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Glycine-betaine ionic liquid analogues as novel phase-forming components of aqueous biphasic systems

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Abstract

Given the biotechnology advances observed in recent years in terms of upstream, the development of effective downstream processes becomes mandatory to decrease the associated costs of biotechnological-based products. Although a large interest has been devoted to ionic-liquid-based aqueous biphasic systems (IL-based ABS) as tailored separation platforms, imidazolium-based ILs have been the preferred choice as phase-forming agents. To overcome some toxicity and biodegradability issues associated to imidazolium-based ILs, novel ABS composed of ILs analogues of glycine-betaine (AGB-ILs) are here proposed and investigated. Five AGB-ILs were synthesized, characterized in terms of ecotoxicity, and applied toward the development of novel ABS formed with Na₂SO₄. The respective ABS ternary phase diagrams, as well as the tie-lines and tie-line lengths, were determined at 25°C. Finally, their performance as extraction strategies was evaluated with five amino acids (L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine and L-3,4-dihydroxyphenylalanine/L-dopa). In all studied systems amino acids preferentially migrate to the IL-rich phase, with maximum extraction efficiencies of 98.91% for L-tryptophan, 96.95% for L-phenylalanine, 97.40% for D-phenylalanine, 96.70% for L-tyrosine and 94.37% for L-3,4-dihydroxyphenylalanine, achieved in a single-step. In summary, this work demonstrates that novel ABS composed of AGB-ILs can be formed, while corresponding to improved strategies for the extraction of biomolecules, and thus can be envisaged as promising separation platforms for other value-added compounds of biotechnological interest.

Keywords

Aqueous Biphasic Systems; Ionic Liquid; Glycine-Betaine; Amino acids; Extraction

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Introduction

Advances in biotechnology have enhanced the production of a wide variety of value-added biocompounds.¹ Nevertheless, the major drawback still is their recovery and purification from the original complex media by cost-effective techniques, while keeping their biological activity. Therefore, the development of more benign and effective separation techniques has been a hot topic of research in the past years.²

Aqueous biphasic systems (ABS) fit within liquid-liquid extraction/purification techniques. These are formed by two compounds (two salts, two polymers or a polymer and a salt), that when dissolved above given concentrations in aqueous media result in the formation of two (immiscible) liquid aqueous phases, each one enriched in one of the phase-forming components.^{3,4} Due to the large amount of water in their composition, ABS could be seen as more biocompatible extraction platforms when compared to liquid-liquid extraction techniques which employ volatile organic compounds. Although largely exploited in the past decades^{2,5}, conventional polymer-based ABS have coexisting phases of high viscosity, turning the phases separation step difficult to occur in a short time and requiring energetic/centrifugation inputs. Furthermore, the coexisting phases in these more conventional polymer-based ABS display a restricted polarity difference, which hampers enhanced extraction efficiencies and high selectivity to be achieved in a single-step.⁶ In fact, several attempts can be found in the literature to improve the performance of polymer-based ABS, either by introducing additives or by carrying out the functionalization of polymers.^{7,8} However, after demonstrating the possibility of creating ionic-liquid-based ABS⁹, a shift in the ABS research field was noticed. These systems have shown to lead to remarkable extraction efficiencies and are of high selectivity - trends observed with a wide variety of biocompounds and matrices.⁵

Ionic liquids (ILs) are salts, with melting temperatures below 100°C.¹⁰ Due to their ionic nature, most ILs present notable properties, such as a negligible volatility at atmospheric conditions, non-flammability, high thermal and chemical stabilities, and a strong solvation ability for a wide range of compounds.^{10,11} Besides these features, ILs are also labelled as “designer solvents” due to the ability of combining a large number of ions with significantly different chemical structures, allowing the design of improved ILs for particular applications. This characteristic is also reflected in IL-based ABS⁵, and is one of the main reasons behind the outstanding performance of these more recent systems over the conventional polymer-based ones. IL-based ABS have been successfully applied in the separation of antioxidants¹², alkaloids^{13,14}, amino acids¹⁵, proteins/enzymes^{16,17,18}, among others. The largest fraction of IL-based ABS investigated hitherto are based on imidazolium-based ILs and inorganic salts as phase-forming components.⁵ However, imidazolium-based ILs display some toxicity and may compromise the biological activity of several molecules, thus motivating the research on more benign ILs to be applied in ABS. Albeit some reports have appeared in the past few years with this issue in mind, these studies were mainly carried out with cholinium-based ILs, either combined with salts or with polymers to create ABS.^{19–25} Nevertheless, nowadays, there are other ILs derived from natural sources and that could be explored in the creation of ABS. Among these, analogues of glycine-betaine (AGB) can be seen as a promising alternative.²⁶

Glycine-betaine is composed of a zwitterionic quaternary tri(methyl)amine bearing an acetate group. These organic osmolytes accumulate in a wide variety of plant species in response to environmental stress.^{27,28} They have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants growing under stress conditions.^{27,28} ILs analogues of AGB have been recently proposed^{29–32}, whereas hydrophobic ones have been applied in the extraction of pesticides²⁹ and metal ions.^{30–32} However, based on their natural-derived source and if coupled with anions that turn them miscible with water, these ILs can be used in the creation of ABS aiming the separation and recovery of biologically active compounds. Taking into account this possibility and advantages connected to AGB-ILs, this work is focused on the development of more biocompatible IL-based ABS and on the evaluation of their performance to extract amino acids. To this end, five AGB-ILs have been synthesized and characterized, and their ecotoxicity towards the marine bacterium *Vibrio fischeri* and ability to create ABS with Na₂SO₄ addressed. The respective ternary phase diagrams were determined at 25°C to infer the mixture compositions required to form two-phase system, followed by the evaluation of their performance to extract amino acids.

Materials and methods

Materials

AGB-ILs were synthesized by us according to previously reported protocols.²⁶ The ILs triethyl[2-ethoxy-2-oxoethyl]ammonium bromide ([Et₃NC₂]Br), tri(*n*-propyl)[2-ethoxy-2-oxoethyl]ammonium bromide ([Pr₃NC₂]Br), tri(*n*-butyl)[2-ethoxy-2-oxoethyl]ammonium bromide ([Bu₃NC₂]Br), tri(*n*-butyl)[2-ethoxy-2-oxoethyl]phosphonium bromide ([Bu₃PC₂]Br), and N-(1-methylpyrrolidyl)-2-ethoxy-2-oxoethyl]ammonium bromide ([MepyrNC₂]Br) were synthesized by the reaction of ethyl 2-bromoacetate and triethylamine, tri(*n*-propyl)amine, tri(*n*-butyl)amine, tri(*n*-butyl)phosphine or N-methylpyrrolidine, respectively. All ILs were dried under vacuum for at least 72h at 45°C. After this procedure, the purity of each IL was checked by ¹H and ¹³C nuclear magnetic resonance (NMR), being > 98%. All ILs synthesized are solid at room temperature, yet water-soluble. The chemical structures of the synthesized ILs are depicted in Figure 1.

The tertiary amines and the 2-bromoacetic acid ethyl ester were acquired from Sigma-Aldrich. NaSO₄ (anhydrous, 100 wt% pure) was from Prolabo. L-tryptophan (purity = 99.0 wt%) and L-3,4-dihydroxyphenylalanine (L-dopa, purity = 98.0 wt%) were acquired from Sigma-Aldrich. L-tyrosine (purity > 99.0 wt%) and tetrabutylammonium bromide ([N₄₄₄₄]Br, purity > 98.0 %) were acquired from Fluka. L-phenylalanine (purity = 99.0 wt %) and D-phenylalanine (purity = 99.0 wt%) were acquired from Alfa Aesar. The chemical structures of the investigated amino acids are also provided in Figure 1.

Microtox® toxicity tests

The Microtox® test was used to evaluate the ecotoxicity of the synthesized AGB-ILs towards the marine bacterium *Vibrio fischeri*, through the determination of its luminescence inhibition in presence of aqueous solutions of ILs. The bacterium was exposed to a series of aqueous diluted solutions of each IL, ranging from 0 to 81.9 wt%, being 100% the

concentration of the stock solution. After 5, 15 and 30 min of exposure of the bacterium to each IL aqueous solutions, the light output of the bacterium was assessed and compared with the light output of the blank control (an aqueous solution without AGB-ILs), enabling the calculation of the EC₅₀ values at 5, 15 and 30 min through the Microtox® Omni™ Software.

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Phase diagrams and tie-lines

Aqueous solutions of each synthesized AGB-IL ([MepyrNC₂]Br, [Et₃NC₂]Br, [Pr₃NC₂]Br, [Bu₃NC₂]Br and [Bu₃PC₂]Br), at concentrations ranging between 50 and 70 wt%, and aqueous solutions of Na₂SO₄ at 17 wt%, were initially prepared and used for the determination of the binodal curves. The phase diagrams were determined through the cloud point titration method at 25°C and atmospheric pressure. Further details are given elsewhere. 34 The system compositions were determined by the weight quantification of all components added ($\pm 10^{-4}$ g).

The experimental solubility data were correlated using Equation (1):

$$[IL] = A \exp \left[\left(B[salt]^{0.5} \right) - \left(C[salt]^3 \right) \right] \quad (1)$$

where $[IL]$ and $[salt]$ are the IL and the salt weight fraction percentages, respectively, and A , B , and C are constants obtained by the regression of the experimental data.

The tie-lines (TLs), which correspond to the composition of each phase for an overall mixture composition at the biphasic region, were determined by a gravimetric method proposed by Merchuk et al.³⁵. Different mixture points at the biphasic region (total weight of 3 g) were prepared in glass tubes (ca. 5 mL), vigorously stirred, centrifuged for 10 min, and allowed to reach equilibrium by the separation of the two phases for at least more 10 min at 25°C. After separation of the two phases, both the top and bottom phases were weighted. Each individual TL was determined by application of the lever-arm rule to the relationship between the top phase composition and the overall system composition. Further details are given in the Supporting Information, as well as for the determination of the tie-lines length (TLL). In all systems investigated the top phase is majorly composed of IL and water, whereas the bottom phase is enriched in the inorganic salt and water.

Extraction of amino acids

The ternary mixtures compositions used in the partitioning experiments were chosen based on the phase diagrams determined in this work, i.e. at compositions which lead to the formation of two-phase systems. Aqueous solutions of amino acids were prepared at a concentration of 5 g.L⁻¹ for L-tryptophan, 15 g.L⁻¹ for L-phenylalanine, 15 g.L⁻¹ for D-phenylalanine, 2.5 g.L⁻¹ for L-tyrosine and 1.5 g.L⁻¹ for L-dopa, and used as the aqueous solution in each ABS preparation. Each mixture was vigorously stirred, centrifuged for 10 min, and left to equilibrate for more 10 min at 25°C to reach the amino acids complete partitioning between the coexisting phases. After, a careful separation of the phases was performed and the amount of each amino acid in each phase was quantified by UV-

spectroscopy, using a BioTeck Synergy HT microplate reader, at a wavelength of 280 nm (for L-tryptophan, L-tyrosine and L-dopa) or 260 nm (for L-phenylalanine and D-phenylalanine), using calibration curves previously established. At least three independent ABS were prepared and three samples of each phase quantified. The percentage extraction efficiency ($EE_{AA}\%$) of L-tryptophan L-phenylalanine, D-phenylalanine, L-tyrosine and L-dopa corresponds to the percentage ratio between the weight amount of each amino acid in the IL-rich phase to that in the total mixture, defined according to Equation (2):

$$EE_{AA} \% = \frac{w_{AA}^{IL}}{w_{AA}^{IL} + w_{AA}^{Salt}} \times 100 \quad (2)$$

where w_{AA}^{IL} and w_{AA}^{salt} are the total weight of amino acids in the IL-rich and in the salt-rich aqueous phases, respectively.

The pH of the IL- and Na_2SO_4 -rich aqueous phases containing the amino acids was measured at 25°C using a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity equipment within ± 0.02 . The calibration of the pH meter was carried out with two buffers (pH values of 4.00 and 7.00, acquired from Metrohm).

Results

Ecotoxicity of AGB-ILs

The evaluation of the AGB-ILs toxicity was determined by exposing the marine bacterium *Vibrio fischeri* to several aqueous solutions of each IL at different concentrations, for which the EC_{50} values were determined after 5, 15 and 30 min of exposure. The EC_{50} data at 15 min are presented in Figure 2. The corresponding EC_{50} values for 5 and 30 min are presented in the Supporting Information, as well as the detailed data for 15 min of exposure. The higher the EC_{50} values the less ecotoxic is the IL toward the marine bacterium *Vibrio fischeri*. According to the EC_{50} values at 15 min, the toxicity of the synthesized AGB-ILs increases according to the rank: $[\text{Et}_3\text{NC}_2]\text{Br} < [\text{Pr}_3\text{NC}_2]\text{Br} < [\text{Bu}_3\text{PC}_2]\text{Br} < [\text{MepyrNC}_2]\text{Br} < [\text{Bu}_3\text{NC}_2]\text{Br}$. All ILs investigated share the common bromide anion; however, they comprise cations with different central atoms (N vs. P), with different alkyl side chains length, and with cyclic vs. non-cyclic cationic structures. The results obtained reveal that an increase in the alkyl side chain length increases the IL ecotoxic nature, according to the rank: $[\text{Et}_3\text{NC}_2]\text{Br} < [\text{Pr}_3\text{NC}_2]\text{Br} < [\text{Bu}_3\text{NC}_2]\text{Br}$. Longer aliphatic moieties strongly interact (or penetrate better) with the phospholipid bilayer of membranes cells, as reflected by the stronger inhibition of the bacterium luminescence. ILs with longer alkyl side chains are thus more ecotoxic, in good agreement with the literature.^{36,37}

Taking into account the cation central atom, the EC_{50} values decrease considerably from $[\text{Bu}_3\text{PC}_2]\text{Br}$ (2141.9 mg. L^{-1}) to $[\text{Bu}_3\text{NC}_2]\text{Br}$ (340.0 mg. L^{-1}), meaning that the phosphonium-based IL is less toxic than its ammonium-based counterpart. This trend is the opposite to what has been found in the literature, for which the EC_{50} values are higher in

ammonium-based ILs compared to their phosphonium counterparts.³⁸ This result indicates that the presence of an ethoxy group may change the ILs ecotoxicity.

Since the [MepyrNC₂]Br presents a cyclic cation structure, it is expected to be more toxic than the linear-based counterparts.^{38–41} In fact, this IL is more toxic than [Et₃NC₂]Br, even with a lower total number of carbons in the aliphatic part connected to the N central atom.

In Figure 2 are also provided the EC₅₀ values for commercial tetrabutylammonium- and phosphonium-based ILs, namely for tetrabutylammonium bromide and tetrabutyl phosphonium bromide ([N₄₄₄₄]Br and [P₄₄₄₄]Br), and for an imidazolium counterpart (1-butyl-3-methylimidazolium bromide, [C₄mim]Br⁴²) for comparison purposes. The EC₅₀ values of [N₄₄₄₄]Br were determined in this work, whereas those for [P₄₄₄₄]Br and [C₄mim]Br were taken from the literature.⁴² In summary, it is here demonstrated that the AGB-ILs synthesized in this work display a lower toxicity for *V. fischeri* than those commercially available and commonly used for the preparation of ABS. All the prepared AGB-ILs are classified as harmless or practically harmless according to Passino and Smith.⁴³

Phase diagrams and tie-lines

Novel ternary phase diagrams for five ILs ([MepyrNC₂]Br, [Et₃NC₂]Br, [Pr₃NC₂]Br, [Bu₃NC₂]Br and [Bu₃PC₂]Br) + Na₂SO₄ + water were determined at 25°C and atmospheric pressure. The respective phase diagrams are displayed in Figure 3. In all ABS, the top phase corresponds to the IL-rich phase while the bottom phase is mainly constituted by Na₂SO₄ and water.

In all phase diagrams represented in Figure 3, the monophasic and the biphasic areas are localized below and above each solubility curve, respectively. Phase diagrams with a larger area above the solubility curve present a higher ability to form two phases, *i.e.* require a lower amount of salt or IL to create two-phase systems. Overall, the ability of AGB-ILs to form ABS at 25°C follows the order: [Bu₃PC₂]Br > [Bu₃NC₂]Br > [Pr₃NC₂]Br > [Et₃NC₂]Br > [MepyrNC₂]Br. It was previously shown that tetralkylammonium- and tetralkylphosphonium-based ILs present a higher ability to create ABS with a given salt, requiring thus a lower amount of salt or IL to undergo liquid-liquid demixing when compared to imidazolium-based ILs.⁴⁴ This trend was also verified in this work with AGB-ILs (*cf.* the Supporting Information). This feature is a major advantage since lower amounts of IL or salt are required to create ABS, meaning that a higher amount of water will be present - an advantage when envisioning their use as separation platforms of biologically active compounds.

In Figure 3 are given the phase diagrams at 25°C for the systems composed of [Bu₃NC₂]Br, [Pr₃NC₂]Br, [Et₃NC₂]Br and [MepyrNC₂]Br, allowing to address the effect of the IL cation alkyl chain length through the ABS formation ability. In general, ILs with longer alkyl side chains are more hydrophobic and more easily salted-out by the inorganic salt from aqueous media. Accordingly, [Bu₃NC₂]Br requires a lower amount of Na₂SO₄ to form ABS than [Pr₃NC₂]Br and [Et₃NC₂]Br. This trend is in accordance with the literature⁴⁵, where it has been reported that an increase in the IL cation alkyl side chain leads to a higher ability to

create ABS. On the other hand, [MepyrNC₂]Br is the IL with the lowest ability to create ABS. Although this ILs comprises a cyclic ring contrarily to the remaining ILs, this IL presents a higher affinity for water due to the lower number of methyl groups in its structure, therefore requiring higher amounts of salt to be salted-out from aqueous media.

The phase diagrams for the systems constituted by [Bu₃PC₂]Br and [Bu₃NC₂]Br are also depicted in Figure 3. Although with a similar chemical structure, [Bu₃PC₂]Br presents a better phase separation performance in presence of aqueous solutions of Na₂SO₄. The higher ability of phosphonium-based ILs to create ABS when compared with their ammonium counterparts was previously demonstrated with other salts.^{44,46,47} In general, phosphonium-based ILs are more hydrophobic than ammonium-based ones as a result of the cation central atom charge distribution.⁴⁸

All experimental data corresponding to the binodal curves were fitted using Equation 1, with the respective fitting also presented in Figure 3. The regression parameters estimated by least-squares regression, standard deviations (σ), and correlation coefficients (R^2) are given in the Supporting Information. The experimental TLs determined for each ABS, along with their respective length (TLL), as well as the compositions of each phase for the mixtures corresponding to the extraction studies, are also given in the Supporting Information, as well as the graphical representation of the TLs for all the systems investigated.

Extraction of amino acids

The extraction efficiency of the investigated systems for five amino acids, namely L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine and L-dopa, was determined in a common mixture composition (≈ 40 wt% of IL, ≈ 7.5 wt% of Na₂SO₄ and ≈ 52.5 wt% of an aqueous solution containing the amino acids) in order to address the potential of the investigated systems to act as separation platforms. The results obtained at 25°C are displayed in Figure 4. In all systems, amino acids preferentially migrate to the most hydrophobic phase, *i.e.* the IL-rich phase, with extraction efficiencies higher than 60%, and up to 98%, achieved in a single-step. These high values support the influence of salting-out effect exerted by Na₂SO₄. In general, the extraction efficiency of the studied ILs as phase-forming components of ABS for all amino acids follows the rank: [MepyrNC₂]Br > [Et₃NC₂]Br > [Pr₃NC₂]Br > [Bu₃NC₂]Br > [Bu₃PC₂]Br. This trend correlates with the IL hydrophobicity, as given by their ability to form ABS discussed before. The higher the IL hydrophobicity, which also corresponds to an IL-rich phase with a lower water content— *cf.* the Supporting information with the TL data, the lower the extraction efficiencies of the respective ABS for amino acids.

In general, the amino acids partition in IL-based ABS is a result of different forces, including the ability of ILs to establish specific interactions with amino acids, the water content at a given phase and the salting-out strength of the salt used to create ABS. In IL-based ABS constituted by imidazolium-based ILs, it was already reported that electrostatic, $\pi \dots \pi$ and H-bonding interactions play a pivotal role.^{34,45,49,50} Furthermore, the partition of L-tryptophan, L-phenylalanine, L-tyrosine, L-leucine, and L-valine in pH-controlled IL-based ABS (constituted by [C₄mim]Br and K₃C₆H₅O₇/C₆H₈O₇) allowed to conclude that

hydrophobic interactions also are of significant relevance, although electrostatic interactions and salting-out effects cannot be discarded.⁵⁰

Depending on the pH, different chemical species of amino acids are predominantly present due to their functional groups ionization/protonation (-COOH and -NH₂ groups). According to the pH measurements of the IL- and Na₂SO₄- rich phase, which range between 3.1 and 6.2, as shown in the Supporting Information, all amino acids are predominately in their zwitterionic form.⁵¹ Therefore, in the studied ABS, electrostatic interactions are not playing the major role toward the preferential migration of all amino acids to the IL-rich phase.

The extraction efficiencies of the studied ABS for amino acids, as displayed in Figure 4, decrease according to the following order: L-tryptophan > L-tyrosine > L-phenylalanine ≈ D-phenylalanine > L-dopa. This rank closely correlates with the amino acids octanol-water partition coefficients (log(K_{ow})) of L-tryptophan: -1.06, L-phenylalanine: -1.38, D-phenylalanine: -1.38, L-tyrosine: -2.26, L-dopa: -2.74).⁵² Although more hydrophobic ILs display a lower performance to extract amino acids, as discussed above, IL-based ABS display a better performance to extract more hydrophobic amino acids to the IL-rich phase, being a main result of the salting-out effect exerted by the inorganic salt. L-tyrosine appears however as an exception to this trend, and where specific IL-amino-acid interactions may occur. Furthermore, no significant differences are seen in these systems capability to extract L-phenylalanine vs. D-phenylalanine, meaning that the studied ABS display a similar performance to extract enantiomers.

The extraction efficiencies of the amino acids obtained here range between 60% and 98% in ABS formed by 40 wt% of AGB-IL + 7.5 wt% of Na₂SO₄, which are superior to those obtained by more conventional polymer-based ABS.^{53–55} Extraction efficiencies for L-tryptophan ranging between 72% and 99% with ABS composed of imidazolium-based ILs and potassium citrate (K₃C₆H₅O₇) have been reported, yet using imidazolium-based ILs, a strong salting-out species, and higher amounts of salt (20 wt%).³⁴ ABS formed by imidazolium-based ILs and organic biological buffers have also been investigated for the extraction of L-phenylalanine and L-tryptophan, leading to extraction efficiencies to the IL-rich phase ranging between 22 and 100%, still requiring the use of 30–49 wt% of IL + 9–36 wt% of biological buffer.⁵⁶ These results confirm thus the potential of IL-based ABS as effective extraction strategies, as well as the potential of AGB-ILs to substitute the largely investigated imidazolium-based ILs. In summary, the systems investigated display a good performance to extract amino acids from aqueous media, with the additional advantage of using lower amounts of salt in the ABS composition and more biocompatible ILs.

Conclusions

In order to develop more biocompatible ABS, in this work, a series of AGB-ILs (based on glycine-betaine analogues) were synthesized, characterized in terms of ecotoxicity towards the marine bacterium *Vibrio fischeri*, applied in the creation of novel ABS, and evaluated to extract amino acids, namely L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine and L-dopa. The systems investigated demonstrated to be effective extraction routes for amino acids, with extraction efficiencies higher than 60%, and up to 98%, achieved in a

single-step. Besides the negligible ecotoxicity of AGB-ILs, the extraction efficiencies obtained are competitive compared to those reported using ABS formed by imidazolium-based ILs, which present some toxicity and biodegradability issues concerns, while using lower amounts of salt. Thus, the systems investigated here contribute towards the development of more biocompatible ABS, which should be investigated for the extraction/separation of other added-value biological compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.
Chemical structures and abbreviations of the AGB-ILs and amino acids studied.

Figure 2.

EC₅₀ values, in mg.L⁻¹, for 15 min of exposure of the marine bacteria *Vibrio fischeri* to IL aqueous solutions: (■) measured in this work; and (■) values obtained from Ventura *et al.* 42.

Figure 3.

Phase diagrams for the systems composed of AGB-IL + Na₂SO₄ + H₂O at 25 °C:

[MepyrNC₂]Br (◆); [Et₃NC₂]Br (▲); [Pr₃NC₂]Br (●); [Bu₃NC₂]Br (■); [Bu₃PC₂]Br (▲).

The lines correspond to the fitting by Equation 1.

Figure 4.

Extraction efficiency ($EE\%$) of the studied ABS (composed of 40 wt% of AGB-IL + 7.5 wt % Na_2SO_4 at 25 °C) for L-tryptophan (■), L-phenylalanine (■), D-phenylalanine (■), L-tyrosine (■), and L-dopa (■).