

Journal Pre-proofs

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PII: S1383-5866(20)31524-0
DOI: <https://doi.org/10.1016/j.seppur.2020.117050>
Reference: SEPPUR 117050

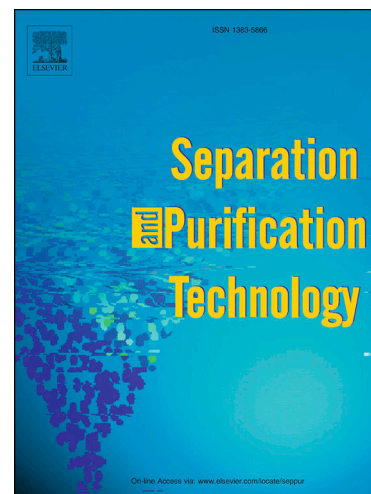
To appear in: *Separation and Purification Technology*

Received Date: 31 January 2020
Revised Date: 17 April 2020
Accepted Date: 18 April 2020

Please cite this article as: A. Dimitrijević, A. Paula M. Tavares, A. Jocić, S. Marić, T. Trtić-Petrović, S. Gadžurić, M.G. Freire, Aqueous biphasic systems comprising copolymers and cholinium-based salts or ionic liquids: insights on the mechanisms responsible for their creation, *Separation and Purification Technology* (2020), doi: <https://doi.org/10.1016/j.seppur.2020.117050>

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Aqueous biphasic systems comprising copolymers and cholinium-based salts or ionic liquids: insights on the mechanisms responsible for their creation

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Abstract

Aqueous biphasic systems (ABS) formed by copolymers and ionic liquids (ILs) have demonstrated to be effective separation platforms, but there is still a gap on the complete understanding of the molecular-level mechanisms ruling the two-phase formation for this type of systems. This work addresses the determination of the liquid-liquid equilibrium of ABS composed of cholinium-based salts or cholinium-based ILs and the triblock copolymer Pluronic PE6200 (PL6200). It is demonstrated that PL6200 can form ABS with all investigated cholinium-based salts or ILs, contrarily to most poly(ethylene)glycol polymers, which is due to the presence of hydrophobic propylene oxide (PO) blocks. From the phase diagrams behavior and IL/salt anions properties, it is shown that the formation of ABS with cholinium-based salts is ruled by the anions polar surface and ability to be hydrated, whereas in systems comprising ILs van der Waals interactions between the copolymer and the IL cannot be discarded. The partition of a series of alkaloids in these systems, namely caffeine, nicotine, theophylline, and theobromine, was additionally appraised. It is shown that caffeine, theophylline, and theobromine preferentially migrate to the more hydrophobic PL6200-rich phase, and that their partition depends on the water content in the respective phase, being ruled by the phases' hydrophobicity. On the other hand, nicotine, with the most prominent hydrophobic character amongst the studied alkaloids, preferentially migrates to the salt- or IL-rich phase, in which interactions occurring between this alkaloid and the IL/salt cannot be discarded. The ABS formed by cholinium dihydrogenphosphate is the most selective system identified to separate nicotine from the remaining alkaloids, giving some insights into their investigation as separation platforms for alkaloids from natural extracts.

Key words: Cholinium-based; ionic liquids; Pluronic; molecular-level mechanisms; alkaloids; aqueous biphasic system.

1. Introduction

Liquid-liquid extraction techniques have several advantages to be applied in separation processes. However, volatile organic solvents immiscible with water are commonly used for this purpose. To overcome the drawbacks associated to volatile organic solvents, aqueous biphasic systems (ABS) based on polymer-polymer or polymer-salt combinations in aqueous media were proposed by Albertsson [1], who demonstrated their potential in the separation of biological products. In 2003, Rogers and co-workers [2] demonstrated that new ABS can be formed by the combination of ionic liquids (ILs) and inorganic salts. In the following years, IL-based ABS formed with carbohydrates, amino acids, and polymers have been described [3–5], as well as by using ILs as adjuvants in traditional polymer-salt ABS [6]. One of the most important attractiveness of IL-based ABS is related with the ILs designer solvents ability, by the combination of different cations and anions chemical structures, allowing higher extraction efficiencies and improved selectivity [7].

IL-based ABS have demonstrated to be effective separation platforms for many biomolecules, such as proteins, enzymes, antioxidants, pharmaceuticals and alkaloids [7–12], through the wise selection of the phase-forming components and their composition. Recent research has demonstrated that cholinium-based ILs paired with appropriate anions present outstanding biodegradable and low toxicity properties, in which proteins structure and enzymes activity can be maintained or even increased [13]. In addition, this class of ILs is generally accessible, easy to handle, and cheaper than the commonly studied ILs (e.g. imidazolium-based). Due to these and other exceptional properties, interest in these compounds as phase-forming components of ABS has increased in the past few years [14]. Cholinium-based ILs have been combined with salts or polymers to create ABS [13,15]. In what particularly concerns ABS formed by ILs and polymers, there is still a gap in the complete understanding of the molecular-level mechanisms ruling the two-phase formation. It has been shown that ABS formed with ILs and polyethylene glycol (PEG) and polypropylene glycol (PPG) can be created. This type of ABS can be formed when the solubility of the polymer or IL in water is limited [16]. However, ABS formed by ILs and polymers completely miscible in water, in which the polymer and IL are also completely miscible, leading to type 0 ternary phase diagrams, also have been demonstrated [17]. Overall, cholinium-based ILs can form ABS with PEG or PPG polymers; nevertheless, the ABS formation phenomenon is

complex since it also depends on the balance of the binary interactions of all constituents in equilibrium [18].

In addition to PEG and PPG, block copolymers have received increasing attention because their assemblies have potential applications in drug delivery and other medical applications, imaging and sensing, and catalysis [19,20]. Block copolymers consist of incompatible polymer pairs (hydrophobic and hydrophilic blocks) linked by covalent bonds. Pluronics comprise two hydrophilic ethylene oxide (EO) and hydrophobic propylene oxide (PO) blocks arranged in a triblock structure, $\text{EO}_x\text{-PO}_y\text{-EO}_x$, resulting in an amphiphilic nature. The characteristics of Pluronics can be controlled by variation of their block polymeric chains length, i.e. the number of monomer units (x and y) and their molecular weights. Therefore, Pluronics can be seen as potential alternatives as phase-forming components of IL-based ABS, offering a higher degree of tailoring ability to create ABS and to tune the phases' characteristics. Pluronics present additional advantages, such as their amphiphilic nature that allows the solubilization of hydrophobic molecules [21], thermo-sensitivity behavior [22], and capacity to be designed with intermediate degrees of hydrophobicity and high biocompatible nature.

Pluronic PE6200 (PL6200) is a triblock copolymer with the molecular weight $2450 \text{ g}\cdot\text{mol}^{-1}$, with a PEG content of 20% ($\text{EO}_8\text{-PO}_{30}\text{-EO}_8$) [23]. Even though ABS formed by ILs and copolymers can lead to a new plethora of separation systems with relevant properties, research on this particular set of ABS is still scarce [24–26]. Silva et al. [24] investigated ABS formed by a series of inorganic salts or cholinium-based ILs and Pluronic L35, while demonstrating their outstanding ability to separate naringin and rutin in two steps. Liu et al. [25] determined the liquid-liquid equilibrium data of ABS composed of copolymers (poly(ethylene glycol)-block-poly(propylene glycol)-poly(ethylene glycol) (EO10PO90)) and cholinium-based ILs and evaluated the impact of the triblock polymer nature on the ABS formation.

In this work, we aim to extend the characterization of ABS formed by ILs and copolymers while gathering evidences that could contribute to the understating of the molecular-level mechanisms that rule phase separation. To this end, ABS formed by Pluronic PE6200 and different cholinium-based salts or ILs were investigated. ILs are here considered as all compounds studied with a melting temperature below 100°C , whereas cholinium-based salts correspond to those with a higher melting point. The respective phase diagrams were determined at 25°C , allowing to address the effects of the IL/salt anion on phase separation based on their hydrophobicity and

charge density. These ABS were then investigated to extract a series of alkaloids, namely caffeine, theophylline, theobromine, and nicotine. These compounds can be naturally obtained from tea, coffee or cocoa seeds and are commonly known for their diuretic, muscle relaxant, bronchial dilation, cardiac and central nervous system stimulant activities, being used in the treatment of asthma or influenza symptoms [27]. Although some insights could be taken for the use of ABS to extract this series of alkaloids from biomass samples, this series of structurally similar alkaloids was here used as relative hydrophobicity probes, allowing to infer the main mechanisms that rule their partition in a series of ABS composed of Pluronic PE6200 and different cholinium-based compounds. Finally, the selectivity of these systems to separate caffeine, theophylline and theobromine from nicotine was determined.

2. Experimental

2.1. Materials

Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) - Pluronic PE6200 - was acquired from BASF. Cholinium chloride ([Ch]Cl), cholinium dihydrogen citrate ([Ch][DHCit]), cholinium bitartrate ([Ch][Bit]), cholinium hydrogencarbonate (80 wt% in H₂O), propanoic acid, butanoic acid, sulfuric acid, hydrochloric acid, and inorganic salts (Na₂CO₃, NaHCO₃) were supplied by Sigma-Aldrich. Cholinium dihydrogen phosphate ([Ch][DHP]) and cholinium acetate ([Ch][Ac]) were purchased from Iolitec. Lactic acid was acquired from Riedel-de-Haën. Cholinium propionate ([Ch][Pro]), cholinium lactate ([Ch][Lac]) and cholinium butanoate ([Ch][But]) were synthesized using a metathesis reaction, according to literature protocols [28]. Experimental details of the ionic liquid synthesis are given in the Supporting Information, as well as the chemical structure and properties of ionic liquids (Table S1).

The alkaloids caffeine (99 wt% pure), nicotine (99 wt% pure), theophylline (99 wt% pure) and theobromine (99 wt% pure) were supplied by Sigma-Aldrich. The molecular structure of the targeted alkaloids and corresponding properties are shown in Table S2 in the Supporting Information. Aqueous solutions of the studied alkaloids were prepared with the following concentrations: 4.28 mmol.dm⁻³ of nicotine, 2.67 mmol.dm⁻³ of caffeine, 2.80 mmol.dm⁻³ of theophylline and 1.64 mmol.dm⁻³ for theobromine. These solutions were used in the ABS formation regarding the extraction experiments. Ultrapure deionized water obtained by a Milli-Q plus water purification system was used in all experiments.

2.2. Determination of the ABS Phase Diagrams

The ABS ternary phase diagrams, composed of water, cholinium-based ILs or salts and Pluronic PE6200, were initially determined by the cloud point titration method at $25 (\pm 1) ^\circ\text{C}$ and atmospheric pressure ($0.10 \pm 0.01 \text{ MPa}$) using the procedure reported elsewhere [29]. This method is based on the dropwise addition of an IL or salt aqueous solution ($\sim 60 \text{ wt}\%$) to a known mass of polymer aqueous solution ($\sim 55 \text{ wt}\%$) until the mixture becomes turbid, representing the compositions at which the two-phase system occurs. Then, water was added until the solution becomes clear, fitting the monophasic regime. This procedure was repeated to obtain enough experimental data points for the design of the respective binodal curves [29]. The composition of each system was calculated by the weight quantification of all components added ($\pm 10^{-4} \text{ g}$).

Although tie-lines (TLs) are most of the time determined by applying the gravimetric method described by Merchuk et al. [30], which demand careful separation of the top and bottom phases, in this study, TLs were determined by an analytical approach because the set of equations and mass balance approaches proposed by Merchuk et al. [30] do not satisfactorily describe the solubility data for the studied ABS. Mixtures at the biphasic region were prepared by addition of a known amount of PL6200 and IL/salt into Eppendorf tubes, vigorously stirred using a vortex agitator (Reax Top, Heidolph) at 2500 rpm, and allowed to reach equilibrium by the separation of both phases for 12 h at $25 (\pm 1) ^\circ\text{C}$. After the separation of the phases, fractions of each phase were weighted ($\sim 50 \text{ mg}$) and diluted with the appropriate amount of water. The content of [Ch][DHP], [Ch][Bit], [Ch][Ac], [Ch]Cl, [Ch][Lac], [Ch][Pro] and [Ch][But] in the top and bottom phases was determined using an ion chromatograph, 861 Advanced Compact IC (Metrohm, Switzerland), with an anion self-regenerating suppressor, conductivity detector and sample loop of $100 \mu\text{L}$. Separation of anions was carried out on a column Metrosep A supp 5 ($100 \text{ mm} \times 4 \text{ mm i.d.}$) with packing material constituted of polyvinyl alcohol with quaternary ammonium groups and particle size of $5 \mu\text{m}$. The suppressor was periodically regenerated using water and 5 mmol.dm^{-3} sulfuric acid solutions. The mobile phase was constituted by $3.2 \text{ mmol.dm}^{-3} \text{ Na}_2\text{CO}_3$ and $1 \text{ mmol.dm}^{-3} \text{ NaHCO}_3$ solutions, with a flow rate of 0.7 ml.min^{-1} .

Since [Ch][DHCit] could not be quantified by IC due to presence of broad peaks in the chromatogram, the composition of this salt was determined using a Shimadzu UV-1800 spectrophotometer with quartz cells [31]. The spectrum of [Ch][DHCit] has a maximum

wavelength at 209 nm corresponding to carbonyl groups (C=O) of unionized or ionized carboxyl groups (electronic n/p* transitions). Sample preparation consisted on the addition of 100 mg of the [Ch][DHCit]-rich phase to 9.9 mL of deionized water. Then, an aliquot was mixed with 0.25 mol.dm⁻³ HCl solution in the ratio 1:1 (v:v) to shift the dissociation of citrate towards a less dissociated species to move the absorption maximum to higher wavelengths [32]. The water content was determined by a drying procedure; an appropriate amount of each phase was weighted and dried at 70°C under constant stirring until constant weight. The amount of PL6200 was determined by difference based on mass balance.

2.3. Partitioning of alkaloids

The separation ability of the studied ABS for alkaloids was carried out at a fixed mixture point at the biphasic region of the ternary phase diagrams: 30 wt% PL6200 + 25 wt% IL + 45 wt% aqueous solution containing the alkaloid. These mixtures were vigorously agitated and left for 12 h for equilibration at 25°C. After careful separation, the phases were weighed, diluted and the alkaloids content in the phases quantified by UV spectroscopy using a Shimadzu UV-1800 spectrophotometer at the wavelengths of 272 nm for Caf, 259 nm for Nic, 270 nm for Tph and 272 nm for Tbr.

The partition coefficient (K) was calculated as the ratio between the equilibrium concentration of each alkaloid in the PL6200-rich phase ($[Alk]_{PL}$) to that in the IL/salt-rich phase ($[Alk]_{IL}$):

$$K = \frac{[Alk]_{PL}}{[Alk]_{IL}} \quad (1)$$

The selectivities of Caf, Tbr, and Tph in respect to Nic was calculated according to the following equation:

$$S_{Alk/Nic} = \frac{K_{Alk}}{K_{Nic}} \quad (2)$$

where subscript Alk stands for Caf, Tbr or Tph.

3. Results and discussion

3.1 ABS ternary phase diagrams

ABS formed by a common polymer (Pluronic PE6200) and different cholinium-based salts or ILs (described in Table S1 in the Supporting Information) were investigated in this work, allowing

to evaluate the effect of the IL/salt anion on the ability to form two-phase systems. The respective phase diagrams were determined at room temperature (~ 25 °C) and atmospheric pressure (0.1 MPa). Cholinium-based salts with melting temperatures above 100 °C ([Ch][DHCit], [Ch][DHP], [Ch][Bit], and [Ch]Cl), and cholinium-based ILs ([Ch][Ac], [Ch][Pro], [Ch][Lac], and [Ch][But]) with melting temperatures below 100°C, by considering the general definition of ILs, were used. Among those that fit within the ILs category, [Ch][Ac] has a melting temperature of 85 °C, whereas [Ch][Pro], [Ch][Lac], and [Ch][But] are liquid at room temperature.

The respective ternary phase diagrams determined by cloud-point titration are represented in Figure 1, with the respective experimental weight fraction data provided in the Supporting Information (Tables S3 and S4). The ternary phase diagrams in Figure 1 are given in molality units to avoid the effects derived from the IL/salt molecular weight. Figure 1 provides the phase diagrams in an orthogonal representation, where the amount of water is not shown, corresponding to the amount required to reach 100 wt% (cf. Tables S3 and S4 with detailed data). By IC or spectrophotometric quantification, it was found that the systems containing [Ch][DHP], [Ch][Bit], Ch[DHCit], or [Ch][Lac], have a bottom phase enriched in the IL or salt and a top phase majorly constituted by PL6200. On the other hand, when ABS are prepared with [Ch]Cl, [Ch][Ac], [Ch][Pro], and [Ch][But], an inversion in the phases' densities occurs, being the top phase the cholinium-rich phase and the bottom phase the PL6200-rich phase. TLs were analytically determined for all systems, being given in Table S5 in the Supporting Information, together with the pH value of each phase.

For the sake of comparison of different salts/ILs to induce phase separation, the PL6200 concentration of 0.15 mol.kg^{-1} (27 wt%) was chosen. At this value the tendency of the cholinium based-salts/ILs to form ABS follows the order: [Ch][DHP] > [Ch][Bit] > [Ch][Ac] > ChDHCit > [Ch][Lac] > [Ch][Pro] > [Ch]Cl \approx [Ch][But]. [Ch][DHP] shows the highest ability to promote two phase formation with PL6200, whereas [Ch]Cl and [Ch][But] exhibit the lowest. In other studies involving ABS and polymers, such as poly(ethylene)glycol (PEG), [Ch][DHP] is usually the cholinium-based salt with the highest affinity to create two-phase systems [13,18, 33]. It was previously demonstrated that the cholinium counterion role is predominant in what concerns interactions with water and dehydration of the copolymer to create ABS [24]. In this work, when preparing mixtures of Pluronic and higher melting cholinium based-salts ([Ch][DHP] > [Ch][Bit] > [Ch][DHCit] > [Ch]Cl), there is the need of a simultaneous high anion polar surface and low

anion hydrophobicity (appraised by the octanol-water partition coefficients ($\log K_{ow}$) given in Table S1 in the Supporting Information). This fact suggests that the aptitude of the higher melting cholinium salts to induce ABS with PL6200 is governed mainly by their ability to form ion-water complexes that are related to the Gibbs free energy and entropy of hydration of the respective ions. This behavior is in close agreement with that previously observed in conventional PEG–inorganic salt ABS, in which anions with higher charge density are more able to create ion–water complexes and have low tendency to interact with the ether oxygens of PEG [34]. On the other hand, the odd behavior of [Ch][DHCit], i.e. a lower ability to form ABS given its high anion polar surface, was also previously observed for other polymer-based ABS, being justified in the light of anion-anion interactions due to the [DHCit]⁻ self-aggregation [18,35].

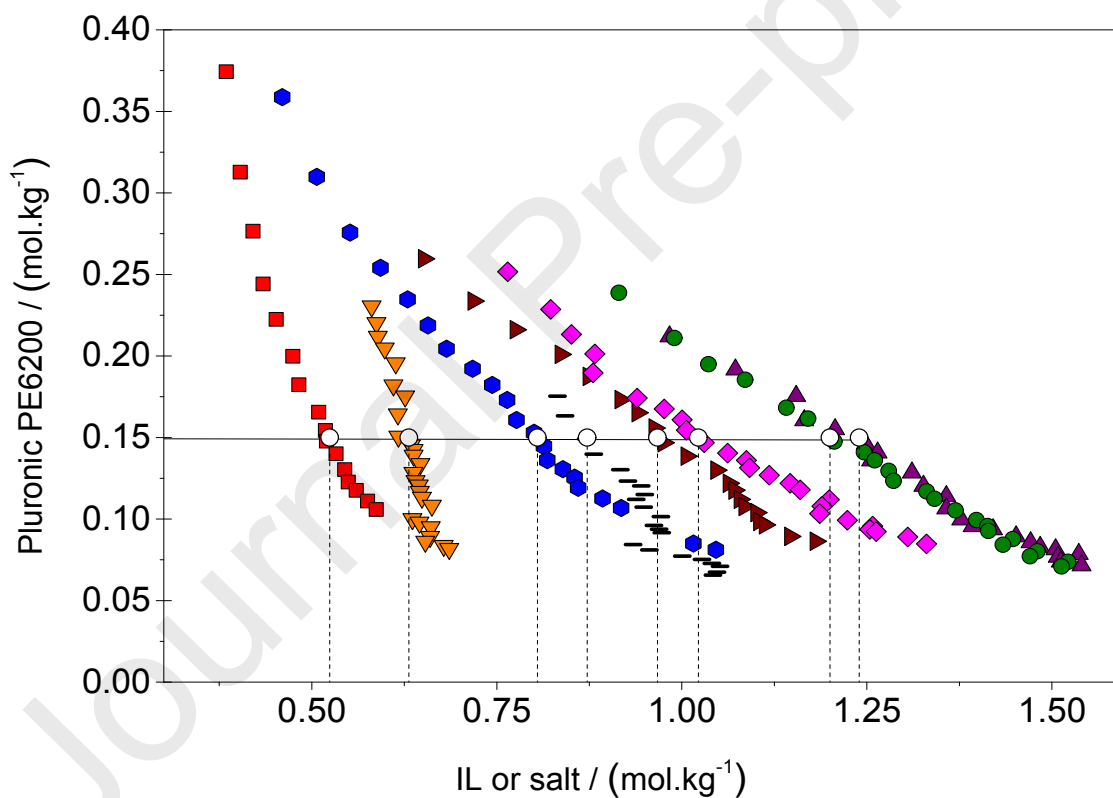


Figure 1. Ternary phase diagrams composed of PL6200 + IL + H₂O, at 25°C and atmospheric pressure (0.1 MPa). Legend: (■) [Ch][DHP]; (▼) [Ch][Bit]; (●) [Ch][Ac]; (◄) ChDHCit; (►) [Ch][Lac]; (◆) [Ch][Pro]; (●) [Ch]Cl; (▲) [Ch][But]; (○) systems composed 0.15 mol.kg⁻¹ of PL6200 and respective concentrations of the IL required to form ABS.

When considering the ability of cholinium-based ILs to undergo phase separation in presence of PL6200 aqueous solutions, they follow the order: [Ch][Ac] > [Ch][Lac] > [Ch][Pro] > [Ch][But]. This trend is in accordance with the $\log K_{ow}$ values of these ILs, but does not follow the anion polar surface trend. These results allow to conclude that the ILs hydrophobicity plays a major role to create ABS with polymers, being mediated by their ability to interact with the polymer. The formation of ABS containing ILs and polymers are far more complex than those in salt-IL or salt-polymer systems [17,34,36,37]. It was previously shown that the ions' hydration ability is not the main driver of phase separation, where the specific interactions occurring between the IL and the polymer play a significant role, which further depend on the polymer nature and chemical structure of the IL ions [18,36].

It was shown that neither [Ch][Pro] nor [Ch][But] induce two-phase formation with several PEGs (PEG200, PEG600, and PEG1000), while [Ch][Lac] forms ABS only with higher molecular weight PEGs [18]. On the other hand, all these ILs are able to create ABS with poly(propylene)glycol with a molecular weight of 400 g.mol⁻¹ (PPG 400) [33], a more hydrophobic polymer than PEGs of similar molecular weight. According to the structure of PL6200 (EO₈-PO₃₀-EO₈), eight units of ethylene oxide is equivalent to PEG 400 and thirty units of propylene oxide to PPG 2000, meaning that PL6200 is more hydrophobic than PEG, afforded by the propylene oxide units, and has lower ability to interact with water. On the other hand, and although hydrogen bonds between the proton accepting sites of the ILs anions and the hydrogens from the terminal hydroxyl groups of EO may occur, the overall results obtained suggest that van der Waals interactions occurring between the aliphatic part of the IL anions and PL6200 seem to be more relevant. The higher the possibility of van der Waals interactions between PL6200 and IL anions with longer alkyl side chains at the anion, representing a high solubility and affinity between the copolymer and the IL, the lower the ability of the respective IL to create ABS. This fact is confirmed by the lower ability of [Ch][Lac] to create ABS when compared to [Ch][Ac]. The first has an additional hydroxyl group, but a lower alkyl side chain at the anion, reinforcing the relevance of the aliphatic part of the IL anion to create ABS with PL6200. Furthermore, results obtained from the literature [38], using Fourier-transform infrared spectroscopy, confirm that the EO blocks from copolymers undergo only a small degree of dehydration by the addition of

cholinium-based ILs in aqueous solution, reinforcing that in the current case salting-out phenomenon does not play a pivotal role.

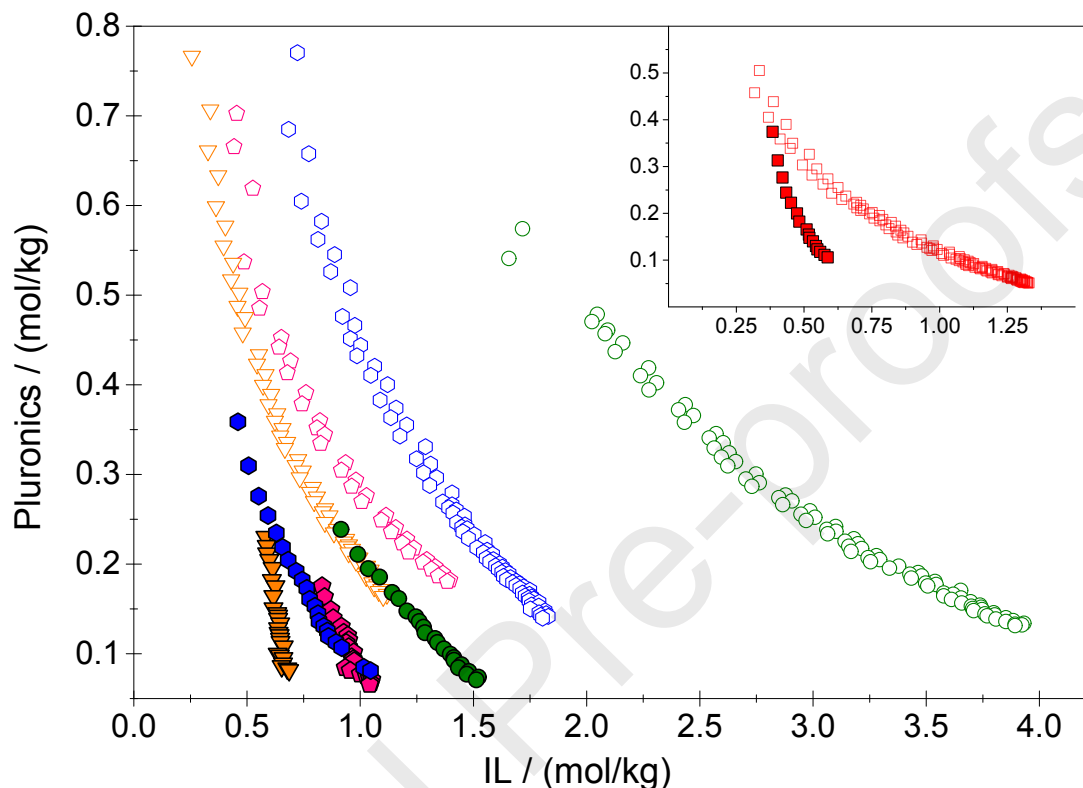


Figure 2. Ternary phase diagrams composed of Pluronic PE6200 (closed symbols) and L35 [24] (open symbols) at 296.15 K and atmospheric pressure (0.1 MPa). Legend: (■) [Ch][DHP]; (▼) [Ch][Bit]; (●) [Ch][Ac]; (◆) [Ch][DHCit]; (○) [Ch]Cl.

When comparing the ternary phase diagrams obtained in this work to those published by Silva et al. [24] regarding ABS formed by Pluronic L35 and high melting temperature cholinium-based salts, it is found that the biphasic regions afforded by PL6200 are larger than those obtained with PL35-based ABS. The comparison of these results is given in Figure 2. These results suggest that two-phase systems can be created more easily with PL6200, i.e. by requiring lower amounts of phase-forming components to create ABS, and have a higher water content in each phase that is advantageous when foreseeing their application in the separation of biologically active products and also from an economic perspective. Compared to PL6200, PL35 (EO₈-PO₂₁-EO₈) has a

smaller PO-block and the same number of EO units, and as such is less hydrophobic than PL6200. Furthermore, an interesting effect is noticed regarding the relative position of the solubility curves corresponding to the same ILs. The higher displacement of the binodal position is observed with [Ch]Cl, the weakest two-phase promoter. As the hydrophilic character of ILs increases along the sequence [Ch]Cl < [Ch][Ac] < ChDHCit < [Ch][Bit] < [Ch][DHP], the shift between the solubility curves corresponding to the ABS formed PL6200 and PL35 by gradually decreases. The reduction in the IL concentration needed to generate two-phase was quantified, being given in Table 1. This phenomenon may be related with the several salts/ILs influence on the copolymer self-assembling in aqueous solutions, being further reflected in the ABS phase behavior. Khan et al. [38] showed that cholinium-based ILs generally increase the stability of micelles, being this phenomenon mainly IL anion dependent, with [Ch]Cl identified as the lowest and [Ch][DHP] as the highest micellization inducers.

Table 1. Composition of IL or salt in PL35-based ABS ($[\text{IL}]_{\text{PL35-ABS}}$) and PL6200-based ABS ($[\text{IL}]_{\text{PL6200-ABS}}$), in molality units, and their difference ($\Delta[\text{IL}] = [\text{IL}]_{\text{PL35-ABS}} - [\text{IL}]_{\text{PL6200-ABS}}$) at 0.17 mol kg^{-1} of polymer.

IL	$[\text{IL}]_{\text{PL35-ABS}}$	$[\text{IL}]_{\text{PL6200-ABS}}$	$\Delta[\text{IL}]$
[Ch][DHP]	0.84	0.52	0.32
[Ch][Bit]	1.07	0.63	0.44
ChDHCit	1.41	0.86	0.55
[Ch][Ac]	1.74	0.76	0.98
[Ch]Cl	3.61	1.15	2.46

3.2 Partitioning of alkaloids

One of the advantages of copolymers over polymers is the fact ABS can be generated with all ILs and salts investigated, unlike what happens with PEG 400 and water-insoluble PPG 2000 (building blocks of PL6200). This possibility is relevant to tailor the performance of polymers to create ABS and to tune the partition of target products when their use in separation processes is envisaged. The studied systems formed by PL6200 and ILs/salts were investigated to separate a series of alkaloids, namely caffeine, nicotine, theophylline, and theobromine. A similar mixture

composition was adopted for this series of experiments: 30 wt % PL6200 + 25 wt % cholinium based-ILs/salts + 50 wt % aqueous solution of each alkaloid. The results obtained are depicted in Figure 3, with detailed results given in Table 2. Results are given as logK values, in which values higher than 0 correspond to a preferential migration of the alkaloids to the PL6200-rich phase, whereas the opposite occurs for values below 0. The pH values of the coexisting phases, ranging between 3.73-7.32, are shown in Table S5 in the Supporting Information. The studied alkaloids may suffer speciation as a function of pH, whose individual speciation curves are presented in Figures S1 to S4 in the Supporting Information. In the investigated ABS, caffeine is present as a neutral molecule; theophylline and theobromine mainly exist in a neutral form; and nicotine is present mostly as a positively charged species.

Table 2. Partition coefficients (*K*) of alkaloids in ABS.

IL	Theobromine	Caffeine	Theophylline	Nicotine
[Ch][DHCit]	1.16	1.29	1.55	0.42
[Ch][But]	1.13	1.36	1.65	1.23
[Ch][Lac]	1.23	1.58	1.98	1.09
[Ch][Pro]	1.36	1.70	2.22	0.94
[Ch]Cl	1.24	1.83	2.30	0.52
[Ch][Bit]	1.39	2.11	2.35	0.37
[Ch][Ac]	1.35	2.19	2.90	0.86
[Ch][DHP]	2.43	4.30	4.96	0.31

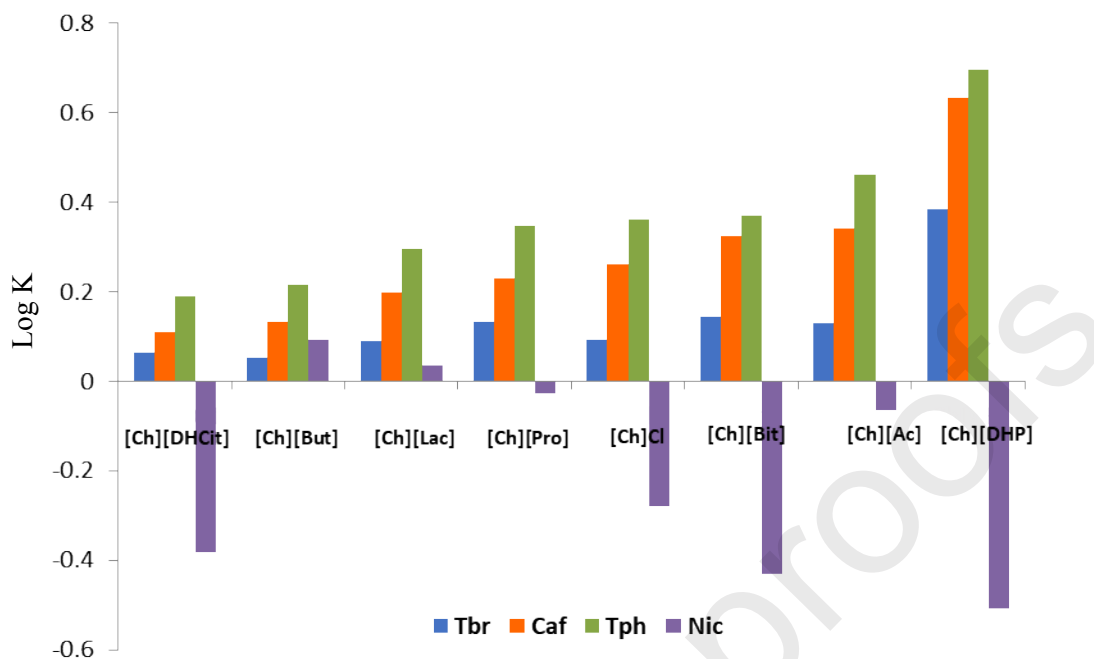


Figure 3. Partition coefficients of alkaloids in ABS composed of 30 wt % PL6200 + 25 wt % cholinium based-ILs/salts + 50 wt % aqueous solution of each alkaloid, at 25°C and atmospheric pressure.

The partition coefficients (K) for caffeine, theophylline and theobromine are higher than 1 for all investigated ABS, showing that these alkaloids preferentially partition to the PL6200-rich phase (Figure 3). These three alkaloids partition to the most hydrophobic phase, the PL6200-rich phase, in an extent that follows their $\log K_{ow}$ values (cf. Table S2 in the Supporting Information). Furthermore, the alkaloids partition more extensively to the PL6200-rich phase according to the following ILs/salts order: ChDHCit < [Ch][But] < [Ch][Lac] < [Ch][Pro] < [Ch]Cl < [Ch][Bit] < [Ch][Ac] < [Ch][DHP]. This trend is in close agreement with the IL and water contents at the PL6200-rich phase, as summarized in Figure 4. Detailed TLs data are given in Table S5 in the Supporting Information.

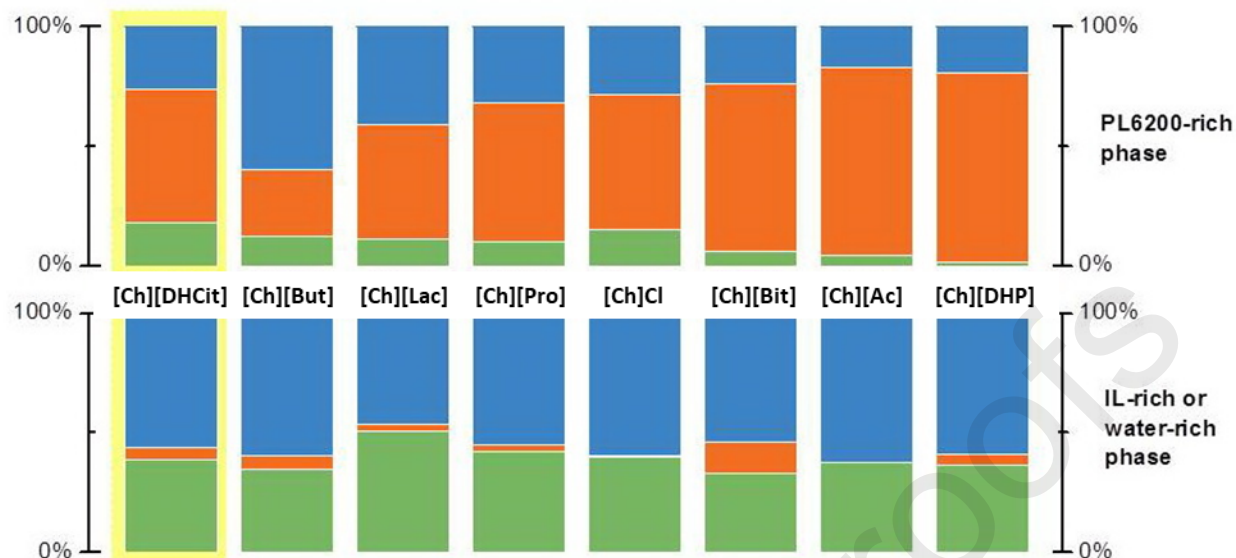


Figure 4. Graphical representation of phases' compositions in weight percentage; IL (green), PL6200 (orange) and water (blue).

Taking into account the data depicted in Figure 4 it is evident that the PL6200-rich phase of all investigated ABS has relatively low amounts of ILs/salts (< 18. wt%). Regarding the IL/salt-rich phase, it is largely constituted by water (> 62 wt%). ABS comprising PL6200-rich phases with higher IL/salt or water contents are those that lead to lower partition coefficients of caffeine, theophylline and theobromine. These results suggest that the partition of these three alkaloids follow the phase's hydrophobicities, and as such can be used as hydrophobicity probes as suggested in previous works [39]. However, an exception was observed in the system composed of [Ch][DHCit]. In this system, the distribution coefficients are the lowest despite the fact that the water content in the the PL6200-rich phase is not significantly higher amongst the studied systems and it is comparable to the ABS formed by [Ch][Bit]. The odd trend observed with [Ch][DHCit] could be a direct result of specific intramolecular interactions occurring between the [DHCit]⁻ anions as discussed before.

Pereira et al. [39] computed the energetic contributions to the excess enthalpies of PEG + cholinium-based salts/ILs in aqueous media, suggesting that the repulsive dispersion interactions between non-charged alkaloids and [DHP]⁻, a bulky and disperse charged anion, are highly pronounced. These results also explain the preferential partition of caffeine, theobromine and

theophylline into the PL6200-rich phase since no specific interactions between the IL/salt ions and these alkaloids seem to occur in an extent able to control their partitioning behavior.

With an opposite behavior, with the highest hydrophobic character amongst the studied alkaloids ($\log K_{ow} = 1.163$), nicotine preferentially partitions to the IL-rich phase (except in ABS formed by [Ch][Lac] and [Ch][But]). Contrarily to methylxanthines, nicotine is present mostly as a positively charged species in all investigated systems, preferentially partitioning to the most hydrophilic and charged IL/salt-rich phase. This behavior suggests that the partition of nicotine may involve specific interactions with the ILs/salts, namely hydrogen-bonding and electrostatic interactions. Furthermore, the extent of the nicotine partitioning to the IL/salt-rich phase is different when compared to the remaining alkaloids, according to the following order: [Ch][DHP] > [Ch][Bit] > [Ch][DHCit] > [Ch]Cl > [Ch][Ac] > [Ch][Pro] > [Ch][Lac] > [Ch][But]. This order is in close agreement with the ABS formation aptitude, which involves the IL/salt ions to establish interactions with water and the copolymer, further contributing to the understanding that specific interactions between the IL/salt ions and nicotine may be present and govern this alkaloid partitioning between the two phases.

ABS formed by [Ch][DHP] allow the highest partition coefficients of caffeine, theobromine and theophylline towards the PL6200-rich phase, while also allowing the lowest partition coefficients of nicotine. It seems that by changing the IL/salt some tailoring to separate the set of the studied alkaloids can be achieved. Figure 5 shows the selectivity of the studied systems to separate caffeine, theobromine or theophylline from nicotine. The [Ch][DHP]-based ABS is, amongst the screened ABS, the most selective system to separate nicotine from the remaining alkaloids: $S_{nic/tph} = 15.97$, $S_{nic/cof} = 13.86$ and $S_{nic/tbr} = 7.81$.

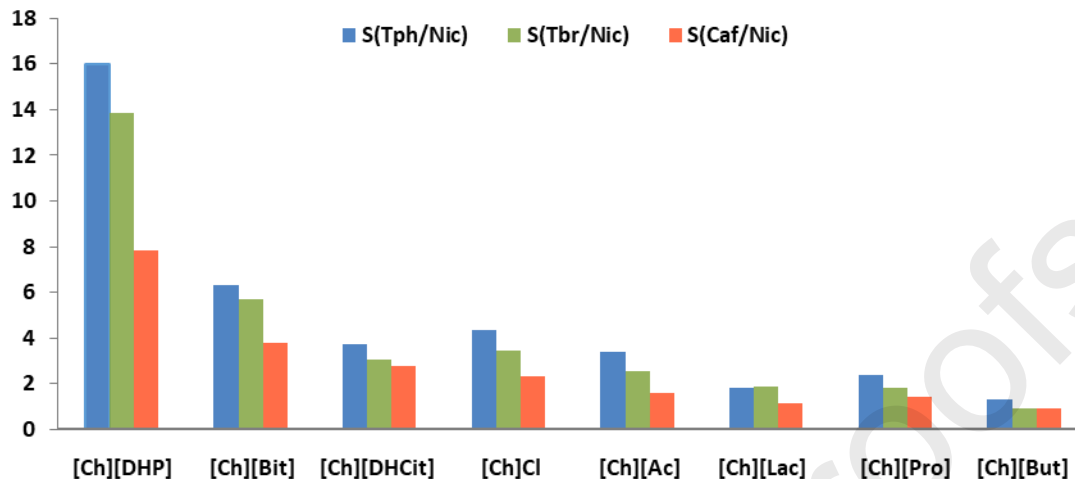


Figure 5. Selectivity parameters in ABS composed of 30 wt % PL6200 + 25 wt % cholinium based-ILs/salts + 50 wt % aqueous solution of each alkaloid, at 25°C and atmospheric pressure.

Conclusions

Novel ABS based on cholinium salts or ILs combined with the triblock copolymer Pluronic PE6200 were investigated. By the determination of the respective phase diagrams it was demonstrated that the molecular-level mechanisms ruling the formation of ABS containing ILs and copolymers is more complex than those formed by salts and ILs or salts and polymers. In particular, and contraly to cholinium-based salts, specific interactions occurring between the IL and the copolymer play an important role in the ABS formation. The ability of cholinium-based salts to form ABS with PL6200 decreased in following order: [Ch][DHP] > [Ch][Bit] > [Ch][DHCit] > [Ch]Cl, being the two-phase formation mainly governed by the salts anion polar surface. The odd behavior observed with [Ch][DHCit] may be related with the anion self-aggregation. The ability of cholinium-based ILs to induce ABS decreased as following: [Ch][Ac] > [Ch][Lac] > [Ch][Pro] > [Ch][But], which is in accordance with the octanol-water partition coefficient values of the corresponding anions. In IL-copolymer ABS, it was found that the IL interactions with the polymer cannot be discarded and play a crucial role in creating two-phase systems.

The partitioning of a series of alkaloids in the investigated ABS was also analyzed to gather further insights on the mechanisms ruling the partition behavior in these systems. Caffeine,

theophylline and theobromine preferentially partitioned into the more hydrophobic PL6200-rich phase, with their partition coefficients increasing in following order [Ch][DHCit] < [Ch][But] < [Ch][Lac] < [Ch][Pro] < [Ch]Cl < [Ch][Bit] < [Ch][Ac] < [Ch][DHP]. Overall, PL6200-rich phases with lower IL/salt or water contents lead to higher partition coefficients of caffeine, theophylline and theobromine. These results suggest that the partition of these three alkaloids follows the phase's hydrophobicities, and as such can also be used as hydrophobicity probes. On the other hand, nicotine, with the most prominent hydrophobic character amongst the studied alkaloids, preferentially migrates to the salt- or IL-rich phase, revealing that interactions between nicotine and ILs may rule the partition behavior of this alkaloid. The ABS formed by [Ch][DHP] was identified as the most selective system to separate nicotine from the remaining alkaloids, which could give some insights on the use of these ABS to extract and separate alkaloids from complex extracts in future investigations.

Acknowledgements

The authors would like to thank Ministry of Education, Science and Technological Development of Republic of Serbia for financial support. Also, this work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. A.P.M. Tavares acknowledges FCT for the Research Contract and Exploratory Project IF/01634/2015.

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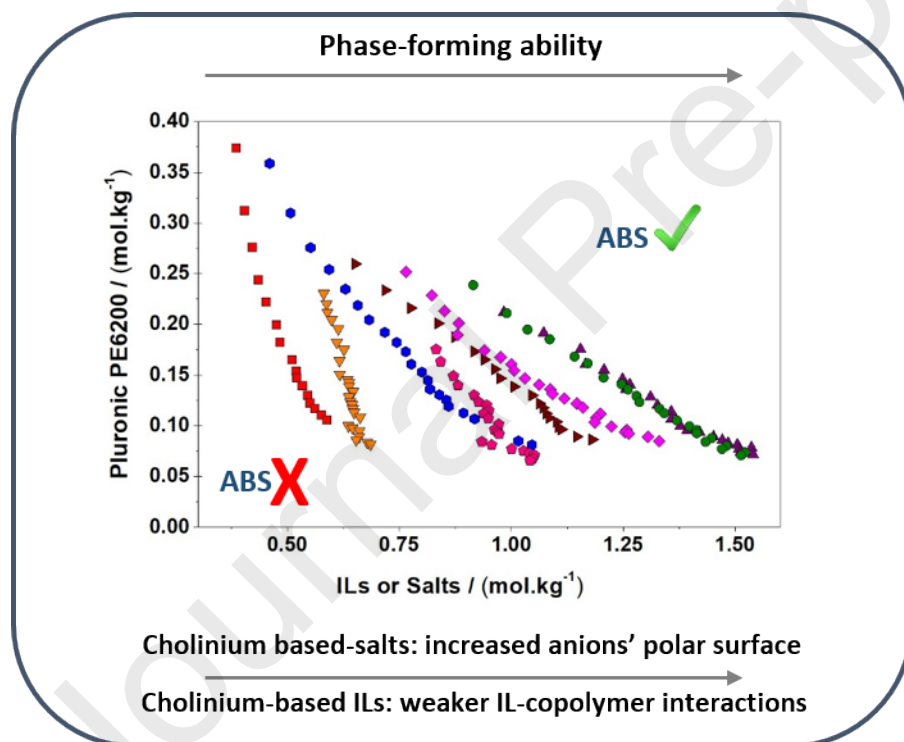
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Highlights

- Cholinium-based ionic liquids or salts and Pluronic PE6200 form aqueous biphasic systems.
- The salts' anion polar surface rules the formation of aqueous biphasic systems.
- The interactions occurring between the ionic liquid and the copolymer contribute to the phase behaviour.
- The partition of most alkaloids follows the phases' hydrophobicity.
- The partition of nicotine is governed by interactions occurring with the ionic liquid or salt.



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Aleksandra Dimitrijević: Conceptualization, Investigation, Visualization, Writing - Original draft preparation; **Ana Paula M. Tavares:** Validation, Writing - Reviewing and Editing; **Ana Jocić:** Investigation, Validation; **Sladana Marić:** Investigation, Validation; **Tatjana Trtić-Petrović:** Conceptualization, Methodology, Supervision, Writing - Reviewing and Editing, Funding acquisition; **Slobodan Gadžurić:** Methodology, Supervision, Writing - Reviewing and Editing; **Mara G. Freire:** Conceptualization, Methodology, Supervision, Writing - Reviewing and Editing, Project administration, Funding acquisition.