{Phe} > K_{Leu} > K_{Val}$. These results agree with the chemical structure of the amino acids. Trp, Tyr and Phe (aromatic model amino acids) have aromatic π systems that allow them to interact with the aromatic π system in the imidazolium cation. Regarding the aliphatic amino acids, hydrophobicity plays a major role; Leu has a longer hydrocarbon chain (one more –CH$_2$ group)
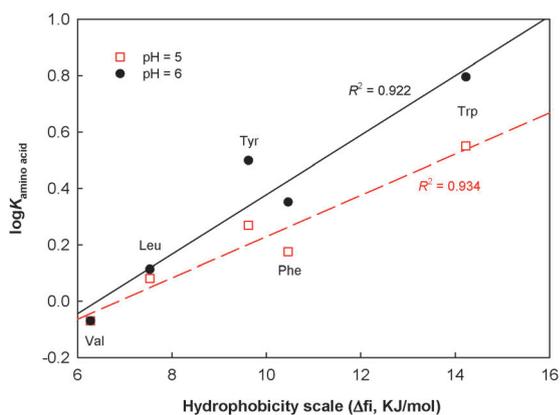
Fig. 24 Partitioning coefficients of the model amino acids for the $[C_4C_1im]Br$ + potassium citrate–citric acid + H_2O ABS at pH = 5 and pH = 6, at 298.15 K, *versus* the hydrophobicity of the amino acids.[95,110]

than Val, which makes the smaller amino acid relatively more hydrophilic and originating a smaller affinity towards the hydrophobic ionic-liquid-rich top phase ($K_{Val} < 1$). As expected, the affinity of amino acids to the preferred phase rises with the growth of the TLL.

The differences in the chemical structure of the amino acids studied allow for the analysis of the effect of the pyrrole ring, –OH and –CH$_2$ groups, and hydrophobicity on their partitioning between the two aqueous phases. Given that the pyrrole ring and the –CH$_2$ group are hydrophobic, and the –OH group is hydrophilic, it can be concluded that hydrophilic groups decrease, while hydrophobic groups increase, the partitioning of the amino acids into the aqueous ionic-liquid-rich phase at a given pH. To further examine this hydrophobic effect, we have plotted in Fig. 24 the logarithm of the partition coefficient as a function of the hydrophobicity scale of the amino acid (defined as the free energy change when a specific amino acid is transferred from water to ethanol)[110] for the ionic liquid $[C_4C_1im]Br$ at selected pH values.[95]

Good correlations have been obtained, which is expectable considering that the polarities of these ionic liquids are comparable to those of the lower alcohols.[111] It is obvious that the extraction effectiveness of amino acids rises with their increasing hydrophobicity, confirming the importance of the hydrophobic interactions as a driving force for their extraction in ionic-liquid-based ABS.

The authors[95] also found that the solution pH may strongly affect the partitioning of amino acids through its impact on the electrostatic and hydrophobicity–hydrophilicity characteristics – *cf.* ESI.† The partition coefficient of tryptophan increases in the order: K_{Trp} (pH = 7) > K_{Trp} (pH = 6, the pH close to its isoelectric point) > K_{Trp} (pH = 5). This trend is the result of several factors: (i) decreasing the pH leads to an increase in the percentage of phase-forming salt ions with minor valence, diminishing their salting-out power; (ii) at lower pH values, the percentage of Trp in its cationic form increases dramatically, which means a lower affinity toward the hydrophobic ionic-liquid-rich phase; (iii) with the increase in the cationic form of Trp, the π···π interactions between the imidazolium cation and the aromatic residue of Trp are

weakened, resulting in a decrease in the partitioning of the amino acid to the aqueous ionic-liquid-rich phase.

Apart from the ionic liquid–salt ABS, ionic liquid–carbohydrate ABS for extractive purposes have also been reported.[17] Carbohydrates are non-charged, biodegradable, non-toxic, and a renewable feedstock with reasonable affinity for water and salting-out aptitude. Freire and co-workers[17] reported on ABS composed of a water-stable ionic liquid and several carbohydrates and evaluated the partitioning of L-tryptophan between the two aqueous phases. Partition coefficients of L-tryptophan in these systems were found to be somewhat higher than in those observed in PEG–polysaccharide systems.[17,106] This fact leads to the conclusion that ionic-liquid–carbohydrate-based systems present improved extraction abilities over polymer-based systems since weaker salting-out species were used (monosaccharides, disaccharides and polyols) instead of polysaccharides. The K_{Trp} values for these systems are depicted in ESI† and are quasi-independent of the carbohydrate used.

Zafarani-Moattar *et al.*[64] investigated the partitioning of two essential amino acids, L-tryptophan and L-tyrosine, in $[C_2C_1im]Br$ + PPG 400 aqueous systems. Depending on the TLL, L-tyrosine preferentially migrates either for the polymer-rich phase or the ionic-liquid-rich phase. In contrast, L-tryptophan always shows a preferential affinity for the $[C_2C_1im]Br$-rich phase.[64] Both amino acids have an aromatic π system that makes possible for them to interact with the imidazolium cation. The preferential migration of L-tryptophan for the imidazolium-rich phase is due to the additional pyrrole ring which further enhance the π···π interactions, while in L-tyrosine the partitioning seems to be governed by the hydrogen-bonding between the –OH group in its side chain and the oxygen atoms contained in the ether groups of the PPG chain. Due to the different behaviour displayed by both amino acids the authors further suggested these systems as viable alternatives for the purification of L-tryptophan from its fermentation broth.[64]

In summary, the extraction aptitude for amino acids of ABS composed of hydrophilic ionic liquids and different salting-out/-in agents, such as inorganic and organic salts, carbohydrates, and PPG 400, was evaluated in the total of seven papers.[17,30,31,35,43,64,95] Based on all these studies, it can be concluded that good results were obtained, especially for aromatic amino acids, and that these novel systems represent enhanced and alternative media to greener separations overall. Furthermore, despite the fact that the mechanism governing the partitioning of amino acids is complex and yet not fully understood, it can be concluded that hydrophobic interactions, hydrogen-bonding, electrostatic interactions and salting-out effects play important roles and cannot be neglected.

Besides the application of ionic liquids as main constituents of ABS, ionic liquids can also be used as adjuvants in typical polymer–salt aqueous systems for the separation and purification of biomolecules. Pereira *et al.*[61] studied the effect of the addition of various imidazolium-based ionic liquids to conventional PEG–inorganic salt ABS on the phase behaviour and extraction capability for L-tryptophan. The obtained results[61] indicate that the addition of small amounts of ionic liquid to classical PEG-based ABS could largely control the extraction efficiency of L-tryptophan, which further indicates that the application of ionic liquids as adjuvants to modify the characteristics of

the polymer-rich phase could be an interesting alternative to the common approach of PEG functionalization.

3.1.4. Proteins.

The growing demand for biotechnological fine chemicals and biological molecules, in particular proteins (which have been increasingly used in therapeutic and diagnostic applications), has led to the development of specific separation and purification methods. Traditional methods for purifying and separating proteins are highly complex, time- and cost-consuming and are not easily scaled-up.[112] Since proteins are easily denatured and lose their biological activity when in contact with most organic solvents, the use of ionic liquids' to design new separation solvents which might be introduced into traditional techniques, such as liquid–liquid extraction, seems to be a natural step towards producing and purifying high-value biotechnological products.

A considerable number of experimental studies of protein behaviour in the presence of ionic liquids have been published[5,15,16,36,38,44,85–87,92,93] and some authors have been trying to identify the major factors ruling the protein–ionic-liquid interactions. Kragl and co-workers[87] recently presented a very interesting study of the phenomena governing the activity and extraction efficiency of proteins in ionic liquids media. The idea that ionic liquids can be tailored to exhibit specific properties is very attractive if the key parameters that define protein stability in ionic liquids can be pointed-out *a priori*.

The authors[87] used four model proteins and performed a statistical analysis of the effect of experimental variables, such as temperature, pH and protein parameters (molecular weight, sphericity, volume, accessible surface area and hydrophobicity), on the protein partitioning. Along the same line, two molecular dynamic studies have also been published shading light on the molecular interactions of enzymes and ionic liquids and also on the crucial role of some experimental parameters, such as the presence of water and pH values.[113,114] However, very few reports can be found in the literature on the extraction of proteins and enzymes using pure ionic liquids.[5,15,16,36,38,44,85–87,92,93] This fact is probably due to the limited solubility of proteins in most hydrophobic ionic liquids. One of the approaches for enhancing protein solubility within hydrophobic ionic liquids is the addition of small amounts of water, giving rise to microemulsions.[115] The use of ABS composed of ionic liquids is a good option for the extraction of enzymes from their production media as well as subsequent media for carrying out biocatalysis. Therefore, ionic-liquid-based ABS offer the opportunity to combine the extraction and concentration of active biocatalysts with the performance of ionic liquids as biocatalytic media. The results found in the literature for the extraction of proteins using ionic-liquid-based ABS are compiled in Table 9.

Bovine Serum Albumin (BSA) has been the most used protein for studying the effect of ions on the protein structure and establishing series of behaviours known as the Hofmeister series. However, only a few studies have been published on the BSA' extraction using ionic-liquid-based ABS.[16,36,38,87] All of them used imidazolium-based compounds, exploring either the increase in the alkyl side chain (ethyl, butyl or hexyl) of the cation[38] or a limited number of anions, such as Br−,[38] Cl−,[16] and [N(CN)$_2$]−.[36] The exception is that by Dreyer *et al.*[87] where an ammonium-based ionic liquid, Ammoeng110[TM], was combined

Table 9 Ionic-liquid-based ABS reported in the literature for the extraction of proteins

Protein	System
BSA	Ammoeng110[TM] + K$_2$HPO$_4$–KH$_2$PO$_4$,[87] [C$_4$C$_1$im]Cl + K$_2$HPO$_4$,[16] [C$_n$C$_1$im]Br (n = 4, 6, 8) + K$_2$HPO$_4$,[38] [C$_4$C$_1$im][N(CN)$_2$] + K$_2$HPO$_4$[36]
Trypsin	Ammoeng110[TM] + K$_2$HPO$_4$–KH$_2$PO$_4$,[87] ([C$_4$C$_1$im]Br, [C$_6$C$_1$im]Br, [C$_8$C$_1$im]Br) + K$_2$HPO$_4$[38]
Cytochrome C	[C$_4$C$_1$im]Cl + K$_2$HPO$_4$,[92] ([C$_4$C$_1$im]Br, [C$_6$C$_1$im]Br, [C$_8$C$_1$im]Br) + K$_2$HPO$_4$[38]
Lysozyme	Ammoeng110[TM] + K$_2$HPO$_4$–KH$_2$PO$_4$[87]
Myoglobin	Ammoeng110[TM] + K$_2$HPO$_4$–KH$_2$PO$_4$,[87] [C$_4$C$_1$im]Cl + K$_2$HPO$_4$[92]
Ovalbumin	[C$_4$C$_1$im]Cl + K$_2$HPO$_4$[92]
Hemoglobin	[C$_4$C$_1$im]Cl + K$_2$HPO$_4$[92]
γ-Globulin	[C$_n$C$_1$im]Br (n = 4, 6, 8) + K$_2$HPO$_4$[38]
TlL	[C$_2$C$_1$im][C$_2$SO$_4$] + K$_2$CO$_3$[85]
CaLA	[C$_2$C$_1$im][C$_4$SO$_4$] + (NH$_4$)$_2$SO$_4$[86]
CaLB	([C$_n$C$_1$im]Cl (n = 2, 4, 6–8), [C$_7$H$_7$C$_1$im]Cl, [C$_4$C$_1$im][CF$_3$SO$_3$], [C$_4$C$_1$im][N(CN)$_2$], [C$_4$C$_1$im][C$_1$SO$_3$], [C$_4$C$_1$pyr]Cl, [C$_4$C$_1$py]Cl, [C$_4$C$_1$pip]Cl, [C$_8$C$_1$py][N(CN)$_2$]) + K$_2$HPO$_4$–KH$_2$PO$_4$[44]
Lipase *Bacillus* sp. ITP-001	[C$_4$C$_1$im]Cl + K$_2$HPO$_4$–KH$_2$PO$_4$[93]
Alcohol dehydrogenases	(Ammoeng100[TM], Ammoeng101[TM], Ammoeng110[TM]) + K$_2$HPO$_4$–KH$_2$PO$_4$[15]
Horseradish Peroxidase	[C$_4$C$_1$im]Cl + K$_2$HPO$_4$[5]

with the salting-out agent K$_2$HPO$_4$–KH$_2$PO$_4$. The selection of the salting-out agent is limited by its effect on pH, which is responsible for the surface charge of the proteins, and its phase forming ability. These four works[16,36,38,87] show that the experimental conditions may be manipulated so that extraction efficiencies of almost 100% can be obtained in just a one-step procedure. UV-Vis and FTIR[38] have been used to confirm that the proteins structure was not affected by the presence of the ionic liquid. However, the main driving force for protein extraction is not clear: while Dreyer *et al.*[87] and Zuo *et al.*[16] stated that the electrostatic interaction between the negatively charged amino acid residues on the protein surface and the cation of the ionic liquid is the main impetus behind extraction, Pei *et al.*[38] suggested that the protein partitioning was favoured by hydrophobic interactions, while electrostatic potential differences and salting-out effects (repulsion by the HPO$_4^{2-}$ anion present in the inorganic salt-rich phase) only assist during extraction.

The presence of other components such as inorganic salts (in urine samples for example)[16] and saccharides[36] towards the BSA extraction has also been tested. Overall, this methodology may prove very useful in the purification of proteins from complex matrices since the free cationic metal species, alkali, alkaline earth, and most transition metals, as well as saccharides, tend to remain in the lower salt-rich phase. In addition, the molecular structure of the saccharides is a factor to consider since it influences the extraction efficiency of BSA from the aqueous saccharide solutions.[36]

Ruiz-Angel *et al.*[92] evaluated the extraction efficiency of four proteins (cytochrome C, myoglobin, ovalbumin, hemoglobin) using ionic-liquid-based ABS and PEG-based ABS. The authors[92] concluded that the partition coefficients using systems composed of ionic liquids are usually 2 to 3 orders of magnitude larger than those obtained with PEG-based

systems, and attributed this favourable partition ratio to the large difference in polarity between the two phases when ionic-liquid-based ABS are used.

Although protein extraction using ionic-liquid-based ABS can serve as a guideline for the use of these systems in the extraction of enzymes, it should be acknowledged that the catalytic activity is the key parameter for the successful extraction of enzymes. To this end, it is mandatory to guarantee that hydrophilic ionic liquids and their aqueous solutions possess balanced ionic-liquid–enzyme interactions: strong capacity to dissolve enzymes but not too strong to disrupt their structure and/or interact with their active sites. Since lipases are the most widely used enzymes in organic synthesis, extensively represented in biotransformations and widely applied in industry, their recovery using ionic-liquid-based ABS have deserved special attention. Three different lipases, *Thermomyces lanuginosus* lipase,[85] CalB,[44] and CalA,[86] have been successfully extracted using this technique with high extraction efficiencies. Furthermore, Ventura *et al.*[93] carried out the pioneer work on the separation and purification of an extracellular lipolytic enzyme produced by *Bacillus* sp. ITP-001, from the fermentation broth at the end of the production phase, with purification factors and enzyme recovery efficiencies above 90%. In all cases, imidazolium-based ionic liquids have proven to be the most efficient cations, and Cl^-,[44,93] $[C_2SO_4]^-$,[85] and $[C_4SO_4]^-$[86] were chosen as the optimal anions. Other works have focused on the extraction of a peroxidase[5] and two different alcohol dehydrogenases,[15] using imidazolium- and ammonium-based ionic liquids, respectively. In the latter work,[15] besides reporting an increased specific activity for the two different alcohol dehydrogenases, it was also shown that the presence of an ionic liquid provided the opportunity to combine an efficient extraction process with the increased performance in the biocatalytic reaction; in general, extraction efficiencies of 90% were obtained.

Despite the fact that it is always possible to find the best ionic liquid and inorganic salt combination, no general conclusions regarding the most appropriate ionic liquid structure for enzyme extraction have yet been found.

3.1.5. Other solutes. Table 10 summarises the ionic-liquid-based ABS found in the literature for the extraction of other solutes than those discussed above. These results are analysed below.

Ruiz-Angel and co-workers[92] compared ionic-liquid-based ABS composed of 1-butyl-3-methylimidazolium chloride, K_2HPO_4, and water with its conventional polymer-based counterpart using polyethylene glycol (PEG 1000), and combined both ABS with the CCC technique to test the liquid phase retention in all possible configurations. Based on the homologous series of linear alcohols (methanol throughout hexanol), the authors[92] have demonstrated the significant polarity difference between PEG- and $[C_4C_1im]Cl$-based systems. The authors have estimated that all compounds with an octanol–water partition coefficient (K_{OW}) higher than 0.22 (log $K_{OW} > -1.7$), which include the vast majority of compounds, will partition preferably into the ionic-liquid-rich phase.

Aromatic compounds are common contaminants in wastewater streams. For instance, phenol derivatives are listed as top pollutants by the US Environmental Protection Agency (EPA)[116] since they are harmful to organisms, even at low concentrations, and many of them have been classified as hazardous pollutants given their potential harm to human health. Strict EPA regulations call for lowering phenol contents in wastewater streams to less than 1 mg L^{-1}.[116] There are many studies handling the treatment processes for wastewater contaminated with aromatic compounds, such as adsorption,[117] reduction–oxidation,[118] membrane separation,[119] biodegradation,[120] and solvent extraction.[121] Despite the potential application value of these techniques, all of them present serious handicaps from the practical point of view. The adsorption processes are effective but the regeneration of the adsorbents is very expensive. The destructive processes based on reduction–oxidation steps and biodegradation often present low efficiency and intricate operation processes. The volatile organic compounds applied in solvent extraction usually involve environmental concerns. Consequently, it is important and advantageous to develop simple and effective methods for extracting aromatic compounds before their disposal into water. Accordingly, some authors[7,96] have explored the potential application of ionic-liquid-based ABS in the extraction of aromatic compounds by investigating the partitioning behaviour of phenol,[7,96] nitrobenzene,[96] 4-nitrophenol,[96] and aniline.[96]

Zhang and co-workers[96] reported the enrichment of nitrobenzene, 4-nitrophenol, phenol and aniline from water, using both hydrophobic and hydrophilic ionic liquids. The results indicate that both $[C_8C_1im][PF_6]$ (hydrophobic) and $[C_4C_1im]Cl$ (hydrophilic) + salting-out inducing salt ABS can efficiently extract those aromatic compounds from aqueous media. However, the implementation of extraction processes based on hydrophobic ionic liquids on a large scale will be limited due to monetary and environmental costs, since these types of ionic liquids usually contain expensive and non-stable fluorinated anions. Given this caveat, the potential to apply hydrophilic ionic liquids in separation schemes is much greater due to the availability of "greener" anions and less expensive materials.

The results assembled in the work of Cláudio *et al.*[68,122] indicated that ionic-liquid-based ABS can be also used for the extraction and purification of added-value phenolic compounds, such as vanillin and gallic acid. Vanillin is currently used in food, beverages, pharmaceutical products, and the cosmetic industry because of its flavour and fragrance. Besides these applications, vanillin is also applied as a chemical intermediate in the production of pharmaceuticals and fine chemicals, such as biocides.[123] Gallic acid is an interesting natural compound because of its antioxidant, anti-inflammatory, antifungal and antitumor properties. It is present in relatively high concentrations in a number of biomass sources and in industrial wastes from where it could be extracted. Despite the variety of applications of both phenolic compounds, their recovery and purification by cost-effective and environmental friendly processes is still a major concern. For all the fourteen studied imidazolium-based ABS, and under all the conditions analysed, vanillin preferentially partitions for the ionic-liquid-rich phase presenting partition coefficients larger than 1.[68] The thermodynamic functions of the partitioning process have also been determined based on the temperature dependence of the partition coefficients.[68] The data revealed that the partition of vanillin results from an interplay between enthalpic and entropic contributions where

Table 10 Ionic-liquid-based ABS reported in the literature for the extraction of other families of compounds

Family	Solute	System
Steroid hormones	Testosterone; epitestosterone	$[C_4C_1im]Cl + (K_2HPO_4, K_3PO_4, K_2CO_3, KOH)$[24]
Short chain alcohols	Methanol; ethanol; propanol; butanol; pentanol; hexanol	$[C_4C_1im]Cl + K_2HPO_4$[92]
Aromatic and phenolic compounds	Vanillin	$([C_nC_1im]Cl$ (n = 2, 4, 6, 7, 10) $[aC_1im]Cl, [OHC_2C_1im]Cl, [C_7H_7C_1im]Cl, [C_4C_1im]Br, [C_4C_1im][C_1SO_3], [C_4C_1im][C_1CO_2], [C_4C_1im][C_1SO_4], [C_4C_1im][CF_3SO_3], [C_4C_1im][N(CN)_2]) + K_3PO_4$[68]
	Gallic Acid	$([C_nC_1im]Cl$ (n = 7, 8), $[C_4C_1im]Br, [C_4C_1im][N(CN)_2], [C_nC_1im][CF_3SO_3]$ (n = 2,4), $[C_4C_1im][C_nSO_4]$ (n = 1, 8)) + $(K_3PO_4, K_2HPO_4–KH_2PO_4, Na_2SO_4)$,[122] $[C_4C_1im][C_2SO_4] + Na_2SO_4$,[122] $[C_4C_1im][CF_3SO_3] + Na_2SO_4 + NaOH$[122]
	Phenol	$[C_4C_1im]Cl + (K_2HPO_4, K_3PO_4, K_2CO_3)$,[96] $[C_nC_1im][BF_4]$ (n = 3–8) + glucose[7]
	Nitrobenzene	$[C_4C_1im]Cl + (K_2HPO_4, K_3PO_4, K_2CO_3)$[96]
	4-Nitrophenol	$[C_4C_1im]Cl + (K_2HPO_4, K_3PO_4, K_2CO_3)$[96]
	β-Carotene	$[P_{1i(444)}][tos] + K_3PO_4$,[31] $[C_4C_1im][CF_3SO_3] + $ (sucrose, D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, D-(+)-xylose, D-maltitol, xylitol, D-sorbitol)[17]
	Capsochrome	$[C_4C_1im][BF_4] + SDBS$[49]
	Rhodamine 6G	$([P_{1i(444)}][tos], [P_{1444}][C_1SO_4], [P_{4444}]Br) + K_3PO_4$[31]
Metals	Cr(IV); Cr(III)	$[N_{4444}]Br + (NH_4)_2SO_4$[84]
	Cd^{2+}	$[N_{4444}]Br + (NH_4)_2SO_4$[83]

both the ionic liquid anion and more complex cations play an essential role.[68]

Regarding the gallic acid extraction, both the ionic liquid structure and salt employed were evaluated.[122] For most of the studied systems, the partition coefficients of gallic acid for the ionic-liquid-rich phase decrease in the following order of employed salt: $Na_2SO_4 \gg K_2HPO_4–KH_2PO_4 > K_3PO_4$.[122] Moreover, both preferentially partitioning for the ionic-liquid-rich or salt-rich phases was observed.[122] Although K_3PO_4 was the strongest salting-out salt studied by the authors,[122] its efficacy in promoting the migration of the phenolic compound for the more hydrophobic phase (ionic-liquid-rich) is only marginal. Therefore, the authors pointed out that the pH of the aqueous media plays a major role in the partition behaviour observed.[122] Indeed, the authors found that at low acidic pH values the non-charged form of gallic acid preferentially migrates for the ionic-liquid-rich phase whereas its conjugate base preferentially partitions to the salt-rich phase.[122] This trend was further supported by the progressive addition of NaOH into a system composed of $[C_4C_1im][CF_3SO_3] + Na_2SO_4$ aiming at increasing the pH of the aqueous phase.

3.1.6. Metals. Depending on their oxidation state most metals present one or more toxic ionic forms. The maximum concentration of these toxic ions is usually regulated by the World Health Organization guidelines for drinking water. Despite this fact, only two articles have been published addressing this subject.[83,84] Akama et al. used ABS composed of tetrabutylammonium bromide and ammonium sulphate to selectively extract Cr(IV) from Cr(III),[84] and Cd^{2+} from Co^{2+}, Cu^{2+}, Fe^{3+} and Zn^{2+},[83] although 24% of the last ion was also extracted to the ionic-liquid-rich phase. It was proposed that the formation of ion pairs between the metal cation and the ionic liquid anion is the extraction mechanism in both cases.[83,84] The authors[84] showed that it is possible to use the above-mentioned ABS combined with atomic absorption analytical methods to determine traces of Cr(IV) in wastewater samples. The determination limit found was 60 μg L^{-1} and recovery efficiencies of 90% have been reported.[84] Compared with the

traditional extractive methods, the proposed method is simpler and does not require the use of organic solvents.

3.2. Recovery/concentration of hydrophilic ionic liquids from aqueous solutions

Although ionic-liquid-based ABS are mostly used in the development and design of extraction processes, their intrinsic nature also provides an effective route for the recovery and/or concentration of hydrophilic ionic liquids from aqueous solutions, as firstly suggested by Gutowski et al.[3]

As the environmental impact of ionic liquids is still an open issue, their removal from water is especially important when dealing with their application on a large scale and in related wastewater streams. In fact, for the industrial application of these liquids, their recovery and recycling need to be addressed. The potential use of ionic-liquid-based ABS for the recycling and recovery of hydrophilic ionic liquids has been clearly demonstrated and a summary of the literature regarding this subject is presented in Table 11.

Deng and co-workers[13] studied ABS composed of $[aC_1im]Cl$ + salt (K_3PO_4, K_2HPO_4, or K_2CO_3) + water with the aim of recovering the ionic liquid from aqueous media. They[13] verified that the recovery efficiency of $[aC_1im]Cl$ increased with the rise of the inorganic salt concentration and that, for the same salt concentration, the recovery efficiencies follow the Hofmeister series: $K_3PO_4 > K_2HPO_4 > K_2CO_3$. A recovery efficiency of 96.80% of ionic liquid was achieved.[13] Li and co-workers[27] also reported that the ionic liquid $[C_4C_1im][BF_4]$ can be recovered from aqueous solutions by the addition of distinct "kosmotropic" salts (Na_3PO_4, Na_2CO_3, Na_2SO_4, NaH_2PO_4, or NaCl) aiming at promoting ABS, and that the maximum recovery efficiency was 98.77%. These authors[27] also found that a stronger salting-out effect allows for higher recovery efficiency of the ionic liquid, and that, for the same amount of salt, the results also agree with the Hofmeister series: $Na_3PO_4 > Na_2CO_3 > Na_2SO_4 > NaH_2PO_4 > NaCl$.

With the aim of proceeding to more environmentally benign ABS, Neves et al.[34] have recently proposed the use of aluminum-based salts to form ABS for removing and recovering ionic liquids from aqueous media. Indeed, aluminum-based salts

Table 11 Ionic-liquid-based ABS reported in the literature for the recycling and recovery of hydrophilic ionic liquids from aqueous solutions

Ionic liquid	Salting-out species
$[aC_1im]Cl$	K_2HPO_4,[13] K_3PO_4,[13] K_2CO_3[13]
$[C_4C_1im]Cl$	K_3PO_4[3]
$[C_4C_1im][BF_4]$	Na_3PO_4,[27] Na_2CO_3,[27] Na_2SO_4,[27] Na_2HPO_4,[27] NaCl,[27] sucrose,[50,51] glucose,[50] xylose,[50] fructose[50]
$[C_nC_1im][CF_3SO_3]$ ($n = 2, 4$)	$Al_2(SO_4)_3$,[34] $AlK(SO_4)_2$[34]
$[C_4C_1im][SCN]$	$Al_2(SO_4)_3$[34]
$[C_4C_1im][tos]$	$Al_2(SO_4)_3$[34]
$[C_4C_1im][N(CN)_2]$	$Al_2(SO_4)_3$[34]
$[C_7H_7C_1im][C_2SO_4]$	$Al_2(SO_4)_3$[34]
$[P_{i(444)1}][tos]$	$Al_2(SO_4)_3$[34]
$[P_{4444}]Br$	$Al_2(SO_4)_3$[34]
$[P_{4444}]Cl$	$Al_2(SO_4)_3$[34]
$[P_{1444}][C_1SO_4]$	$Al_2(SO_4)_3$[34]
$[C_8py][N(CN)_2]$	$Al_2(SO_4)_3$,[34] $AlK(SO_4)_2$[34]

are actually widely used as coagulants in drinking water treatment processes. Neves et al.[34] studied a large array of ionic liquids (imidazolium-, pyridinium- and phosphonium-based) combined with the inorganic salts $Al_2(SO_4)_3$ and $AlK(SO_4)_2$. The minimum recovery efficiency found was 96%, whilst in most of the studied systems recovery efficiencies of 100% were attained.[34]

All of the studies referred above[13,27,34] implemented inorganic salts as salting-out inducing agents, which usually contain highly charged anions (mostly phosphate, sulphate, hydroxide, carbonate) and which entail further environmental risks given the high concentrations of salt required. The introduction of these types of ions also complicates the recycling, since a small amount of undesirable speciation may occur.[3] Alternatively, carbohydrates were introduced for the development of sustainable ionic-liquid-based ABS, since they are non-charged, biodegradable, nontoxic, and a renewable feedstock. Wu and co-workers[50,51] reported that $[aC_1im]Cl$, $[aC_1im]Br$ and $[C_4C_1im][BF4]$ can be recovered from aqueous solutions by application of ionic liquid + carbohydrates (sucrose, glucose, xylose, fructose) ABS. Although the highest recovery efficiency attained only reached 74%,[50,51] these studies show the potential of a more environmentally friendly recovery process which can be further improved by the optimization of a variety of conditions. Nevertheless, we must be aware that on these examples we are adding now a large amount of organic matter to the aqueous medium.

The ability to control the aqueous miscibility of hydrophilic ionic liquids by implementing salting-out phenomena to induce phase separation is of particular importance for the recovery of this type of ionic liquids from aqueous solutions, overcoming wastewater contamination issues and further promoting the industrial application of the novel fluids.

4. Conclusions

Taking into account the vast amount of literature reviewed, ionic-liquid-based ABS show enormous potential for a wide range of applications. The additional advantages shared by ionic liquids and ABS (e.g. enriched aqueous media, no use of volatile organic solvents, low viscosity, and quick phase

separation) contribute to their great potential as novel approaches in extraction and separation processes.

From an experimental point of view, the number of reports dealing with ionic-liquid-based ABS has increased immensely throughout the past few years. This critical review provides a summary of the ionic-liquid-based ABS already investigated and can be viewed as a guide for future research studies. Screening all data from the literature has also enabled us to sort out between high-quality data and those that are doubtful. Furthermore, we have discussed the mechanisms which rule the formation of ionic-liquid-based ABS. The understanding of the effect of the various phase-forming components in the creation of ABS will permit us to conveniently tailor the corresponding phase diagrams in a near future. Ionic-liquid-based ABS have been formed in the presence of a large range of inorganic and organic salts, amino acids, sugars and polyols, and, more recently, polymers. Particularly in this last example, the ionic liquid is the major salting-out species and superb liquid–liquid behaviours have been found. Moreover, the effect of temperature as well as pH on the phase diagrams behaviour has also been discussed.

In order to highlight the potential of the "novel" ionic-liquid-based ABS for practical purposes, additional reports from the literature have been compiled regarding their use in extractions of added-value compounds and in the recovery of ionic liquids from aqueous media. A general overview of these applications has been provided.

Although ionic liquids can be viewed as green solvents due to their negligible volatility, some concerns still exist regarding their seepage into water ecosystems when wide applications are envisaged. Therefore, the use of more benign and biocompatible ionic liquids in ABS formulations is still in demand (such as choline- and amino-acid-based ionic liquids). The use of biodegradable organic salts and/or gentler salting-out species, such as amino acids and carbohydrates, is also clearly recommended.

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