

Understanding the Role of Cholinium Carboxylate Ionic Liquids in PEG-Based Aqueous Biphasic Systems

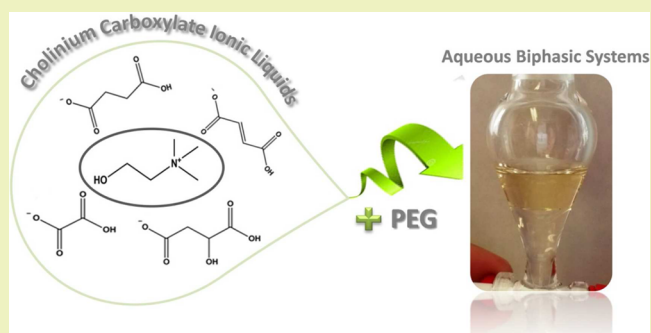
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Supporting Information

ABSTRACT: This work aims at exploring new sustainable separation processes based on ionic liquids. Aqueous biphasic systems (ABS) based on poly(ethylene glycol) (PEG) with low molecular weight (600 and 4000 g mol⁻¹) and cholinium-based ionic liquids and salts containing anions derived from carboxylic acids (oxalate, malonate, succinate, L-malate, fumarate, glutarate and citrate), available in natural compounds, are here presented. Contrary to common ionic liquids, the cholinium-based ionic liquids used in this work are biodegradable, nontoxic, cheap, and simple to prepare, and PEG is also a cheap and nontoxic phase promoter agent. The data reported in this work allows novel insights into the phase splitting mechanism of these ABSs regarding the influence of alkyl chain length of the anion and the presence of substituent groups in the anion. The effect of PEG molecular weight in the ABS was also addressed. Furthermore, the possible application of these systems for the extraction/separation of antioxidants, namely, *tert*-butylhydroquinone (TBHQ), was evaluated.

KEYWORDS: Cholinium carboxylate ionic liquids, Poly(ethylene glycol) (PEG), Aqueous biphasic systems, Antioxidants



INTRODUCTION

In the last decades, investigations dealing with the reduction of the ecological footprint, developing more sustainable (cheaper, greener, and safer) alternatives to the current processes, and fossil-based chemical products have been stimulated.^{1,2} Separation and purification technologies play a central role in the chemical industry not only due to their impact in the quality of the final product but also due to the high level of energy and enormous amounts of solvent mixtures to separate. In this context, aqueous biphasic systems (ABS), largely applied in bioseparation processes, have been proposed as alternatives to other liquid–liquid extraction techniques³ due to their many advantages such as high effectiveness, high yield, high purity degree, low cost, and technological simplicity.⁴ Because these systems are mainly composed of water, in contrast to other liquid–liquid extraction approaches where volatile organic solvents are used, they fulfill the requisites regarding green chemistry principles.⁵

ABS are liquid–liquid extraction systems that are characterized by the formation of two mutually incompatible aqueous phases above the critical concentration of system components. These components are soluble in water, but they have different chemical structures, polarities, and hydration capabilities, allowing for the separation of valuable and chemically diverse molecules such as amino acids,⁶ antibiotics,⁷ enzymes,^{8,9} alkaloids,¹⁰ and natural compounds.¹¹ This separation is a result of direct equilibrium and a selective distribution of the molecule of interest between the two aqueous phases.

Currently, ABS based on polymer–polymer, polymer–salt, and salt–salt combinations have been successfully implemented not only for the separation of biologically relevant molecules but also for other chemicals.^{12,13}

Since 2003,¹⁴ ionic liquids (ILs) have also been proposed as efficient compounds to be used in ABS. These fluids combine interesting properties such as negligible vapor pressure, low flammability, large liquid range, high thermal stability, and above all the ease to tailor their properties by the adequate manipulation of ions arrangement. The combination of ILs with a variety of inorganic salts and polymers allows for specific and efficient separation of a wide diversity of molecules.¹² However, despite ILs being considered “green solvents” essentially due to their low volatility, a large number, especially those based on imidazolium and pyridinium cations combined with fluorinated or other anions, are poorly biodegradable and highly toxic.^{15–18} As a result, again using the highly tunable properties presented by this class of fluids, in recent years, new benign and nontoxic ILs have been synthesized through simple and economical procedures from environmentally friendly precursors,^{19–23} such as cholinium chloride ([Ch]Cl), usually called choline. This salt is known to be biodegradable, nontoxic, and easy to produce and is used as an essential nutrient.²⁴ Cholinium-based ionic liquids have shown low toxicity and good biodegradability.^{22,25}

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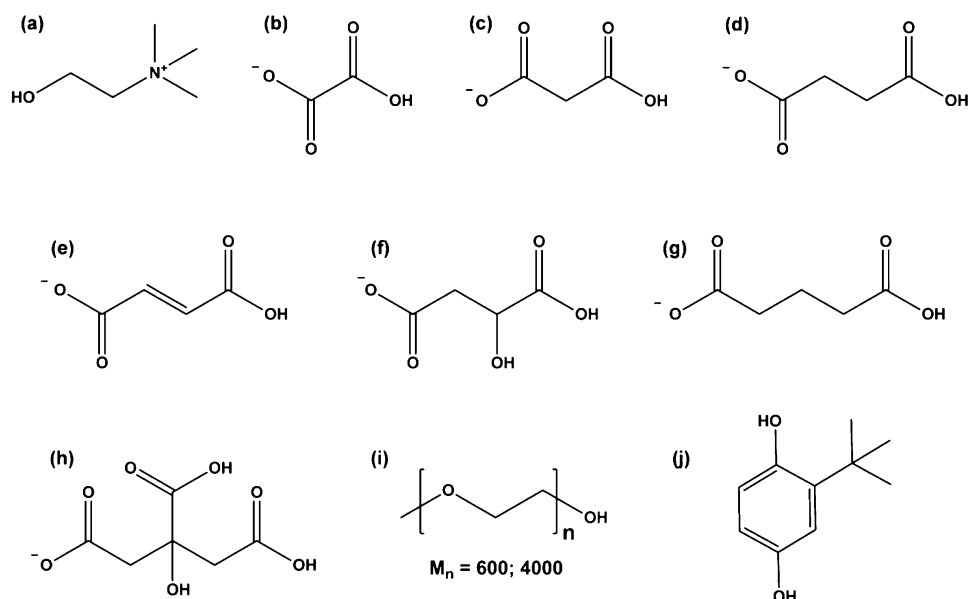


Figure 1. Chemical structure and short names of the cations, anions, polymers, and antioxidants studied: (a) cholinium ([Ch]), (b) oxalate ([Ox]), (c) malonate ([Mal]), (d) succinate ([Suc]), (e) fumarate ([Fum]), (f) L-malate (L-ma), (g) glutarate ([Glu]), (h) dihydrogenecitrate (Cit), (i) poly(ethylene glycol) (PEG 600; PEG 4000), and (j) *tert*-butylhydroquinone (TBHQ).

Therefore, the combination of the cholinium cations with carboxylated anions derived from sustainable organic acids provides a suitable environmentally friendly and nontoxic pathway to develop new benign extraction schemes.

Shahriari et al.²⁶ used cholinium-based ILs combining different anions derived from organic acids to prepare ABS using K_3PO_4 (as the inorganic salt), a strong salting out agent. The overall trend of these ILs in forming ABS is in agreement to what was previously observed for other ionic liquid families, such as imidazolium-based ILs.^{6,27,28} The mechanism behind the phase formation of IL + inorganic salt (IS) systems is the competition between the IL and the IS for the water molecules, which is won by the latter because it is more able to form hydration complexes. Consequently, the ILs structural characteristics, for instance, the number of hydroxyl groups and size of the hydrocarbon moiety of the IL ions, have a large impact on phase behavior.

Even though cholinium-based ILs are able to form ABS in the presence of salting out species,²⁶ their ability to promote phase separation is lower than that of other IL families constituted by different cations, such as dialkylimidazolium, alkylpyrrolidinium, tetralkylphosphonium, or tetralkylammonium,²⁹ because they are more hydrophilic and thus do not undergo phase separation as easily as other hydrophobic ILs. In this way, polymer + cholinium ILs ABS provide an interesting alternative. However, only a few reports on this topic exist in the literature. Li et al.¹⁷ prepared ILs combining the cholinium cation and diverse anions (formate, acetate, propionate, butyrate, glycolate, lactate, benzoate, dicholinium oxalate, and tricholinium citrate). These authors used these ILs and poly(propylene glycol) (PPG) 400 to prepare ABS for protein extraction. Liu et al.³⁰ compared polymer (PPG 400, PPG 1000, and poly(propylene glycol)-*block*-poly(propylene glycol)-*block*-poly(propylene glycol, $EO_{10}PO_{90}$) + IL ABS formed by three different cholinium-based ILs with anions derived from small organic acids (lactate, propanoate, and glycolate). Pereira et al.³¹ reported that for systems containing poly(ethylene glycol) (PEG) polymers and various cholinium-based salts with

different chemical structures, with melting points higher than 100 °C, the phase splitting mechanism is dominated by the solvation of these salts in water, in a similar manner to that of IL + IS or PEG + IS systems. On the other hand, for cholinium-based ILs (with melting points below 100 °C), these authors concluded that the balance between the interactions of PEG–water and ILs–water seems to rule the phase separation mechanism. The most complete study on ABS composed of PEG polymers with a large number of imidazolium-based ILs combining different anions was published by Freire et al.³² These authors show that both the ILs and the PEG polymers can act as salting out species depending on the chemical features of the IL. In conclusion, it can be affirmed that while hydrophilic ILs display two phase formation with either ISs or polymers, such as PPG and PEG, the mechanism of phase formation is quite different in the two cases. In the case of the IL + IS systems, the mechanism is ruled by the formation of hydration complexes and the affinity of the ions for water, while for the polymer + IL systems, a more intricate mechanism has been recently proposed,³³ where both the IL and the polymer become independently solvated by water through the establishment of hydrogen bonds between water and the IL and water and the polymer, separately.

In order to further investigate the ABS formation of very hydrophilic ILs and salts and PEG polymers, the cholinium cation was combined with a variety of anions derived from naturally occurring organic acids (diacids of oxalate, malonate, succinate, fumarate, L-malate, glutarate; triacid of citrate) with variable alkyl chain lengths and different substituent groups. Figure 1 depicts the chemical structures of the cations and the anions contained in the ILs/salts used, which were chosen to provide insights on several structural effects such as the increase in the alkyl chain length and the presence of substituent groups. This careful selection of the anions is very important because it will allow unveiling if the definition of IL (and salt) is or not correlated to the phase splitting mechanism of ABS. Several phase promoting agents, such as inorganic salts and PEG-based polymers, were tested. With the exception of cholinium

glutarate and cholinium succinate previously published,²⁶ no phase splitting was observed when K_3PO_4 , the most powerful salting out inorganic salt, was used indicating that the hydration capacity of this salt is not high enough to compete with the prepared cholinium-based ILs/salts for the available water. On the other hand, PEG polymers (Figure 1) were successful in the formation of two phase systems and are thus explored, as they are interesting polymers from the industry point of view due to their biodegradability, low toxicity, and low cost.

Nowadays, antioxidants are extensively studied and are known as “free radical scavengers”;^{34,35} these compounds interact with free radicals and neutralize them and prevent cellular damage, including damage that may lead to cancer. A large number of chemicals have antioxidant activity,³⁵ and they have many application areas such as dietary supplements, food preservatives, oil additives, and materials preservatives.³⁶ Once antioxidants are widely used, their extraction from natural sources is an important research focus. In this context, the systems with PEG 600 characterized were used as models for the extraction of *tert*-butylhydroquinone (TBHQ) (Figure 1), which is an aromatic molecule derived from hydroquinone with a great antioxidant power. TBHQ is widely used in the biodiesel and oil industries as an additive,³⁷ and also in the food industry as a preservative in vegetable oils and animal fats.³⁸

EXPERIMENTAL SECTION

Materials. PEG polymers of average molecular weights 600 and 4000 g mol⁻¹ (abbreviated as PEG 600 and PEG 4000, respectively), cholinium bicarbonate solution (76% in H₂O), oxalic acid (99%), malonic acid (99%), succinic acid (99%), fumaric acid (99%), L-malic acid (99%), glutaric acid (99%), citric acid (99.5%), and diethyl ether (99.8%) were purchased from Sigma-Aldrich and used as received. *tert*-Butylhydroquinone (TBHQ) with assay ≥98% was supplied by Fluka and was used as partitioning solute. Double distilled water, passed through a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment, was used in all experiments.

Cholinium Carboxylate Ionic Liquids/Salts. The ionic liquids/salts used in this work, namely, cholinium oxalate ([Ch][Ox]), cholinium malonate ([Ch][Mal]), cholinium succinate ([Ch][Suc]), cholinium ([Ch]fum), cholinium L-malate ([Ch]L-ma), cholinium glutarate ([Ch][Glu]), and cholinium citrate ([Ch]Cit), were prepared by dropwise addition of the corresponding acid (1:1) to aqueous cholinium bicarbonate, following an established procedure.^{22,39} The mixtures were stirred at room temperature and pressure for 12 h. The resulting products were washed with diethyl ether to remove unreacted acid. Excess of water and traces of other volatile substances were removed first by rotary evaporation and then by stirring and heating under vacuum. The chemical structures and purities of the synthesized cholinium-based ILs were confirmed by ¹H and ¹³C NMR (see Supporting Information for further details). All the ionic liquid samples were dried prior to their use by stir-heating under vacuum at moderate temperature (40–50 °C, >48 h, ~0.01 mbar). Figure 2 shows the prepared cholinium carboxylate ionic liquids at room temperature after the drying procedure. Their water contents were determined by Karl Fischer titration (831 KF coulometer, Metrohm) and were considered in all experiments.

Thermal Properties Analysis. The thermal stabilities and decomposition temperatures of the prepared cholinium carboxylate ionic liquids and salts were measured using a thermal gravimetric analyzer (TA Instruments Model TGA Q50). The samples were placed inside aluminum pans and heated to 450 °C at a heating rate of 5 °C min⁻¹ until complete thermal degradation was achieved. All samples were recorded under a nitrogen atmosphere. Universal Analysis, version 4.4A software, was used to determine the onset (T_{onset}) and decomposition (T_{dec}) temperatures, as the temperatures at which the baseline slope changes during the heating and at which 50% of weight loss was observed, respectively. The ionic liquids' melting

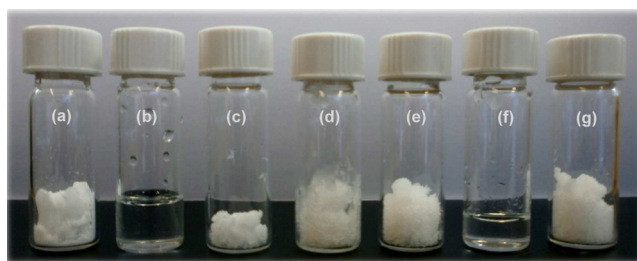


Figure 2. Cholinium carboxylate ionic liquids and salts prepared and used in this work (from left to right): (a) [Ch][Ox], (b) [Ch][Mal], (c) [Ch][Suc], (d) [Ch][Fum], (e) [Ch]L-ma, (f) [Ch][Glu], and (g) [Ch]Cit.

temperatures (T_m) and glass transition (T_g) temperatures were determined by differential scanning calorimetry (DSC) (DSC Q200, TA Instrument). Samples (about 5 to 10 mg) were crimped in aluminum standard pans and analyzed under nitrogen atmosphere by heating and cooling cycles at the rate of 5 °C min⁻¹. T_m was determined as the local maximum of the endothermic peak on heating. T_g was taken as the temperature corresponding to the inflection point of the heat flow versus temperature curve on heating. The uncertainties in the melting point and glass transition temperatures were expressed using the standard deviation of several consecutive measurements of the same sample and were found to be less than 1 °C. All the thermal characterization results are presented in Table 1,

Table 1. Thermal Properties of Cholinium Carboxylate Ionic Liquids and Salts^a

ionic liquid	T_{onset} (°C) ^a	T_{dec} (°C) ^a	T_m (°C)	T_g (°C)
[Ch][Ox]	172	230	57	n.d. ^b
[Ch][Mal]	130	198	n.d. ^b	-65
[Ch][Suc]	198	233	48	-56
[Ch][Fum]	194	235	78	-62
[Ch]L-ma	204	239	106	-35
[Ch][Glu]	186	242	n.d. ^b	-67
[Ch]Cit	165	215	103	n.d. ^b

^a T_{onset} and T_{dec} defined as the temperatures at which the baseline slope changes during the heating, and at 50% weight loss, respectively. Please note that these are from scanning TGA, and do not represent isothermal stabilities. ^bn.d.: not detected. ^aonset (T_{onset}), decomposition (T_{dec}), melting (T_m), and glass transition (T_g) temperatures.

and as observed, some of the prepared compounds fall in the definition of ILs, salts that melt below 100 °C. Thus, only for these, the ILs nomenclature is used, and the anions are represented inside square brackets.

Water Activity. The water activities (a_w) of the studied cholinium carboxylate ionic liquids and salts were measured using a Rotronic HygroPalm AW1. Aqueous solutions of each ionic liquid or salt were prepared, and their water contents were determined by Karl Fisher Titration (831 KF coulometer, Metrohm). Then, the prepared solutions were placed in a sealed container connected to a probe. Each sample slowly exchanges moisture with the air inside the sealed container until the equilibrium was reached. The water activity values were considered when constant values were achieved in the thermohygrometer. The measurements were performed under controlled temperature (25 ± 0.01 °C) with an accuracy of ±0.005 a_w . The water activity results are presented in Figure 3.

Phase Diagrams and Tie-Lines. The phase diagrams were measured in a glass vessel using the cloud point titration method at 25 °C and atmospheric pressure, as previously described.^{27,40} The vessel was provided with an external jacket in which water circulated at the given temperature (±0.01 °C) using a Lauda E200 water thermostat and a Pt 100 probe coupled to a Yokogawa 7561 digital multimeter.

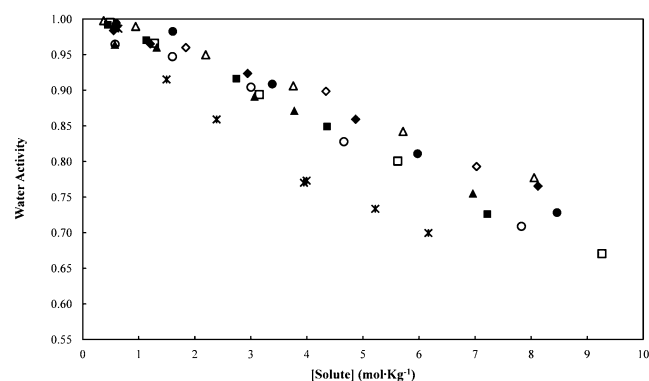


Figure 3. Experimental water activities at 25 °C: * PEG 600, ◇ [Ch][Cl], □ [Ch][Mal], ○ [Ch][Glu], △ [Ch]Cit, ◆ [Ch][Ox], ■ [Ch][Fum], ● [Ch]L-ma, and ▲ [Ch][Suc].

Briefly, ILs aqueous solutions with variable concentrations (ranging from 35 to 100 wt %) were loaded into the test cell. Repetitive dropwise addition of PEG 600 to the ionic liquid aqueous solution was carried out until the appearance of a cloudy solution (biphasic region), followed by the dropwise addition of water until the formation of a clear and limpid solution (monophasic region). In the case of aqueous solution of PEG 4000, the addition was carried out in the reverse order, meaning that an aqueous solution of ionic liquid was added to the PEG. All the additions occurred under constant stirring. The ternary systems compositions were determined by weight quantification of all components within $\pm 10^{-4}$ g. The above procedure was repeated to obtain sufficient data to construct the phase diagrams.

The experimental binodal curves were correlated according to eq 1, where Y and X refer to the PEG and the ionic liquid mass fraction percentages, respectively, and A , B , and C are constants obtained by the regression of the experimental binodal data.

$$Y = A \exp[(BX^{0.5}) - (CX^3)] \quad (1)$$

For the determination of each tie-line (TL), a ternary mixture with specific concentration within the biphasic region was prepared by mixing adequate amounts of water, PEG, and ionic liquid. The mixture was vigorously stirred and allowed to settle for about 12 h at 25 °C (± 1 °C). Then, the top and bottom phases were carefully separated and individually weighed within $\pm 10^{-4}$ g. The TLs were determined by solving the system of four equations, eqs 2–5, and four unknown values (Y_T , Y_B , X_T , and X_B). Where X and Y represent the weight fractions of ionic liquid and PEG, respectively; α is the ratio between the mass of the top phase and the total mass of the mixture; and the subscripts T, B, and M denote the top phase, bottom phase, and mixture, respectively.

$$Y_T = A \exp[(BX_T^{0.5}) - (CX_T^3)] \quad (2)$$

$$Y_B = A \exp[(BX_B^{0.5}) - (CX_B^3)] \quad (3)$$

$$Y_T = \frac{Y_M}{\alpha} - \frac{1 - \alpha}{\alpha} Y_B \quad (4)$$

$$X_T = \frac{X_M}{\alpha} - \frac{1 - \alpha}{\alpha} X_B \quad (5)$$

The tie-line length (TLL) of each tie-line was calculated according to eq 6, where X and Y are the ionic liquid and PEG weight fraction percentages, and the subscripts T and B designate the top phase and bottom phase, respectively.

$$\text{TLL} = \sqrt{(X_T - X_B)^2 + (Y_T - Y_B)^2} \quad (6)$$

Partitioning of TBHQ. Specific mixtures within the biphasic region were selected. For each experiment, the aqueous biphasic system was prepared by mixing the exact amount of each ionic liquid, PEG, and aqueous solution containing approximately 1 g dm⁻³ of

TBHQ. After the complete dissolution of all the components by stirring, the mixture was allowed to settle for about 12 h at 25 °C (± 1 °C). After the phase separation, the TBHQ was quantified using a UV–vis spectrophotometer (SHIMADZU UV-1800) at a wavelength of 288 nm, which corresponds to the TBHQ maximum absorption wavelength. The calibration curve was previously established. Possible interferences of both the PEG and ionic liquid with the analytical method were taken into account and found to be of no significance considering the dilutions and the ionic liquids used in this work.

At least three individual vials were prepared, and three samples of each aqueous phase were quantified, allowing the determination of the average partition coefficient, according to eq 7, where $[\text{TBHQ}]_{\text{PEG}}$ and $[\text{TBHQ}]_{\text{IL}}$ are the concentrations in molarity of TBHQ in the PEG-rich phase and ionic liquid-rich phase, respectively.

$$K_{\text{TBHQ}} = \frac{[\text{TBHQ}]_{\text{PEG}}}{[\text{TBHQ}]_{\text{IL}}} \quad (7)$$

The extraction efficiencies of TBHQ were determined according to eq 8, where $[\text{TBHQ}]_{\text{PEG}}$ and $[\text{TBHQ}]_{\text{IL}}$ are the concentrations of TBHQ in the PEG-rich phase and ionic liquid-rich phase, respectively, and m_{PEG} and m_{IL} are the mass of the PEG-rich phase and ionic liquid-rich phase.

$$\%EE_{\text{TBHQ}} = \frac{[\text{TBHQ}]_{\text{PEG}} \times m_{\text{PEG}}}{[\text{TBHQ}]_{\text{PEG}} \times m_{\text{PEG}} + [\text{TBHQ}]_{\text{IL}} \times m_{\text{IL}}} \quad (8)$$

RESULTS AND DISCUSSION

Phase Equilibria. The ability of the prepared cholinium carboxylate ionic liquids to induce ABS with PEG 600 and 4000 was evaluated. Binodal curves of PEG + cholinium-based IL systems were determined through the cloud point titration method. The experimental weight fraction data of the ternary systems are detailed in the Supporting Information. However, the binodal curves are represented throughout the figures in molality units to remove the influence of the ionic liquids molecular weight and thus provide more suitable comparisons. The phase diagrams are presented in different figures to allow a better evaluation of the influence of different IL features, such as the anion alkyl chain length (Figure 4), hydroxyl substituent groups in the anion alkyl chain (Figure 5), and effect of PEG molecular weight in the ABS formation (Figure 6)

Table 2 presents the parameters obtained by regression of the experimental binodal curves data (in weight fraction) of the studied systems using eq 1. Good correlation coefficients were

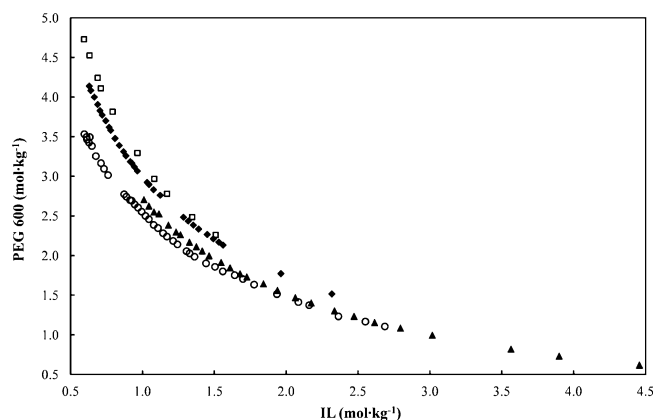


Figure 4. Ternary phase diagrams for systems composed of PEG 600 + IL + water at 25 °C and atmospheric pressure to study the anion alkyl chain length influence on ABS formation: ◆ [Ch][Ox], □ [Ch][Mal], ▲ [Ch][Suc], and ○ [Ch][Glu].

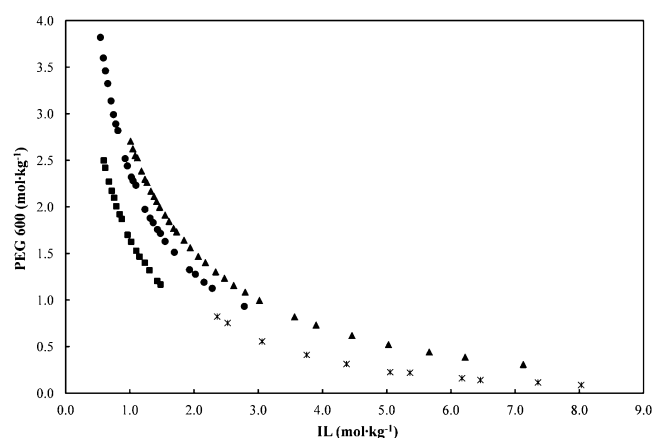


Figure 5. Ternary phase diagrams of systems composed of PEG 600 + IL + water at 25 °C and atmospheric pressure to study the effect of the substituent groups in anion alkyl chain: ▲ [Ch][Suc], ● [Ch]L-ma, ■ [Ch][Fum], and * [Ch]Bit.³¹

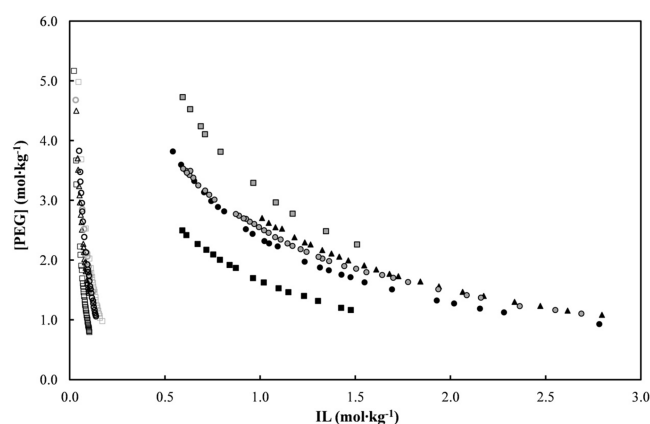


Figure 6. Ternary phase diagrams for systems composed of PEG + IL + water at 25 °C and atmospheric pressure to study the influence of PEG molecular weight on ABS formation: gray ■ PEG 600 + [Ch][Mal], ▲ PEG 600 + [Ch][Suc], gray ● PEG 600 + [Ch][Glu], ● PEG 600 + [Ch]L-ma, ■ PEG 600 + [Ch][Fum], gray □ PEG 4000 + [Ch][Mal], gray ○ PEG 4000 + [Ch][Glu], ○ PEG 4000 + [Ch]L-ma, □ PEG 4000 + [Ch][Fum].

Table 2. Correlation Parameters of Eq 1 Adjusted to Binodal Experimental Data and Respective Standard Deviations (σ) and Correlation Coefficients (R^2)

ionic liquid	$A \pm \sigma$	$B \pm \sigma$	$10^5 (C \pm \sigma)$	R^2
PEG 600 + IL + H ₂ O				
[Ch][Ox]	109 ± 1	-0.128 ± 0.001	0.4 ± 0.01	0.9999
[Ch][Mal]	112 ± 2	-0.124 ± 0.005	0.5 ± 0.06	0.9996
[Ch][Suc]	112 ± 1	-0.134 ± 0.001	0.4 ± 0.01	0.9999
[Ch][Fum]	113 ± 2	-0.184 ± 0.005	0.7 ± 0.06	0.9995
[Ch]L-ma	117 ± 1	-0.153 ± 0.002	0.4 ± 0.01	0.9998
[Ch][Glu]	105 ± 1	-0.123 ± 0.002	0.4 ± 0.01	0.9994
PEG 4000 + IL + H ₂ O				
[Ch][Mal]	137 ± 7	-0.246 ± 0.013	0.9 ± 0.06	0.9985
[Ch][Suc]	112 ± 4	-0.232 ± 0.010	1.2 ± 0.09	0.9988
[Ch][Fum]	113 ± 2	-0.266 ± 0.007	2.4 ± 0.07	0.9990
[Ch]L-ma	131 ± 4	-0.240 ± 0.007	1.0 ± 0.03	0.9995
[Ch][Glu]	86 ± 2	-0.147 ± 0.007	1.1 ± 0.06	0.9995
[Ch]Cit	264 ± 34	-0.341 ± 0.027	0.4 ± 0.06	0.9994

obtained, and consequently, eq 1 provides a good description of the experimental data.

Anion Chemical Structure Influence. It is important to mention at the beginning of this discussion that most of the cholinium monocarboxylate ILs or salts do not undergo phase demixing when combined with PEG with variable molecular weight. Pereira et al.³¹ studied cholinium-based ILs with acetate, lactate, propanoate, and butanoate anions and PEG 400 and observed that only the former IL was capable of phase splitting. In this work, we also attempted, without success, to form ABS using cholinium levulinate and cholinium valproate with PEG 600. This fact motivated us to explore cholinium-based ILs combined with anions derived from di- and tri-carboxylic acids, such as those presented in Figure 1. [Ch][Ox], [Ch][Mal], [Ch][Suc], [Ch][Glu], [Ch]L-ma, and [Ch][Fum] form ABSs in the presence of PEG 600. The phase equilibrium of [Ch]Cit with PEG 600 was not measured because data on this system is already available.³¹

According to literature, for systems containing polymers and ionic liquids,^{17,32} cholinium-based ILs are strong phase separation agents due to their improved affinity for water. Because all the ionic liquids under study have the same cation but different anions, the phase-forming ability of the cholinium carboxylate ionic liquids are determined by the interactions of their anions with the two other components in the ABS, water, or PEG 600. Despite the different phase splitting mechanisms, the salting out ability of an anion has been usually related to its hydration capacity.^{32,41} Anions with higher charge densities, as the case of dicarboxylated anions when compared to monocarboxylated anions, establish stronger interactions with water and consequently have higher hydration ability.

The water activity is defined as the ratio of the vapor pressure of aqueous solution of a given solute and the standard vapor pressure of pure water and is a useful parameter to evaluate the magnitude of the water–solute interactions. Because the water activity describes the energy of water in a given system, the higher the depression of the water activity is, the more stable the hydration complexes are, and thus, the stronger the interactions between water and the solute. Figure 3 shows that the measured water activities decrease in the order [Ch]Cit > [Ch][Ox] > [Ch]L-ma > [Ch][Mal] > [Ch][Suc] > [Ch][Fum] > [Ch][Glu] > PEG 600. Actually, two different types of trends can be observed. In the studied solute concentration range, one corresponds to the studied ILs and salts, with higher water activities, and the other corresponds to PEG 600, with lower water activities. It is clear from these data that PEG 600 establishes preferential interactions with water when compared to the studied ILs and salts. However, it should be kept in mind that all types of interactions need to be considered, not only PEG 600, water and IL, and water, but also PEG 600 and IL.

Figure 4 presents a comparison between the binodal curves of PEG 600 and the prepared [Ch][Ox], [Ch][Mal], [Ch][Suc], and [Ch][Glu], with the objective of understanding the influence of the anion alkyl chain length on ABS formation. The closer to the origin is the binodal curve, the lower is the IL concentration required for ABS formation, and consequently, the stronger is the phase-forming ability of the IL. The ability of these cholinium dicarboxylate ILs to promote a biphasic system with PEG 600 follows the trend of the anions: [Glu]⁻ (C₅) > [Suc]⁻ (C₄) > [Ox]⁻ (C₂) ≈ [Mal]⁻ (C₃). In conclusion, the longer the alkyl chain length of the dicarboxylated anion is, the larger immiscibility region is obtained. As was mentioned before, PEG 600 interactions with water are dominant when

compared to those of the studied cholinium ILs and water, and thus the ILs, should be salted out according to their hydrophobicity. However, the water activities (Figure 3) presented before ($[\text{Ch}][\text{Ox}] > [\text{Ch}][\text{Mal}] > [\text{Ch}][\text{Suc}] > [\text{Ch}][\text{Glu}]$) enables the conclusion that the increase in the alkyl chain of the anions leads to an increase in the hydrophobicity of the IL, with $[\text{Ch}][\text{Glu}]$ being the most hydrophilic and $[\text{Ch}][\text{Ox}]$ the most hydrophobic, when comparing the effect of increasing the alkyl chain only. This fact can probably be explained through the existence of intramolecular interactions between the two carboxylic groups of the anion, which are very close in these molecules, leading to an increase in hydrophobicity. Consequently, the IL displaying the largest biphasic region is the most hydrophilic one, showing that, in this case, the interactions between PEG 600–water and the IL–water are not dominant, and thus PEG–IL interactions need to be taken into consideration in order to provide an explanation of the relative order observed for the phase equilibria data. In general, the larger the immiscibility between the IL and PEG 600, the greater the ability of the ionic liquid to induce the polymer separation from the aqueous media.³² Nevertheless, the influence of the anions alkyl chain on the phase equilibria of these systems is not as pronounced as that found when inorganic salts are used.^{6,28} Furthermore, ILs with smaller anion alkyl chain length, such as $[\text{Ch}][\text{Ox}]$ and $[\text{Ch}][\text{Mal}]$, display very similar behaviors, with a somewhat smaller biphasic region than what could be expected from the results of the higher alkyl chain lengths ILs, $[\text{Ch}][\text{Glu}]$, and $[\text{Ch}][\text{Suc}]$. Again, this lower aptitude to create complexes with water of $[\text{Ch}][\text{Ox}]$ can be attributed to the existence of intramolecular interactions between the two carboxylic groups of the anion.

In order to address the effect of substituent groups in anion alkyl chains, the results obtained for $[\text{Ch}]_{\text{L-ma}}$ (anion alkyl chain of four carbons with a hydroxyl substituent group) and $[\text{Ch}][\text{Fum}]$ (anion alkyl chain of four carbons with a double bond) were compared (Figure 5), with the corresponding cholinium dicarboxylate IL having the same alkyl chain length in the anion without substituent groups, $[\text{Ch}][\text{Suc}]$. The results reported in the literature for cholinium bitartrate ($[\text{Ch}][\text{Bit}]$), containing an anion alkyl chain of four carbons with two hydroxyl substituent groups, was also included for comparison.³¹ The ability of these compounds to induce ABS with PEG 600 follows the trend: $[\text{Ch}][\text{Fum}] > [\text{Ch}][\text{Bit}] > [\text{Ch}]_{\text{L-ma}} > [\text{Ch}][\text{Suc}]$. All these phase diagrams (Figure 5), either those based on cholinium-based ILs ($[\text{Ch}][\text{Fum}]$ and $[\text{Ch}][\text{Suc}]$) or cholinium-based salts ($[\text{Ch}][\text{Bit}]$ and $[\text{Ch}]_{\text{L-ma}}$), present similar shapes and thus do not suggest different mechanisms of two-phase formation for the higher melting cholinium-based salts compared to lower melting cholinium-based ILs (Table 1). However, the obtained trend does not follow the hydrophobicity of the anion order indicated by the water activities ($[\text{Ch}]_{\text{L-ma}} > [\text{Ch}][\text{Suc}] > [\text{Ch}][\text{Fum}]$) either. Again, the only possible explanation for this fact is that PEG 600–ILs interactions are dominant over water–ILs and water–PEG 600.

Gathering all the literature results with those provided for very hydrophilic cholinium-based ionic liquids and polymers, such as PEG or PPG, it can be concluded that the mechanism of phase splitting for these systems is not always as obvious as when other less hydrophilic IL families are used. The solvation of hydrophilic ions in aqueous solutions of PEG polymers is complex, and a delicate balance between all possible interactions (PEG–water, PEG–ion, water–ion) needs to be

considered because their relative contributions to the solvation process can be similar, as referred by Pereira et al.³¹

PEG Molecular Weight. Figure 6 depicts a comparison of the phase behavior obtained when PEG with two different molecular weights, 600 and 4000 $\text{g}\cdot\text{mol}^{-1}$, are used. Since $[\text{Ch}][\text{Ox}] + \text{PEG 4000}$ and $[\text{Ch}]\text{Cit} + \text{PEG 600}$ do not present phase separation, only the other five cholinium-based ILs and salts are represented. The use of PEG polymers offers the opportunity of designing the binodal curve by varying the length of the polymeric chains, in other words, the average molecular weight. The same effect was observed in conventional ABS systems of polymer–inorganic salts,⁴² PEG or PPG – cholinium-based ILs,^{29,31} and also in PEG – imidazolium-based ILs.³² For all cholinium-based ILs and salts studied in this work, with the exception of $[\text{Ch}][\text{Ox}]$, the ability to induce phase separation is higher when PEG 4000 is used than when PEG 600 is added (Figure 6). These results are in agreement with those reported by Liu et al.³⁰ and Pereira et al.³¹ and can be explained by the increase in the hydrophobic character of PEG with the molecular weight.

The results obtained for the PEG 4000-based systems alone are represented in Figure 7. Regarding the alkyl chain length in

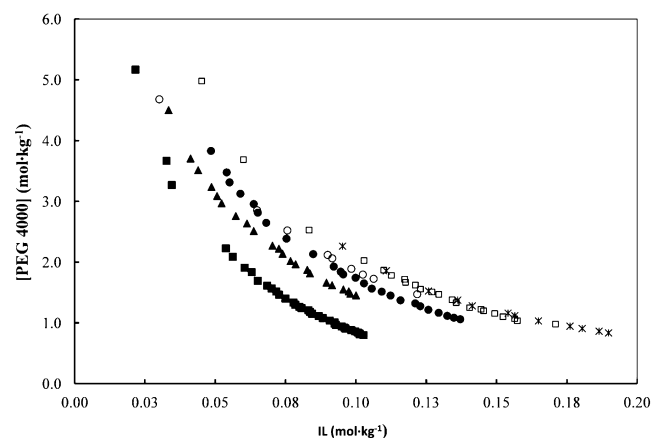


Figure 7. Ternary phase diagrams for systems composed of PEG 4000 + IL + water at 25 °C and atmospheric pressure: \square $[\text{Ch}][\text{Mal}]$, \blacktriangle $[\text{Ch}][\text{Suc}]$, \circ $[\text{Ch}][\text{Glu}]$, \bullet $[\text{Ch}]_{\text{L-ma}}$, \blacksquare $[\text{Ch}][\text{Fum}]$, and $*$ $[\text{Ch}]\text{Cit}$.

the anions, the ability to phase splitting follows the order of $[\text{Ch}][\text{Suc}]$ (C_4) $>$ $[\text{Ch}][\text{Glu}]$ (C_5) $>$ $[\text{Ch}][\text{Mal}]$ (C_3). Despite the relative order between $[\text{Ch}][\text{Suc}]$ and $[\text{Ch}][\text{Glu}]$, the other results ($[\text{Ch}][\text{Suc}]$ (C_4) $>$ $[\text{Ch}][\text{Mal}]$ (C_3) and $[\text{Ch}][\text{Glu}]$ (C_5) $>$ $[\text{Ch}][\text{Mal}]$ (C_3)) are in agreement to what was observed previously in this work for PEG 600, indicating that the longer is the alkyl chain length of the dicarboxylated anion, the larger is the immiscibility region obtained.

As shown in Figure 7, concerning the effect of the introduction of substituent groups in the anion, two comparisons can be established for the systems based on PEG 4000: $[\text{Ch}][\text{Fum}]$ (C_4 with a double bond) $>$ $[\text{Ch}]_{\text{L-ma}}$ (C_4 with an $-\text{OH}$ as substituent group) $>$ $[\text{Ch}][\text{Suc}]$ (C_4) and $[\text{Ch}][\text{Glu}]$ (C_5) $>$ $[\text{Ch}]\text{Cit}$ (C_5 with $-\text{COOH}$ and $-\text{OH}$ substituent groups). Again, the results for the anion with four carbons follow the same trend observed when PEG 600 is used. In relation to the anions with five carbon atoms, the relative increase in the hydrophobicity when the anion of the IL changes from glutarate to citrate is probably due to establishment of intramolecular interactions between the $-\text{COOH}$ and

Table 3. Weight Percentage of PEG (w_{PEG}) and Cholinium Carboxylate Ionic Liquid (w_{IL}) in the Total Mixture (M), Top Phase (T), and Bottom Phase (B) at 25 °C and Respective Values of Tie-Line Lengths (TLL)

ionic liquid	weight percentage (wt %)						TLL
	$w_{\text{IL},\text{M}}$	$w_{\text{PEG},\text{M}}$	$w_{\text{IL},\text{T}}$	$w_{\text{PEG},\text{T}}$	$w_{\text{IL},\text{B}}$	$w_{\text{PEG},\text{B}}$	
PEG 600 + IL + H ₂ O							
[Ch][Ox]	39.9393	42.0537	75.1491	6.5839	10.7920	71.4163	91.35
	42.9623	41.8855	83.6805	3.2368	9.1460	73.9831	102.76
[Ch][Mal]	39.8379	42.0163	55.0274	20.5908	1.5997	95.9532	92.38
	42.9045	42.0952	65.3577	11.2310	0.8926	99.8448	109.58
[Ch][Suc]	39.8125	42.0747	7.5698	77.4601	68.9027	10.1491	91.06
	43.8696	41.2609	5.2548	82.4565	77.3242	5.5704	105.38
[Ch][Fum]	36.0939	37.2909	4.7897	75.6868	62.5892	4.7933	91.47
	27.2779	41.2317	11.0446	60.8972	51.9655	11.3245	64.28
[Ch]L-ma	24.9398	60.1046	7.4629	77.2829	82.3424	3.6827	104.99
	39.8539	41.6850	10.7169	70.9306	74.7965	6.6122	90.79
[Ch][Glu]	39.5253	41.9729	80.7042	5.2978	18.8554	60.3820	82.82
	42.1447	42.6200	88.9952	2.6342	12.2791	68.1095	100.86
PEG 4000 + IL + H ₂ O							
[Ch][Mal]	29.7908	34.1737	11.1382	59.4421	47.6832	9.9351	61.53
[Ch][Suc]	30.2772	30.1782	5.3805	65.3788	47.2096	6.2380	72.44
	25.4134	32.2697	6.3184	62.4231	35.7270	15.9832	54.97
[Ch][Fum]	20.0231	35.0215	3.8630	67.0550	31.6630	11.9483	61.72
	17.0097	35.0634	7.4732	54.1971	21.4625	26.1295	31.36
[Ch]L-ma	29.2225	33.3866	10.7173	58.7546	46.7828	9.3139	61.20
	27.4683	34.5344	11.1461	57.7598	42.9570	12.4949	55.32
[Ch][Glu]	30.3247	34.3631	4.7851	62.3144	58.8486	3.1456	80.15
	28.2918	34.8177	5.0167	61.8258	53.2653	5.8389	73.91
[Ch]Cit	30.9888	34.8597	29.5106	37.0321	33.4441	31.2513	6.99
	34.5914	35.5261	20.1966	54.9644	51.4625	12.7439	52.54

the –OH substituent groups with the terminal carboxylic groups, thus hindering the interactions with water and the ILs hydration capacity decreases. Pereira et al.³¹ also noted that cholinium citrate has a more hydrophobic character than was expected from its chemical structure and also attributed this fact to the presence of intramolecular interactions.

Partitioning of TBHQ. The tie-lines (TL) and tie-line lengths (TLL) were determined through the application of eqs 2–6 for all the systems studied. The composition of the initial mixture and phases in equilibrium as well as the respective tie-line lengths (TLL) are reported in Table 3. As an example, Figure 8 depicts the results obtained for the TLs of the system composed of PEG 600 and [Ch][Suc].

In the present work, TBHQ was chosen as the model biomolecule, and its partitioning behavior between the coexisting phases of ABS formed by PEG 600 and the prepared cholinium-based ILs and salts was studied. Despite the larger two phase regions displayed by the systems based on PEG 4000, the choice of PEG 600-based ABS to perform the extractions is due to the lower viscosity that these systems present when compared to those composed of PEG 4000, facilitating the mass transfer operations and reducing the losses. The pH values of the prepared ABS are close to the pK_{a1} of the dicarboxylic acids of the corresponding IL anions between 3.5 and 4.5. Thus, this parameter does not influence the TBHQ partition.

The results obtained for the partition coefficients and the extraction efficiencies for each composition of the coexisting phases are presented in Table 4. The partitioning coefficients of TBHQ range between 3.55 and 11.79, indicating a preferential partitioning of TBHQ for the PEG-rich phase in all the systems studied; the results are in agreement with results reported in

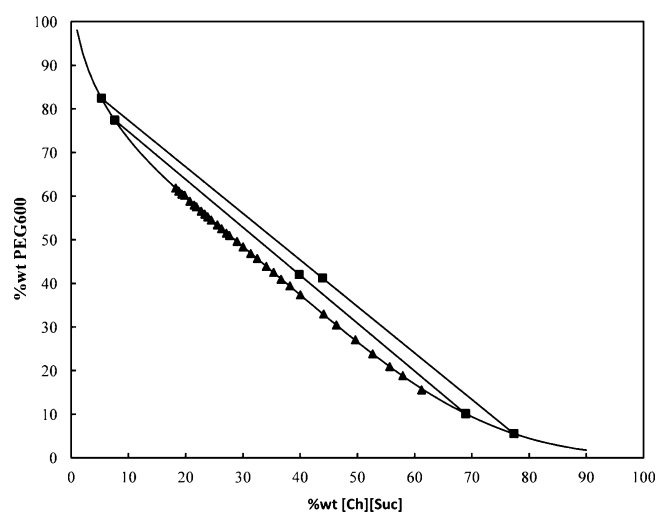


Figure 8. Binodal curve and TLs for the ternary system composed of PEG 600 + [Ch][Suc] + H₂O at 25 °C: ▲ binodal curve data, ■ TL data, and — eq 1.

the literature for extraction tetracycline with systems composed of PEG 600 and cholinium-based salts.⁷ The best partitioning coefficient was obtained for the system of PEG 600 and [Ch][Fum]. The extraction efficiencies of TBHQ range between 83% and 93%. This result shows us that these systems have not only the potential for the extraction of TBHQ but also for other antioxidants presenting a similar structure.

Table 4. Partition Coefficients and Extraction Efficiencies of TBHQ in ABS Composed of PEG 600 + cholinium Carboxylate ILs and Salts

IL + PEG 600 + H ₂ O	weight fraction percentage		$K \pm \sigma$ (PEG)	%EE $\pm \sigma$
	Ionic liquid	PEG 600		
[Ch][Mal]	40.03	41.95	3.55 \pm 0.40	90.79 \pm 0.98
	43.08	41.98	4.16 \pm 0.81	88.21 \pm 1.40
[Ch][Suc]	40.04	41.87	8.10 \pm 1.24	87.24 \pm 1.53
	43.59	41.51	5.23 \pm 2.18	83.08 \pm 2.41
[Ch][Fum]	35.86	37.39	11.79 \pm 0.18	91.79 \pm 0.82
	27.42	41.43	8.70 \pm 0.25	92.86 \pm 0.03
[Ch]l ₁ -ma	39.97	41.75	5.43 \pm 0.24	85.36 \pm 1.82
	24.82	60.32	3.73 \pm 0.08	92.78 \pm 0.22

CONCLUSIONS

Novel, sustainable, cheap, and biodegradable ABS were developed in this work by combining cholinium carboxylate ILs and salts with PEG 600 and PEG 4000 polymers. The chemical structures of ILs and salts used were carefully selected by using very hydrophilic anions derived from natural acids, which specifically address, in a clear way, the effect of the alkyl chain length and the influence of substituent groups in ABS formation. The phase forming ability of the prepared ILs with anions based on diacids and alkyl chains between 2 and 5 carbon atoms increases with increasing hydrophylicity of the anion. The effect of the presence of hydroxyl substituents in the alkyl chain of the anion was also studied through a series of compounds (ILs and salts) derived from succinic acid, where a double bond or a hydroxyl group were introduced. Despite their classification as ILs or salts, no significant change in the phase equilibria curves was observed. In both cases, PEG 600 and ILs interactions seem to have a significant contribution to the phase separation mechanism. The novel results gathered in this work clearly confirm the complex mechanisms underlying the PEG-based ABS phase separation.

ASSOCIATED CONTENT

Supporting Information

Experimental weight fraction data of the ternary systems. ¹H and ¹³C NMR spectra of the synthesized cholinium carboxylate ionic liquids and salts. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

Teresa Mourão and Liliana C. Tomé contributed equally to this work.

Notes

The authors declare no competing financial interest.

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