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Characterization of cholinium-carboxylate-based aqueous biphasic systems

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Abstract

In the past years large efforts have been placed in the development of novel separation techniques with improved resolution, simplicity, speed and easy to scale-up. Among these, ionic-liquid-based (IL-based) aqueous biphasic systems (ABS) have been broadly proposed for the separation of high-value compounds, allowing improved extraction performance and purification. More recently, significant efforts have been placed on the synthesis and use of novel ILs with both an acceptable environmental footprint and enhanced biocompatibility. In this sense, this work aims to characterize ABS composed of cholinium carboxylate ILs ([Ch][CnO2], with n = 2 to 7),
K$_3$PO$_4$ and water. The respective ternary phase diagrams, including binodal curves, tie-lines and tie-line lengths, were determined at (298 ± 1) K and at atmospheric pressure. The ability to form ABS (or of the IL to be salted-out) increases with the increase of the alkyl chain length of the IL anion, up to [Ch][C$_5$O$_2$]; nevertheless, for longer anion alkyl chain lengths ([$\text{Ch}$][C$_6$O$_2$] and [Ch][C$_7$O$_2$]) the ILs self-aggregation leads to a decrease of the ILs ability to form ABS. The liquid–liquid equilibrium data experimentally determined were modeled using the local composition activity coefficient model NRTL (Non-Random Two Liquid). Finally, the partition behavior of three alkaloids (nicotine, caffeine and theobromine), used here as hydrophobicity probes, was evaluated. In all studied systems, alkaloids preferentially migrate to the IL-rich phase, with partition coefficients (K) ranging between 2.23 and complete extraction in a single-step. Furthermore, the set of ILs investigated allowed identifying an odd-even effect in the alkaloids partitioning derived from the IL anion alkyl chain length. These results support the salting-out effect exerted by K$_3$PO$_4$ and favorable dispersive interactions established between the IL-rich phase-forming components and the alkaloids.

**Keywords:** Aqueous biphasic systems; phase diagrams; NRTL; cholinium carboxylate; ionic liquids; alkaloids

1. **Introduction**

The need to develop novel separation techniques with improved resolution, simplicity, speed, easy to scale-up and able to operate in continuous mode is an important topic of modern bioseparation [1,2]. This is particularly relevant in fields involving biomolecules usually present in complex media, whose current separation and purification techniques require several steps, with high energy and chemical demands
Techniques like chromatography, commonly applied in the purification of biomolecules, lead to high purity, but as well as to high purification costs [2,3]. Aiming at minimizing operation costs in this field, different techniques have been proposed, namely cross-flow electrofiltration, reversed micelles, centrifugal partitioning, membrane chromatography and liquid-liquid extraction with aqueous biphasic systems [1].

Aqueous biphasic systems (ABS) fall within the liquid–liquid extraction techniques, and may lead to high yields, high selectivity and improved purification, and a good combination between the recovery and purification steps, while keeping technological simplicity and a low associated cost [3,4]. These systems are formed when at least two water-soluble components, such as polymers, ionic liquids (ILs), alcohols, salts, sugars, among others, are mixed in water at given concentrations [5]. Polymer-based ABS usually generate systems with high viscosity, which can be a drawback in extraction processes, and may have their selectivity compromised due to their low tunable nature. Therefore, IL-based ABS are preferable choices since they allow to decrease the phases’ viscosity and have a tunable character [6]. In the past years, significant efforts have been placed to synthesize and apply novel ILs with both an acceptable environmental footprint and enhanced biocompatibility [7]. Among these, ILs comprising the 2-hydroxyethyl-N,N,N-trimethylammonium (cholinium) cation stand out due to their high biodegradability and marginal toxicity if correctly designed [8]. However, cholinium-based ILs have a tendency to be highly hydrophilic and only form ABS with polymers (e.g., polypropylene glycol (PPG) 400 and 1000 [9–12], polyethylene glycol (PEG) 400, 600 and 1000 [13]), in which the IL may act as the salting-out species. On the other hand, more hydrophobic (still water miscible) cholinium-based ILs can be
designed to create ABS with strong salting-out salts (e.g., $K_3PO_4$ [6,14,15] and $K_2CO_3$ [7,14]).

Although Berton et al. [6] previously characterized ABS composed of cholinium carboxylate ILs and $K_3PO_4$ at controlled pH values, most of the investigated ILs comprise an even alkyl side chain and other species were added to control the pH. With this work we aim to enlarge the characterization of ABS formed by cholinium-based ILs, combining cholinium carboxylate ILs (with a relevant number of both odd and even alkyl side chain lengths) with $K_3PO_4$ in aqueous media, without adding other species or pH control. To this end, ternary phase diagrams, including binodal curves and tie-lines, were determined at (298 ± 1) K and at atmospheric pressure. The liquid-liquid equilibrium data experimentally determined were modeled using the local composition activity coefficient model NRTL (Non-Random Two Liquid) [16]. Finally, a study on the anion alkyl side chain length effect on the partition of a series of alkaloids (caffeine, nicotine and theobromine) of variable hydrophobicity was performed. This group of alkaloids was selected since it allows to study a family of molecules with different hydrophobicity, and to identify differences in partitioning that could arise from the dispersive interactions established with the IL anion aliphatic moieties (and identify odd-even effects). To the best of our knowledge, several works reporting alkaloids partitioning in IL-based ABS can be found in the literature [3,17–19], but only applying imidazolium-based ILs.
2. Materials and methods

2.1. Materials

The cholinium carboxylate ILs evaluated in this study, namely cholinium propanoate ([Ch][C₃O₂]), cholinium butanoate ([Ch][C₄O₂]), cholinium pentanoate ([Ch][C₅O₂]), cholinium hexanoate ([Ch][C₆O₂]) and cholinium heptanoate ([Ch][C₇O₂]), were synthetized via neutralization, following reported methods [20]. Cholinium acetate ([Ch][C₂O₃]) was supplied by Lolitec (>99 wt%). For the ILs synthesized by us, the synthesis started with a solution of cholinium bicarbonate, to which the selected carboxylic acid was added in a molar ratio of 1:1.1:1. The mixture was agitated overnight at room temperature. Then, the water was removed in a rotary evaporator, and the IL was washed with ethyl acetate to remove unreacted compounds. Finally, ethyl acetate and residual water were removed under high vacuum (1 Pa), moderate temperature (343 K) and continuous stirring for at least 72 h. All ILs showed high purity (>97 wt%), confirmed by \(^1\)H and \(^{13}\)C NMR (spectra given in the Supporting Information, with a maximum uncertainty of 10%). The required precursors, namely cholinium bicarbonate (80 wt% in water, Sigma-Aldrich) and propanoic (99 wt%, Acros Organics), butanoic (99 wt%, Acros Organics), pentanoic (99 wt%, Riedel-de Haen), hexanoic (99.5 wt%, Sigma-Aldrich) and heptanoic acids (99 wt%, Acros Organics) were used as received. The IL-based ABS studied in this work were prepared with potassium phosphate, K₃PO₄ (>97 wt%), purchased from Alfa Aesar. For the extraction studies, caffeine anhydrous (99 wt%, Sigma-Aldrich), nicotine (99 wt%, Alfa Aesar) and theobromine (99 wt%, Sigma-Aldrich) were commercially acquired and used as received. The compound name, CAS number, supplier and mass fraction purity of all compounds used are reported in Table
1. The chemical structures of the studied ILs and alkaloids are depicted in Figure 1. The water used was double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

![Chemical structures of studied ILs and alkaloids](image)

Figure 1. Chemical structures of the studied cholinium-based ILs and alkaloids.
Table 1. Compound name, CAS number, supplier and mass fraction purity of all compounds used.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS Reg. No.</th>
<th>Supplier</th>
<th>Mass fraction purity (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinium bicarbonate</td>
<td>78-73-9</td>
<td>Sigma-Aldrich</td>
<td>80(^a)</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>79-09-4</td>
<td>Acros Organics</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>107-92-6</td>
<td>Acros Organics</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Pentanoic acid</td>
<td>109-52-4</td>
<td>Riedel-de Haen</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>142-62-1</td>
<td>Sigma-Aldrich</td>
<td>99.5(^a)</td>
</tr>
<tr>
<td>Heptanoic acid</td>
<td>111-14-8</td>
<td>Acros Organics</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>7778-53-2</td>
<td>Alfa Aesar</td>
<td>&gt;97(^a)</td>
</tr>
<tr>
<td>[Ch][C(_2)O(_2)]</td>
<td>14586-35-7</td>
<td>Iolitec</td>
<td>&gt;99(^a)</td>
</tr>
<tr>
<td>[Ch][C(_3)O(_2)]</td>
<td>n.a.</td>
<td>Synthetized in this work</td>
<td>&gt;97(^b)</td>
</tr>
<tr>
<td>[Ch][C(_4)O(_2)]</td>
<td>n.a.</td>
<td>Synthetized in this work</td>
<td>&gt;97(^b)</td>
</tr>
<tr>
<td>[Ch][C(_5)O(_2)]</td>
<td>n.a.</td>
<td>Synthetized in this work</td>
<td>&gt;97(^b)</td>
</tr>
<tr>
<td>[Ch][C(_6)O(_2)]</td>
<td>n.a.</td>
<td>Synthetized in this work</td>
<td>&gt;97(^b)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>58-08-2</td>
<td>Sigma-Aldrich</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Nicotine</td>
<td>54-11-5</td>
<td>Alfa Aesar</td>
<td>99(^a)</td>
</tr>
<tr>
<td>Theobromine</td>
<td>83-67-0</td>
<td>Sigma-Aldrich</td>
<td>99(^a)</td>
</tr>
</tbody>
</table>

\(^a\)reported by the supplier; \(^b\)determined by \(^1\)H and \(^13\)C Nuclear Magnetic Resonance with a maximum uncertainty of 10%; n.a. not available
2.2. Phase diagrams and NRTL modeling

The binodal data were determined through the cloud point titration method at \( T = (298 \pm 1) \) K and atmospheric pressure [21,22]. An aqueous solution of \( \text{K}_3\text{PO}_4 \) at 50 wt% and aqueous solutions of the different ILs at 60 wt% were prepared and used for the determination of the binodal curves. The repetitive dropwise addition of the salt aqueous solution into the aqueous solutions of each IL was carried out until the detection of a cloudy (biphasic) solution, followed by the dropwise addition of ultrapure water until the observation of a limpid solution (monophasic region). All additions were made under continuous stirring. The binodal curves were determined by the weight quantification of all components added (\( u(m) = 10^{-4} \) g), in which both the weight mass fractions corresponding to the cloud and limpid points were used to describe each binodal curve. The experimental solubility curves were correlated using Eq. (1) [23]:

\[
[\text{IL}] = A \exp\left( B \cdot [\text{Salt}]^{1.5} \right) - (C \cdot [\text{Salt}]^3)
\]

(1)

where IL and Salt represent the IL and salt weight fraction percentages, respectively, and \( A, B, \) and \( C \) are constants obtained by regression.

Tie-lines (TLs) associated with each binodal curve, i.e., the composition of each phase at equilibrium for a specific mixture composition, were additionally determined. To this end, ternary mixtures composed of IL + \( \text{K}_3\text{PO}_4 \) + water were gravimetrically prepared (\( u(m) = 10^{-4} \) g) in sealed glass vials at compositions within the biphasic region. Upon preparation of the selected mixture point, vigorous stirring was applied. After stirring, the system was centrifuged for 30 min at (298 ± 1) K and both phases were
carefully separated, using syringes with 21G syringe needles, which were weighted \(u=m=10^{-4}\) g before and after sampling. Each TL was determined by a mathematical approach (mass balance and lever-arm rule), applying a system of six equations (Eqs. (2) to (7)). Eqs. (2) and (3) correspond to the description of the binodal curve given by Eq. (1), yet describing the top and bottom phases, respectively; Eqs. (4) and (5) correspond to the lever-arm rule; and Eqs. (6) and (7) correspond to the mass balance of the two ABS phase-forming components, namely the IL and salt. Eqs. (1) to (5) were previously proposed by Merchuk et al.[23], whereas Eqs. (6) and (7) are the salt and IL mass balance added by us to improve the TL data accuracy. The system of equations used for the TLs determination has four unknown parameters \([\text{IL}^T], [\text{IL}^B], [\text{Salt}^T], [\text{Salt}^B]\) and is defined as follows:

\[
[\text{IL}]^T_T = A \exp[(B \cdot [\text{Salt}]^T_T^{0.5}) - (C \cdot [\text{Salt}]^T_T^3)] \tag{2}
\]

\[
[\text{IL}]^B_B = A \exp[(B \cdot [\text{Salt}]^B_B^{0.5}) - (C \cdot [\text{Salt}]^B_B^3)] \tag{3}
\]

\[
[\text{Salt}]^T_T = \frac{[\text{IL}]^T_T}{\alpha} - \frac{1-\alpha}{\alpha} \cdot [\text{IL}]^B_B \tag{4}
\]

\[
[\text{Salt}]^B_B = \frac{[\text{Salt}]^B_B}{\alpha} - \frac{1-\alpha}{\alpha} \cdot [\text{Salt}]^T_T \tag{5}
\]

\[
m_M^{\text{IL}} = m_T \cdot [\text{IL}]^T_T + m_B \cdot [\text{IL}]^B_B \tag{6}
\]

\[
m_M^{\text{Salt}} = m_T \cdot [\text{Salt}]^T_T + m_B \cdot [\text{Salt}]^B_B \tag{7}
\]

where \(m\) is the mass of the components (IL or salt) and the subscripts \(M, T\) and \(B\) designate, respectively, the initial mixture, the top and bottom phases. The variable \(\alpha\) is the ratio between the mass of the top phase and the total mass of the mixture experimentally determined. Finally, the tie-line length \((TLL)\) was determined through the application of the following equation:
To ascertain the reliability of the TLs determined by the application of Eqs. (1) to (7) and to address the possibility of ion exchange in the investigated systems, the IL cation ([Ch]+) and anion ([CnCO2]−) in the IL-(top)-rich phase were quantified by 1H NMR, as described by Almeida et al.[7].

The liquid-liquid equilibrium data experimentally determined was modeled using the local composition activity coefficient model NRTL (Non-Random Two Liquid) [16]. The NRTL model is known to be robust and flexible enough for modeling both ternary LLE data and binary subsystems using a common parameter set – the binary interaction parameters τij. Several works have shown the adequacy of using the NRTL model to describe salt-IL ABS [7,24]. However, determining these parameters set by fitting the model to ternary experimental TLs may lead to poor descriptions of the binary subsystems. To the best of our knowledge no binary interaction parameters exist for the studied binary subsystems.

Aiming at minimizing the loss of the model parameters physical meaning, NRTL parameters were optimized by the simultaneous regression of the following equations to all the experimental data available (six ternary systems and three tie-lines per system) using the objective function, OF, reported in Eq. (12).

\[
TLL = \sqrt{([\text{Salt}]_T - [\text{Salt}]_B)^2 + ([\text{IL}]_T - [\text{IL}]_B)^2} \tag{8}
\]

\[
x_i^T \cdot y_i^T = x_i^B \cdot y_i^B \tag{9}
\]

\[
\ln(y_i) = \frac{\Sigma j \tau_{ij} G_{ij}}{\Sigma_k G_{ki} x_k} + \Sigma j \left[ \frac{x_i G_{ij}}{\Sigma_k G_{ki} x_k} \times \left( \tau_{ij} - \frac{\Sigma_m \tau_{mj} G_{mj} x_m}{\Sigma_k G_{ki} x_k} \right) \right] \tag{10}
\]

\[
G_{ij} = \exp(-\alpha_{ij} \tau_{ij}) \tag{11}
\]

\[
OF = \sum_p \sum_n \sum_k \left( x_{p,n,k}^{\text{exp}} - x_{p,n,k}^{\text{calc}} \right)^2 \tag{12}
\]
where $\tau_{ij}$ are the $i$-$j$ interactions parameters, $\alpha_{ij}$ is related to the non-randomness of the mixture and the common value of 0.3 was taken as default for all systems.

2.3. Alkaloids extraction

Ternary mixtures with compositions selected based on the phase diagrams determined were selected to carry out the extraction of alkaloids. All extraction studies were performed at a similar $TLL$ (ca. 54 ± 2) to avoid differences that could arise from the difference in the coexisting phases' compositions. Aqueous solutions of caffeine and theobromine were prepared at a concentration of 0.060 mg·mL$^{-1}$, while for nicotine an aqueous solution of 150 mg·L$^{-1}$ was prepared. Each mixture was vigorously stirred and centrifuged for 30 min at (298 ± 1) K to reach the alkaloids partitioning between the coexisting phases. Then, a careful separation of the phases was performed and each alkaloid in each phase was quantified by UV-spectroscopy, using a BioTeck Synergy HT microplate reader at a wavelength of 260 nm for nicotine and 270 nm for caffeine and theobromine, using calibration curves previously established. At least three independent ABS were prepared, and three samples of each phase quantified. Control or “blank” solutions at the same mixture point used for the extraction studies (with no alkaloid added) were used.

The partition coefficient of each ABS to each alkaloid, $K_A$, corresponds to the ratio of the concentration of each alkaloid in the phase enriched in IL to that in the salt-rich phase, as described by Eq. (14):
where \([\text{Alkaloid}]_{IL}\) and \([\text{Alkaloid}]_{Salt}\) are the concentration of each alkaloid in the IL-rich and in the salt-rich aqueous phases, respectively.

The pH of each ABS phase was measured at \((298 \pm 1)\) K using a Mettler Toledo S47 Seven MultiTM dual meter pH equipment.

### 3. Results and discussion

#### 3.1. Phase diagrams and NRTL modeling

Ternary phase diagrams for six ILs ([Ch][C_2O_2], [Ch][C_3O_2], [Ch][C_4O_2], [Ch][C_5O_2], [Ch][C_6O_2] and [Ch][C_7O_2]) + K_3PO_4 + water were determined at \((298 \pm 1)\) K and atmospheric pressure, being depicted in Figure 2 in an orthogonal representation. The ILs selection comprises anions with odd and even alkyl chains, allowing a better understanding of the effect of the IL anion on ABS formation. The experimental weight fraction data of each phase diagram are reported in the Supporting Information (Tables S1 and S2). The experimental data were fitted by Eq. (1) and the regression parameters estimated by the least-squares regression method, with their values and corresponding standard deviations (\(\sigma\)) are reported in the Supporting Information (Table S3). The fitting of the experimental data by Eq. (1) is given in Figure 2. The ability of cholinium-based ILs to form ABS with K_3PO_4 at \((298 \pm 1)\) K at 20 wt\% of K_3PO_4 follows the rank: [Ch][C_2O_2] < [Ch][C_3O_2] ≈ [Ch][C_5O_2] < [Ch][C_6O_2] < [Ch][C_4O_2] < [Ch][C_7O_2]. Phase diagrams with a larger immiscibility region display a higher ability to form two phases. Accordingly, this trend shows that increasing the alkyl chain length of the IL anion, a higher capability to form ABS (or of the IL to be salted-out) is attained. This trend is observed up to [Ch][C_5O_2]; however, for ILs with anions with longer alkyl...
chain lengths the self-aggregation of the IL ([Ch][C₆O₂] and [Ch][C₇O₂]) occurs, thus decreasing their ability to create ABS [25,26]. Similar trends have been recently observed for ABS composed of cholinium-carboxylate-based ILs and different salts, such as K₂HPO₄ [27] and K₂CO₃ [7], showing that this behavior is independent of the salt used.

Figure 2. Orthogonal phase diagrams representation of the ABS formed by IL + K₃PO₄ + water at (298 ± 1) K and atmospheric pressure.

To determine the TLs of each phase diagram along with their respective length (TLLs), three mixture compositions at the biphasic region were prepared and Eqs. (1) to (7) were applied. The compositions of the top and bottom phases and TLLs are reported in Table 2. The IL cation and anion in the IL-rich phase of two different TLs were quantified by ¹H NMR to address the reliability of applying Eqs. (1) through (7) by assuring that no ion exchange occurs. The results obtained and the ¹H NMR spectra used to evaluate the ion exchange are given in the Supporting Information (Table S4). Although two salts are used in the investigated ABS, the results obtained demonstrate that the IL cation and anion at the IL-rich phase exist in a ratio of 1:1, proving that ion exchange in these systems does not exist or, if it exists, it is of low significance and below the detection limit of the analytical technique. These results confirm the reliability of the TLs given in Table 2.
Table 2. Weight Fraction Compositions (wt%) of the Initial Mixture point (M), compositions of the Top (T) and Bottom (B) phases, and TLLs of the ABS composed of water + cholinium-based ILs + K$_3$PO$_4$ at 298 K and atmospheric pressure (0.1 MPa).\(^\text{a}\)

<table>
<thead>
<tr>
<th>IL</th>
<th>weight fraction composition/wt%</th>
<th>TLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ch][C$_2$O$_2$]</td>
<td>34.88</td>
<td>11.43</td>
</tr>
<tr>
<td></td>
<td>38.66</td>
<td>9.06</td>
</tr>
<tr>
<td></td>
<td>41.78</td>
<td>7.33</td>
</tr>
<tr>
<td>[Ch][C$_3$O$_2$]</td>
<td>40.78</td>
<td>9.21</td>
</tr>
<tr>
<td></td>
<td>43.28</td>
<td>8.11</td>
</tr>
<tr>
<td></td>
<td>47.40</td>
<td>6.55</td>
</tr>
<tr>
<td>[Ch][C$_4$O$_2$]</td>
<td>35.75</td>
<td>8.88</td>
</tr>
<tr>
<td></td>
<td>37.91</td>
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<td></td>
<td>40.80</td>
<td>6.38</td>
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<tr>
<td>[Ch][C$_5$O$_2$]</td>
<td>41.71</td>
<td>7.31</td>
</tr>
<tr>
<td></td>
<td>49.36</td>
<td>4.66</td>
</tr>
<tr>
<td></td>
<td>53.55</td>
<td>3.56</td>
</tr>
<tr>
<td>[Ch][C$_6$O$_2$]</td>
<td>39.65</td>
<td>9.82</td>
</tr>
<tr>
<td></td>
<td>44.20</td>
<td>7.89</td>
</tr>
<tr>
<td></td>
<td>45.60</td>
<td>7.37</td>
</tr>
<tr>
<td>[Ch][C$_7$O$_2$]</td>
<td>41.04</td>
<td>11.16</td>
</tr>
<tr>
<td></td>
<td>49.49</td>
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</tr>
<tr>
<td></td>
<td>52.79</td>
<td>6.14</td>
</tr>
</tbody>
</table>

\(^a\) The combined standard uncertainty for the weight fraction $u_w(100 \text{ wt})$ is 0.01, the standard uncertainty for the temperature $u(T)$ is 1 K, and the standard uncertainty for pressure $u(p)$ is 10 kPa.

The NRTL activity coefficient model was applied to the TL' data allowing to determine binary interaction parameters able of describing the studied systems. Using ternary LLE data to determine binary interaction parameters may lead to poor descriptions of the binary subsystems. Thus, aiming at minimizing the loss of the model parameters physical meaning, the NRTL parameters were optimized by simultaneously regressing all the experimental data (six ternary systems and three TLs per system) using the objective function given by Eq. (12). To the best of our knowledge no binary interaction parameters for the NRTL exist for the studied binary subsystems.
As depicted in Figure 3, the set of optimized binary interaction parameters allows an excellent description of the experimental data with \( \%\text{AAD} \) (average absolute deviation

\[
\%\text{AAD} = \sum_i^N \left| \frac{w_{\text{exp}, i} - w_{\text{calc}, i}}{w_{\text{exp}, i}} \right| \cdot 100 / N
\]

of 1.99 \cdot 10^{-2}. Furthermore, the non-ideality of both phases is governed by the water and salt interactions - as demonstrated by the activity coefficients given in the Supporting Information (Table S5) and the binary interaction parameters described in Table 3. The non-ideality observed is a clear indication that upon mixing of the three components the interactions established between the salt and water compete with those present in the IL + water binary system, thus promoting liquid-liquid equilibrium.

Figure 3. Phase diagrams for the studied ternary systems and TLs description through the optimized NRTL binary interaction parameters: Solid lines - fitting by equation 1; black empty circles - experimental TLs; and dash – TLs obtained from the NRTL fitting.
Table 3. Optimized NRTL binary interaction parameters (J mol$^{-1}$) using $\alpha_{ij} = 0.3$.

<table>
<thead>
<tr>
<th></th>
<th>$\text{H}_2\text{O}$</th>
<th>$\text{K}_3\text{PO}_4$</th>
<th>[Ch][C$_2$O$_2$]</th>
<th>[Ch][C$_3$O$_2$]</th>
<th>[Ch][C$_4$O$_2$]</th>
<th>[Ch][C$_5$O$_2$]</th>
<th>[Ch][C$_6$O$_2$]</th>
<th>[Ch][C$_7$O$_2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>-</td>
<td>0.7814</td>
<td>0.0941</td>
<td>0.8860</td>
<td>0.2575</td>
<td>0.4635</td>
<td>0.3308</td>
<td>0.7568</td>
</tr>
<tr>
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<td>-</td>
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<td>0.8031</td>
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<td>0.1501</td>
<td>0.5886</td>
<td>0.3210</td>
<td>0.3519</td>
<td>0.5944</td>
<td>0.2067</td>
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<td>[Ch][C$_3$O$_2$]</td>
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<td>0.2622</td>
<td>0.0626</td>
<td>0.0986</td>
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</table>

3.2. Alkaloids extraction

In Figure 4 are depicted the results obtained for the partition of nicotine, theobromine and caffeine using ABS composed of the different cholinium-based ILs + $\text{K}_3\text{PO}_4$ + water. These alkaloids were chosen due to their range of hydrophobicity and to identify differences in partitioning that could arise from the dispersive interactions established with the IL anion aliphatic moieties, and thus identify possible odd-even effects derived from the IL anion. All extraction studies were carried out at a common TLL (ca. 54 ± 2). The mixture compositions, respective phases' compositions and TLLs are presented in Table 2, while the partition coefficient ($K_A$) of each alkaloid, respective standard deviations and pH values of the coexisting phases are reported in the Supporting information (Table S6).

In all studied systems it is observed the preferential partitioning of the alkaloids to the IL-rich aqueous phase (the most hydrophobic phase) with partition coefficients of nicotine, theobromine and caffeine ranging between 2.23 and complete extraction to the IL-rich phase (∞) in one step (Figure 4 and Table S6 in the Supporting Information). These high values support the salting-out effect exerted by $\text{K}_3\text{PO}_4$ and possible
favorable interactions established between the IL-rich phase-forming components and alkaloids.

All ABS investigated lead to the complete extraction of caffeine, while an odd-even effect is shown with the remaining alkaloids. Higher partition coefficients are observed for nicotine and theobromine using ABS composed of ILs with an odd alkyl side chain at the anion. In general, ABS with ILs with an odd alkyl side chain led to IL-rich phases with a higher water content (TL data, Table 2), and consequently to higher partition coefficients for nicotine and theobromine. These results reinforce the water content relevance in the partition of alkaloids in the studied ABS. These findings are in agreement with by those previously shown by Belchior et al. [27], who determined the Setschenow salting-out coefficients, $k_s$, for ABS composed of cholinium-based ILs + $K_3HPO_4 +$ water, showing an odd-even effect in these values along the size of the alkyl side chain length. The authors found slightly higher $k_s$ values for ILs comprising anions with even alkyl chains, meaning that these are more prone to be salted-out by the salt or more easily undergo phase separation in aqueous solutions.

In addition to the salting-out effects exerted by $K_3PO_4$ and water content relevance, specific interactions established between the IL-rich phase-forming components and alkaloids should be considered. These interactions in different ABS have found to be important in the partition of different biomolecules, namely amino acids [27], nitrogenous bases [7] and even alkaloids [3]. Although no major differences are observed with the ABS comprising ILs with even alkyl side chains, the partition coefficients of the ABS constituted by ILs with odd alkyl chains at the anion ([Ch][C$_3$O$_2$], [Ch][C$_5$O$_2$] and [Ch][C$_7$O$_2$]) (Figure 4) decrease in the following order: caffeine > nicotine > theobromine. This rank closely follows the logarithm of the octanol–water
partition coefficients (log($K_{ow}$)) of the investigated alkaloids (nicotine, 10.47; caffeine, 0.25; and theobromine, 0.14) [3]. Furthermore, the studied alkaloids may suffer speciation according to the ABS pH values. The pH values of both top and bottom phases are given in the Supporting Information, ranging between 12.7 and 13.1. The dissociation curves of nicotine, caffeine and theobromine as a function of pH are also provided in the Supporting Information (Figures S1 to S3). According to the pH values of the IL and $K_3$PO$_4$-rich phases, nicotine and caffeine are neutral molecules, while theobromine has a negative charge. However, in general, lower partition coefficients are attained for theobromine (as described before), meaning that electrostatic interactions do not play a major role. Overall, this behavior shows that the main forces acting in the alkaloids partitioning correspond to dispersive forces combined with salting-out effects exerted by the salt.

Other works on the partitioning of alkaloids using ABS have been reported in the literature. For example, Freire et al. [17], Passos et al. [3] and Jamehbozorget al. [19] studied the partitioning of alkaloids (caffeine and nicotine; nicotine, caffeine, theophylline and theobromine; and codeine and caffeine, respectively) using ABS composed of imidazolium-based ILs. Although excellent results were achieved and, in some cases, the complete extraction of alkaloids was demonstrated to the IL-rich phase, the mentioned authors reported the use of imidazolium-based ILs instead of cholinium-based counterparts. Additionally, Pereira et al. [18] applied ABS composed of cholinium-based ILs and polyethylene glycol (PEG) to extract caffeine. It was found that in all systems, caffeine preferentially concentrates in the lower water content PEG-rich phase, showing the difference in behavior between ABS composed of ILs and salts and ILs and polymers.
4. Conclusions

In this work, ternary phase diagrams for ABS composed of cholinium carboxylate ILs, K₃PO₄ and water at (298 ± 1) K and atmospheric pressure were determined. In addition to each binodal curve, TLS and TLLs have been determined, and the NRTL model applied. The ability to form ABS increases with the increase of the alkyl chain length of the IL anion, up to [Ch][C₅O₂]; nevertheless, for longer anion alkyl chain lengths ([Ch][C₆O₂] and [Ch][C₇O₂]), the self-aggregation of the ILs leads to a decrease of their ability to form ABS. The set of optimized binary interaction parameters allowed an excellent description of the experimental data by the NRTL activity coefficient model. Furthermore, it is shown that the phase equilibria non-ideality is governed by water-salt interactions.
In addition to the characterization of the studied ABS, their performance to extract a series of alkaloids of different hydrophobicity was evaluated, namely with nicotine, caffeine and theobromine, at a fixed TLL (54 ± 2). The systems investigated allow partition coefficients ranging from 2.23 to ∞ complete extraction to the IL-rich phase. In general, ABS constituted by ILs with even alkyl chains at the anion ([Ch][C₂O₂], [Ch][C₄O₂] and [Ch][C₆O₂]), which correspond to those with a higher water content in the IL-rich phase, lead to higher partition coefficients. These results suggest the occurrence of an odd-even effect in the alkaloids extraction along the size of the anion aliphatic moiety. Overall, factors ruling the alkaloids partitioning in the studied ABS correspond to the salting-out effect exerted by the salt and dispersive forces. The of results here provided contribute to a better characterization of IL-based ABS composed of cholinium carboxylate ILs and salts, while foreseeing their application in the separation of added-value compounds.

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**Diana C. V. Belchior**: Investigation; Writing - review & editing  
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**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.
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5. References


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