

Ionic Liquid-Based Aqueous Biphasic Systems as a Versatile Tool for the Recovery of Antioxidant Compounds

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*The comparative evaluation of distinct types of ionic liquid-based aqueous biphasic systems (IL-ABS) and more conventional polymer/salt-based ABS to the extraction of two antioxidants, eugenol and propyl gallate, is focused. In a first approach, IL-ABS composed of ILs and potassium citrate ($C_6H_5K_3O_7/C_6H_8O_7$) buffer at pH 7 were applied to the extraction of two antioxidants, enabling the assessment of the impact of IL cation core on the extraction. The second approach uses ABS composed of polyethylene glycol (PEG) and potassium phosphate (K_2HPO_4/KH_2PO_4) buffer at pH 7 with imidazolium-based ILs as adjuvants. Their application to the extraction of the compounds allowed the investigation of the impact of the presence/absence of IL, the PEG molecular weight, and the alkyl side chain length of the imidazolium cation on the partition. It is possible to maximize the extractive performance of both antioxidants up to 100% using both types of IL-ABS. The IL enhances the performance of ABS technology. The data puts in evidence the pivotal role of the appropriate selection of the ABS components and design to develop a successful extractive process, from both environmental and performance points of view. © 2014 American Institute of Chemical Engineers *Biotechnol. Prog.*, 31:70–77, 2015*

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Introduction

In many bioengineering and biotechnological processes, the high cost of downstream processing for the purification of the target product is a key issue limiting the viability of novel processes.¹ Recent advances in biotechnology have opened up numerous possibilities for the large-scale production of many bioproducts and more recently, for the recovery of valuable bioactive compounds from various complex matrices (such as fermentation broths, wastes, or raw materials).² The downstream processing of biological or bioactive materials requires a purification platform technology that preserves the chemical structure and the biological activity of the target compounds.³ In this context, liquid–liquid extraction techniques are good choices for bioseparation processes⁴ because they generally provide low interfacial tension, high yields, enhanced selectivity, low processing times, and minimized energy consumption. Moreover, they are operationally simple, cost effective, and easy to scale up.¹ However, the first steps of the liquid–liquid extraction are usually carried out using well-known water immiscible organic solvents,⁴ which are falling out from favor at the biotechnological industry, not only because of the high envi-

ronmental footprint, but also due to the low solubility and potential denaturation of the biomolecules of interest.^{1,4}

Since the pioneering work of Albertsson,⁵ aqueous biphasic systems (ABS) have been the subject of a generous amount of research as promising alternatives to the conventional liquid–liquid extraction in several separation and purification processes.⁶ ABS consist of two immiscible aqueous-rich phases promoted by two incompatible water soluble solutes that can be two polymers,^{7,8} a polymer and a salt^{9,10} or two salts.^{11,12} Nowadays, the range of options regarding the ABS promoting pairs is higher and some alternatives comprising the use of alcohol/salt,^{13,14} acetonitrile/carbohydrates,¹⁵ acetonitrile/polyols,¹⁶ and others including ionic liquids (ILs)¹⁷ are being considered. ILs are in these days known as promising phase-forming agents¹⁸ because they possess versatile chemical structures, due to the possibility to combine their features (cation core, alkyl side chain length or functionalization, and anion moiety) with a wide range of polarities, which is an advantageous characteristic for designing selective extraction technologies.

IL/salt-based ABS were first reported by Rogers and coworkers¹⁸ and since then, several works have dealt with the study of ABS formation using a wide variety of ILs' families with distinct "salting-out" species.¹⁷ Some promising examples on the application of IL/salt-based ABS to the extraction of (bio)molecules have been reported, comprising amino acids,^{19,20} pharmaceuticals,^{21,22} alkaloids,^{23,24} phenolic

Additional Supporting Information may be found in the online version of this article.

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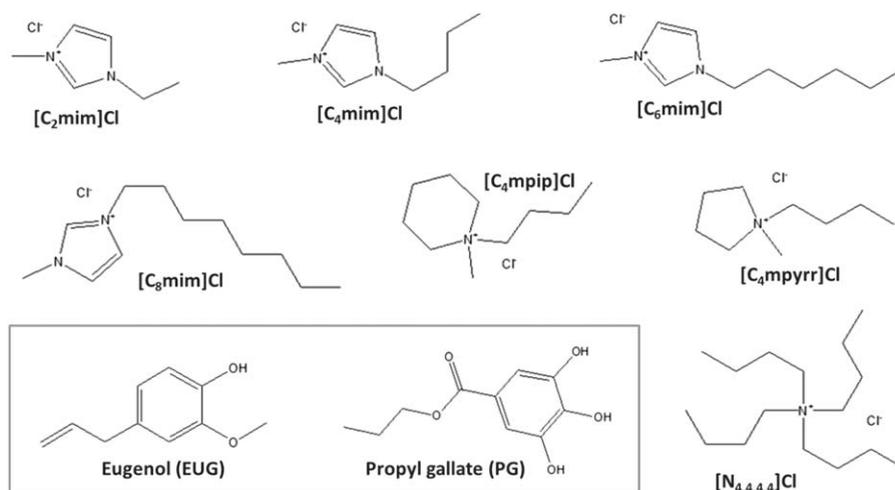


Figure 1. Chemical structure and abbreviated name of the ILs, eugenol, and propyl gallate studied in this work.

compounds,²⁵ natural colorants,²⁶ and enzymes.^{27,28} Recently, Coutinho and coworkers²⁹ proposed the use of ILs as phase separation promoters or adjuvants, introducing the concept of polymer/salt/IL-ABS. The authors developed an alternative approach to manipulate the intrinsic characteristics of the polymer-rich phase adding small amounts of different ILs (≈ 5 wt% in mass fraction composition of the quaternary mixture).²⁹ Such characteristics are a key problem when the target compounds present a more hydrophobic nature. Using such kind of innovative systems, it was possible to boost the extraction of L-tryptophan (an hydrophobic amino acid)²⁹ and, more recently, of some dyes³⁰ and antioxidants³¹ through the accurate selection of the IL characteristics. Despite the large number of works (more than 260 articles in the ISI Web of Knowledge³²) dealing with ILs being studied as ABS separation agents and also ABS used as extraction platforms for the concentration/purification of diverse biomolecules (close to 100 articles in the ISI Web of Knowledge³³), the comparative evaluation of the performance of these systems is still scarce.

Most works in this field apply just one type of IL-ABS to determine the extraction/purification behavior,¹⁷ whereas some of them compare the extraction and/or purification performance of the IL-ABS with the most common polymeric ABS^{26,27,34}; however, the comparative study of different IL-ABS platforms has been poorly explored. In fact, more than the description of the partitioning of various molecules, the specific choice of the most appropriate technology for a certain compound is of utmost importance to achieve a successful extraction and purification from more complex matrices (e.g., human fluids, fermentation media, biomass, and/or residues).^{21,26,27,34,35}

Given the plethora of ABS reported up to date and the inherent advantages of using ILs in their creation, the objective of this work is to provide a survey of the main advantageous characteristics of distinct ABS platforms. For comparison purposes, three types of systems (polymer/salt-based, polymer/salt/IL-based, and IL/salt-based ABS) were chosen to perform a systematic study about the partitioning of two antioxidants widely used in cosmetics, pharmaceutical, and food industries among others—eugenol (EUG)³⁶ and propyl gallate (PG),³⁷ herein adopted as model compounds. At the end, important insights for IL-ABS appropriate design can be ascertained, bringing new perspectives for their application in the extraction of more valuable compounds from

complex matrices (e.g., pharmaceutical and food wastes, various biomass sources, or reaction media).

Materials and Methods

Chemicals

EUG with purity of 99 wt% and PG with purity ≥ 98 wt% were both purchased at Sigma-Aldrich[®]. The series of 1-alkyl-3-methylimidazolium chloride ILs [C_{*n*}mim]Cl, including the 1-ethyl-3-methylimidazolium chloride, [C₂mim]Cl (>98 wt%), 1-butyl-3-methylimidazolium chloride, [C₄mim]Cl (99 wt%), 1-hexyl-3-methylimidazolium chloride, [C₆mim]Cl (>98 wt%), 1-methyl-3-octylimidazolium chloride, [C₈mim]Cl (99 wt%), and the remaining nitrogen-based cyclic ILs, 1-butyl-1-methylpiperidinium chloride, [C₄mpip]Cl (99 wt%), and 1-butyl-1-methylpyrrolidinium chloride, [C₄mpyrr]Cl (99 wt%), were acquired from IoLiTec (Ionic Liquids Technology). The tetrabutylammonium chloride, [N_{4,4,4,4}]Cl (≥ 97 wt%), was purchased from Sigma-Aldrich[®]. The chemical structures of all compounds used in this work are depicted in Figure 1. The purity of each IL was further checked by ¹H and ¹³C NMR and found to be in close agreement with the purity level indicated by the suppliers.

The components of the potassium citrate buffer (C₆H₅K₃O₇/C₆H₈O₇), potassium citrate tribasic monohydrate, C₆H₅K₃O₇·H₂O (≥ 99 wt%), and citric acid monohydrate, C₆H₈O₇·H₂O (100 wt%), were purchased from Acros Organics and Fisher Scientific, respectively. The salts composing the potassium phosphate buffer (K₂HPO₄/KH₂PO₄), potassium phosphate monobasic, KH₂PO₄ (99.5 wt%), and potassium phosphate dibasic, K₂HPO₄ (>98 wt%) were supplied by Sigma-Aldrich[®] and JMVP, respectively. The polyethylene glycol (PEG) polymers with average molecular weights of 1500 and 8000 g mol⁻¹ were both purchased from Sigma-Aldrich[®]. The water used was double distilled, passed by a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification apparatus.

Experimental phase diagrams, tie-lines (TLs), and tie-line lengths (TLLs)

In order to complete the series of IL/salt-based ABS studied in this work, the experimental phase diagrams for the

systems composed of $[C_4\text{mpyrr}]Cl$ and $[C_4\text{mpip}]Cl + C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O were determined, using the cloud point titration method at 298 (± 1) K and atmospheric pressure. The experimental procedure adopted follows a general protocol already established in previous works.³⁸ Aqueous stock solutions of IL at ca. 70 wt% and $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 at ca. 50 wt%³⁹ were prepared. Drop-wise addition of the buffer solution was performed to each IL aqueous solution until the detection of a cloudy point (biphasic region) alternately with the drop-wise addition of water until the appearance of a clear solution (monophasic region).

For most systems, information on the TLs and TLLs was already reported in literature.^{21,23,40} Still, some TLs were determined for this work. These were measured through a well-established gravimetric method⁴¹ previously validated by us.³⁸ A mixture at the biphasic region was prepared, vigorously stirred, and allowed to reach the equilibrium by phase separation for at least 18 h at 298 (± 1) K, using small glass vials (10 mL) specially designed for this purpose. The phases were separated and each one was carefully weighed within $\pm 10^{-4}$ g. The TLs were calculated by the application of the lever-arm rule, being their lengths (i.e., TLLs) described as the Euclidean distance between the top and bottom phase compositions as denoted in Supporting Information Eq. A1. The experimental solubility curves were correlated using the Merchuk equation,⁴¹ which is provided in the Supporting Information Eq. A2.

Partitioning studies of EUG and PG in IL/salt-based ABS

The ABS were prepared gravimetrically using glass vials by adding the appropriate amounts of each IL, of a buffer solution of $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 (at ca. 50 wt%)³⁹ and of an aqueous solution containing ≈ 1 g L^{-1} of either EUG or PG (with an uncertainty of $\pm 10^{-4}$ g). At the end, ABS composed of 40 wt% of IL + 20 wt% of $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + 40 wt% of H_2O with a total weight of 5 g and ≈ 0.2 g L^{-1} of final concentration of the antioxidants in the entire ABS were obtained. Considering the experimental phase diagrams here determined ($[C_4\text{mpyrr}]Cl$ and $[C_4\text{mpip}]Cl + C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O) and those reported elsewhere ($[N_{4,4,4,4}]Cl^{21}$ and $[C_4\text{mim}]Cl^{23} + C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O), the selected mixtures were able to be separated into two clear phases, the IL-rich phase being the top and the $C_6H_5K_3O_7/C_6H_8O_7$ -rich phase being the bottom layer. At this point, the phases were carefully separated and collected for the measurement of their weight (uncertainty of $\pm 10^{-4}$ g), volume (uncertainty of ± 0.1 mL) and pH. The pH of both IL- and salt-rich phases was measured using a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity equipment with an uncertainty of ± 0.02 .

Partitioning studies of EUG and PG in polymer/salt-based and polymer/salt/IL-based ABS

The binodal data for the polymer/salt/IL-based ABS here selected to carry out the optimization study of EUG and PG partitioning are already published elsewhere.³⁰ From the array of experimental conditions investigated by the authors, only the most relevant for the present work were adopted, namely PEGs of two distinct molecular weights, 1500 and 8000 g mol^{-1} , the K_2HPO_4/KH_2PO_4 buffer at pH 7, and the series of $[C_n\text{mim}]Cl$ (n varying from 2 to 8) ILs as adju-

vants. The quaternary ABS were gravimetrically prepared (with an uncertainty of $\pm 10^{-4}$ g) using glass vials by adding the appropriate amounts of PEG, K_2HPO_4/KH_2PO_4 at pH 7 (at ca. 26 wt%) buffer solution, $[C_n\text{mim}]Cl$ and distinct aqueous solutions containing ≈ 2 g L^{-1} of EUG or PG. Biphasic mixtures composed of 15 wt% of PEG + 15 wt% of K_2HPO_4/KH_2PO_4 pH 7 + 5 wt% of $[C_n\text{mim}]Cl$ + 65 wt% of H_2O were obtained at the end. In the specific case of the conventional polymer/salt-based ABS, no IL was added to the mixture, being the ternary system prepared with 15 wt% of PEG + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + 70 wt% of H_2O . For each system, a final weight of 5 g and antioxidant concentration at ca. 0.3 g L^{-1} (considering the total mixture) were obtained. The mixture compositions were selected taking into account the experimental phase diagrams reported elsewhere.³⁰ Two clear phases with a well-defined interface were observed. The phases were then separated and collected to determine their weight (uncertainty of $\pm 10^{-4}$ g) and volume (uncertainty of ± 0.1 mL). In this case, the top was a PEG-rich or PEG/IL-rich phase and the bottom is defined as the salt-rich phase as previously reported³⁰ and suggested by the partition coefficients of the IL (K_{IL} , defined as the ratio between the absorbance output of the IL at 211 nm in the top and the bottom phases).

Antioxidants quantification and determination of the extraction efficiency

The quantification of each antioxidant, PG and EUG, was assayed using a SHIMADZU UV-1700 Pharma-Spec Spectrophotometer at 272 and 279 nm, respectively (based on calibration curves previously determined). The quantification of each molecule was performed in triplicate and at least three different assays for each system were carried out, being further reported the average values and the respective standard deviations associated. Any possible interference of the ABS components (IL, salt, and/or polymer) within the quantification method was eliminated by the systematic application of blank controls, being in these systems the antioxidant aqueous solution replaced by water.

The extraction efficiencies of each antioxidant (EE_{AO} , %) were calculated for each system according to Eq. (1),

$$EE_{AO}, \% = \frac{[AO]_T \times V_T}{[AO]_i \times V_i} \times 100 \quad (1)$$

where V_i and $[AO]_i$ are the initial volume and the initial antioxidant concentration (being AO substituted by EUG or PG, depending of the antioxidant studied) of the whole ABS, whereas V_T and $[AO]_T$ represent the volume of the top phase and the antioxidant concentration in the same phase (IL-rich, PEG-rich and PEG/IL-rich phase in IL/salt-based, PEG/salt-based and PEG/salt/IL-based ABS, respectively).

Results and Discussion

Phase diagrams and tie-lines of IL/salt-based ABS

Aiming at completing the series of ILs to be studied in this work, the experimental solubility curves of the systems composed of $[C_4\text{mpyrr}]Cl + C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O and $[C_4\text{mpip}]Cl + C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O were determined. The data of the phase diagrams in mass fraction units as well as the respective regression parameters (A , B ,

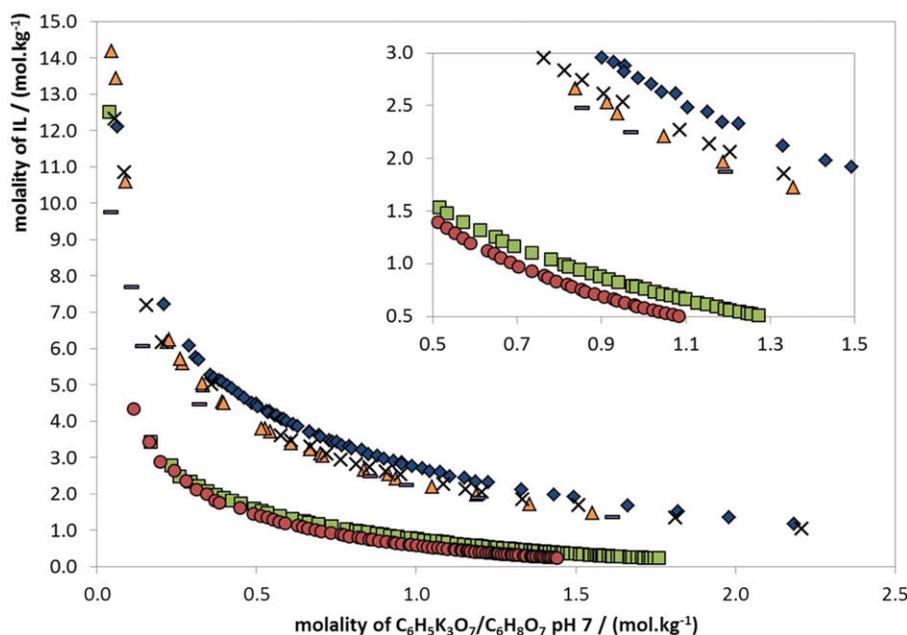


Figure 2. Binodal curves for the systems composed of IL + $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O at $298 (\pm 1)$ K and atmospheric pressure: $[P_{4,4,4,4}]Cl$ (●),⁴⁰ $[N_{4,4,4,4}]Cl$ (■),²¹ $[C_4mpyr]Cl$ (×), $[C_4mpip]Cl$ (▲),⁴⁰ $[C_4mpip]Cl$ (◐), and $[C_4mim]Cl$ (◆).²³

and C), obtained by applying Merchuk Equation (Eq A2 in Supporting Information)⁴¹ standard deviations (std) and correlation coefficients (R^2) are provided in Supporting Information (Table A1).

Using experimental data reported elsewhere,^{21,23,40} it was possible to analyze the ability of ILs with different cation cores, namely $[C_4mim]Cl$, $[C_4mpyr]Cl$, $[C_4mpyr]Cl$, $[C_4mpip]Cl$, $[N_{4,4,4,4}]Cl$, and $[P_{4,4,4,4}]Cl$, to induce ABS formation. As depicted in Figure 2, their ability increases following the order $[C_4mim]Cl < [C_4mpyr]Cl < [C_4mpyr]Cl < [C_4mpip]Cl \ll [N_{4,4,4,4}]Cl < [P_{4,4,4,4}]Cl$. The behavior of the series of IL + $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + H_2O systems here assessed is in good agreement with the results of previous studies where other “salting-out” agents, as K_2HPO_4/KH_2PO_4 buffer³⁸ or $C_6H_5K_3O_7$ salt¹⁹ were applied. In general, the nitrogen-based cyclic ILs, $[C_4mim]Cl$ and $[C_4mpyr]Cl$ (both with 5-atom rings), and $[C_4mpyr]Cl$ and $[C_4mpip]Cl$ (both with 6-atom rings), are less prone to induce phase separation when compared with the acyclic ones ($[N_{4,4,4,4}]Cl$ and $[P_{4,4,4,4}]Cl$), being this behavior closely correlated with the molar volume of the ILs.³⁸

All information on the TLs and respective TLLs further used in the partitioning tests is represented in Supporting Information (Table A2), along with the pH of both top and bottom phases.

Optimization study of the antioxidants partitioning in IL/salt-based ABS

The optimization of the partition of EUG and PG by applying IL/ $C_6H_5K_3O_7/C_6H_8O_7$ -based ABS was carried out considering the most representative ABS of those analyzed above, namely composed of three nitrogen-based cyclic ILs, $[C_4mpyr]Cl$, $[C_4mpip]Cl$ (both nonaromatic), and $[C_4mim]Cl$ (aromatic), and one quaternary ammonium compound, $[N_{4,4,4,4}]Cl$.

The results obtained are shown in Figure 3 and reveal that the total partition of the two antioxidants toward the IL-rich phase

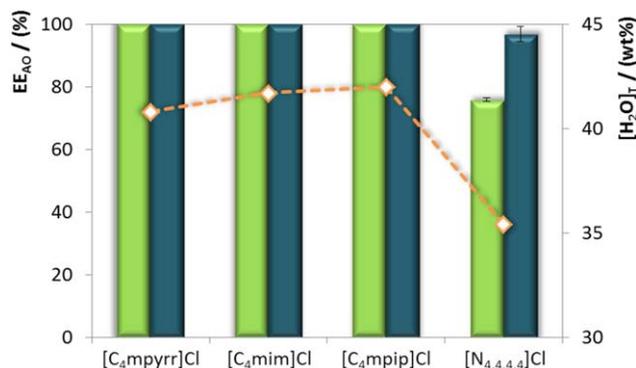


Figure 3. Extraction efficiency data for EUG (■) and PG (■) achieved with IL/salt-based ABS composed of 40 wt% of IL + 20 wt% of $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 + 40 wt% of H_2O at $298 (\pm 1)$ K and atmospheric pressure along with the weight fraction of H_2O present in the top phase ($[H_2O]_T$, ◇).

was achieved for systems containing nitrogen-based cyclic ILs (EE_{EUG} and $EE_{PG} = 100\%$). Having into account the ABS containing the quaternary ammonium $[N_{4,4,4,4}]Cl$, it is observed that PG completely migrates for the more hydrophobic phase ($EE_{PG} = 96.8 \pm 2.4\%$), whereas EUG partitions more similarly between the two phases, although with a preferential migration for the IL-rich phase ($EE_{EUG} = 75.9 \pm 0.6\%$). The fact that both molecules are more likely to be concentrated in the IL-rich phase is easily justified by their octanol–water partition coefficient ($\log P_{EUG} = 2.99$ ⁴² and $\log P_{PG} = 0.97$ ⁴³) indicating their higher preference for more hydrophobic phases ($\log P > 0$).

Despite its lower hydrophobicity, slightly higher EE_{PG} than EE_{EUG} values were noticed. This phenomenon seems to be related with the higher number of hydroxyl groups of PG, allowing stronger interactions with the water molecules present in the top phase via hydrogen bonds. The picture emerging from these data indicates that not only “antioxidant-IL” interactions contribute to the partitioning phenomenon but also the

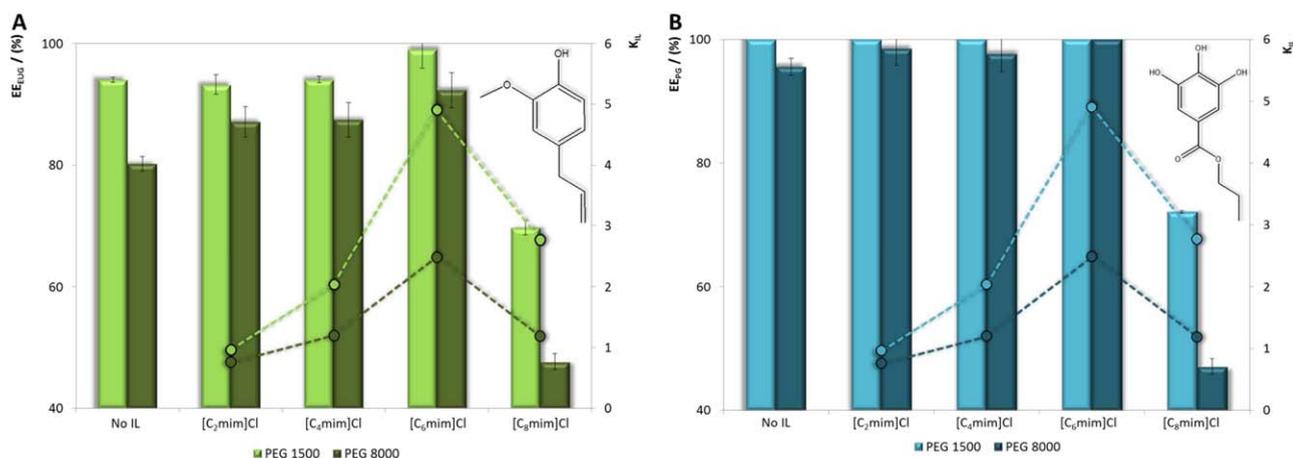


Figure 4. Extraction efficiency data for EUG (EE_{EUG} , %)—(A)—and PG (EE_{PG} , %)—(B)—obtained for different PEG/salt/IL-based ABS composed of 15 wt% of PEG + 15 wt% K_2HPO_4/KH_2PO_4 + 70 wt% of H_2O (no IL) and 15 wt% of PEG + 15 wt% of K_2HPO_4/KH_2PO_4 pH 7 + 5 wt% of $[C_n\text{mim}]Cl$ + 65 wt% of H_2O at $298 (\pm 1)$ K and atmospheric pressure. The EE_{AO} results are represented by the bars and the K_{IL} values are described by the circles with different colors. The partition coefficients of each one of the ILs (K_{IL}) toward the top (PEG-rich) phase applied as adjuvants are indicated as reported elsewhere.³⁰

“antioxidant- H_2O ” interactions have a significant impact (Figure 3). Furthermore, this also justifies the slightly lower EE_{AO} obtained for the ammonium-based ABS ($EE_{EUG} = 75.9 \pm 0.6\%$ for EUG and $EE_{PG} = 96.8 \pm 2.4\%$ for PG) when compared with those gauged using the remaining systems ($EE_{AO} = 100\%$). Actually, the $[N_{4,4,4,4}]Cl$ -rich phase is poorer in water (35.4 wt%) than the remaining systems (40.8 wt% for the $[C_4\text{mpyr}]Cl$ -rich phase; 41.7 wt% for the $[C_4\text{mim}]Cl$ -rich phase; 42.0 wt% for the $[C_4\text{mpip}]Cl$ -rich phase), which is decreasing the “antioxidant- H_2O ” interactions—Figure 3. Evidences related with this joint contribution of “molecule-IL” and “molecule- H_2O ” interactions in the partitioning of compounds between the coexisting phases of IL/salt-based ABS were also established in previous works using paracetamol²¹ and natural red colorants.²⁶

Optimization study of the antioxidants partitioning in polymer/salt-based and polymer/salt/IL-based ABS

The application of ILs as adjuvants in polymer/salt-based ABS in the partition of PG and EUG was investigated by varying the following parameters: the presence/absence of IL as adjuvant; the increase in the alkyl side chain of the $[C_n\text{mim}]^+$ cation ($n = 2, 4, 6,$ and 8); and finally, the variation of the PEG molecular weight (1500 and 8000 $g\ mol^{-1}$). The experimental data obtained for the EE_{EUG} and EE_{PG} with PEG/salt/IL-based ABS are represented in Figures 4A,B, respectively, and in Supporting Information Table A3. As shown, the presence of these ILs in the polymeric ABS here discussed in terms of partition coefficient data of the different ILs studied (K_{IL} represented by the circles in Figure 4), boosts the EE_{AO} for both compounds. The presence of some ILs in the top layer ($K_{IL} > 1$) is likely incrementing and allowing some specific interactions of the antioxidant with the polymer-rich phase, consequently inducing their concentration into the upper phase (both with $\log P > 0$ ^{42,43} as discussed above). Actually, the addition of small amounts of ILs in the polymer/salt systems is allowing the creation of some specific interactions between the IL, the polymer, and the salt, responsible for some changes regarding the polarity of both top and bottom phases. These results are in agreement with the evidences described in Pereira et al.²⁹ work,

where the ability to extract the tryptophan (a hydrophobic amino acid) using the conventional PEG/salt-based ABS was improved by using some ILs as adjuvants. Furthermore, the presence of aromatic rings in the antioxidant molecules explains the increased affinity for the polymer/IL-rich phase, due to the $\pi \cdots \pi$ interactions that may take place between the antioxidant and the imidazolium cation. The effect of the alkyl side chain length of the $[C_n\text{mim}]^+$ cation in the partitioning of both target antioxidant molecules is given by the following trend: $[C_8\text{mim}]^+ < [C_2\text{mim}]^+ \approx [C_4\text{mim}]^+ < [C_6\text{mim}]^+$. For ILs up to $[C_6\text{mim}]Cl$, i.e., with a crescent hydrophobicity nature, the EE_{AO} are improved, being these parameters the highest for the IL $[C_6\text{mim}]Cl$ and both target molecules (Figure 4). This phenomenon seems to be related with the most extensive partition of the $[C_6\text{mim}]Cl$ toward the top phase when compared with the remaining three ILs³⁰ (as indicated by the K_{IL} data represented in Figure 4 and Supporting Information Table A3). Meanwhile, $[C_8\text{mim}]Cl$ is inducing a trend-shift in this behavior, which may be related with the formation of aggregates for ILs with longer alkyl chains (more than six carbons) in aqueous environment⁴⁴ as recently discussed by Passos et al.²³

The impact of the PEG molecular weight, 1500 and 8000 $g\ mol^{-1}$, on the partitioning of these two compounds was also assessed. Higher EE_{AO} were achieved and adopted for the polymer with the lower molecular weight (PEG 1500): $69.7 \pm 1.2\% < EE_{EUG} < 99.0 \pm 3.1\%$ —Figure 4A—and $72.1 \pm 0.2\% < EE_{PG} < 100\%$ —Figure 4B. The higher K_{IL} obtained for PEG 1500 when compared with PEG 8000 is again governing the partitioning phenomenon (Supporting Information Table A3). Other factor that may be influencing the EE_{AO} parameters is the so-called “volume exclusion effect.”^{45,46} In literature, the influence of the polymer molecular weight on the partitioning of various biomolecules, namely enzymes and proteins, in conventional PEG/salt-based ABS has been intensively justified based on it, suggesting that for higher molecular weights of PEG, lower volume is available for the molecules.^{45,47–49}

Comparative assessment: IL-based vs. polymeric-based ABS

Taking into account the results described for the three ABS approaches investigated, it is concluded that both the

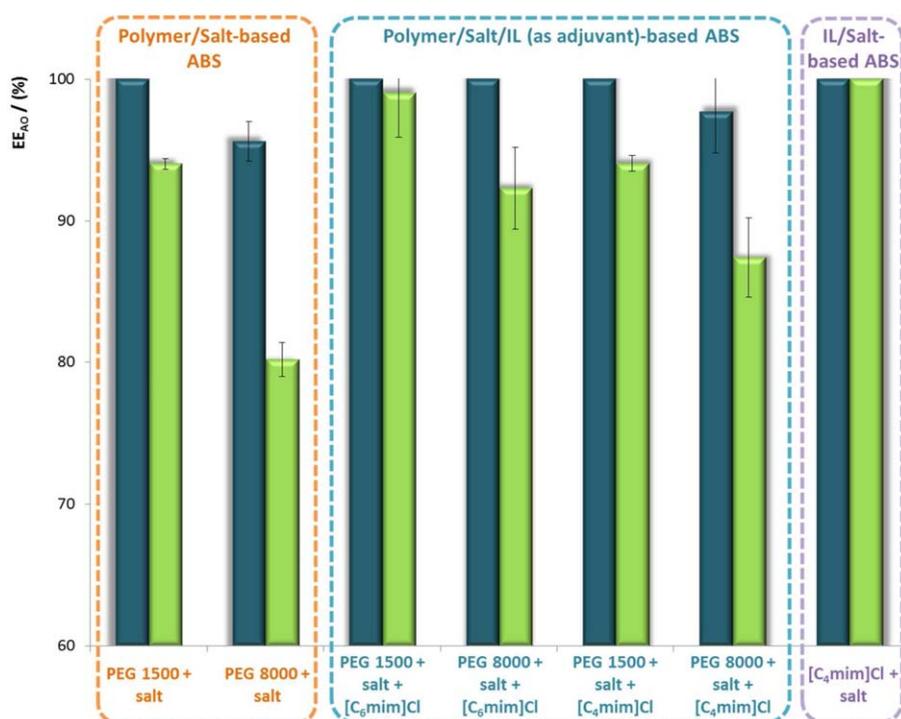


Figure 5. Compilation and comparison of the extraction efficiency data for both antioxidant models (EE_{AO} , %), EUG (■) and PG (■), obtained for the different extraction technologies investigated in this work: polymer/salt-based, polymer/salt/IL-based, and IL/salt-based ABS.

PEG/salt/IL- and the IL/salt-based ABS are more successful in achieving a complete partition of PG than EUG. The weaker efficiencies attained for EUG when common polymeric-based ABS are applied (mainly for PEG 8000) could be explained by the occurrence of less tuned, thus nonspecific interactions, when compared with systems where ILs are present. Again, it seems that the $\pi\cdots\pi$ stacking interactions (between imidazolium cation and EUG aromatic rings) and other “EUG–IL” interactions are decisive for the maximization of the extractive performance. Moreover, the higher number of hydroxyl groups present in the PG seem to create additional “antioxidant–H₂O” and “antioxidant–IL” interactions, which drive its migration toward the phases enriched in IL. By analyzing the results of EUG, the differences in its preferential reconcentration in one ABS phase are clear. The IL-ABS are more efficient than polymer-based ones, because they can achieve a stronger migration of EUG for the top (IL)-rich phase, which is not observed for the remaining systems. From the results in Figure 5, and to summarize these effects, it is possible to draw a tendency considering the crescent reconcentration profile for both model antioxidants, described by:

PG: PEG 8000/salt < PEG 8000/salt/[C₄mim]Cl < PEG 1500/salt/[C₄mim]Cl \approx PEG 1500/salt \approx PEG 8000/salt/[C₆mim]Cl \approx PEG 1500/salt/[C₆mim]Cl \approx [C₄mim]Cl/salt

EUG: PEG 8000/salt < PEG 8000/salt/[C₄mim]Cl < PEG 8000/salt/[C₆mim]Cl < PEG 1500/salt \approx PEG 1500/salt/[C₄mim]Cl < PEG 1500/salt/[C₆mim]Cl \approx [C₄mim]Cl/salt

The results here reported show that to advance in the study of IL-ABS considering their use as a platform for extractive technologies, the comparison between the performances of different systems and approaches is required. The adequate screening of various ABS technologies involving ILs is the key for an accurate optimization of the extrac-

tion systems for further scale-up, together with detailed studies considering the extraction performance of these technologies focused in target biomolecules from complex matrices, the evaluation of their environmental impact, economic profile (including losses of the separation agents, loss of stability of the target molecules, or even loss of their activity), then progressing for the scale up in laboratory and pilot scales.

Conclusions

A comparison between the extractive performances of distinct types of IL-ABS was carried out in this work. The IL/salt-based ABS were able to extract both antioxidants, revealing EE_{AO} ranging from $75.9 \pm 0.6\%$ up to 100%. The polymer/salt/IL-based ABS revealed also a high extractive performance, ranging the EE_{AO} from $47.1 \pm 1.3\%$ to 100%. From the gathered data, it is possible to identify the most advantageous ABS to extract PG and EUG: for the IL/salt-based ABS, the nitrogen-based cyclic ILs are more effective candidates, when compared to the quaternary ammonium compounds; for the ABS using ILs as adjuvants, the [C₆mim]Cl and the polymer with the lower molecular weight are the most beneficial conditions. Based on the reported data, it is possible to regard IL-ABS as promising techniques to be applied in extraction processes. It is possible to manipulate their performance and cost-effectiveness by the appropriate choice of the IL structural features and application mode (either as main component or adjuvant). Moreover, it seems to be possible to achieve EE_{AO} around 100%, not only with the IL/salt-based ABS but also using polymer/salt/IL-based systems in which only 5 wt% of IL are added, meaning that it is possible to simultaneously maintain a higher extraction performance reducing the environmental

impact of the technology, and also the costs associated to the IL. However, it was also shown on this study that, for certain molecules, the extraction performance could be decreased and thus, a careful and through analysis must be carried out, taking into account the cost of ILs, value of the molecules to be purified, extractive performance of the purification technologies, and finally, the energy consumption and cost of recycling or recovering the separation agents applied in the extractive technologies.

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