

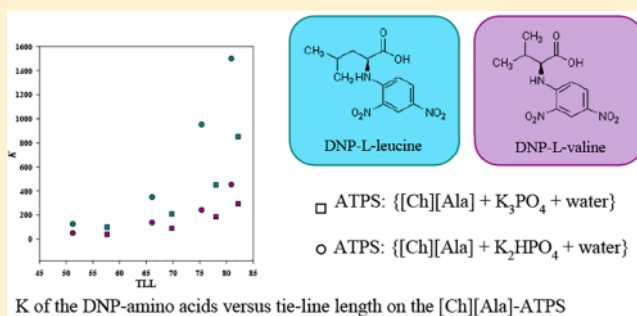
# Partitioning of DNP-Amino Acids in New Biodegradable Choline Amino Acid/Ionic Liquid-Based Aqueous Two-Phase Systems

Patricia F. Requejo Elena Gomez and Eugenia A. Macedo

Associate Laboratory of Separation and Reaction Engineering, Laboratory of Catalysis and Materials (LSRE-LCM), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, Porto 4200-465, Portugal

Supporting Information

**ABSTRACT:** In this work, the partitioning of four *N*-(2,4-dinitrophenyl)-amino acids (DNP-L-leucine, DNP-L-valine, DNP-L-alanine, and DNP-glycine) in aqueous two-phase systems (ATPS) containing a new biodegradable ionic liquid cholinium alaninate, [Ch][Ala], and the inorganic salts  $K_3PO_4$  and  $K_2HPO_4$  was determined at  $T = 298.15$  K and  $p = 101.3$  kPa. The influence of the salt on the partition coefficients was discussed. The distribution coefficients ( $K$ ) of the four studied DNP-amino acids are higher in the ATPS containing the inorganic salt  $K_3PO_4$ . Moreover, the experimental LLE data obtained for both ATPS {[Ch][Ala] (1) +  $K_3PO_4$  or  $K_2HPO_4$  (2) + water (3)} (previously reported) were represented using the nonrandom two-liquid thermodynamic model. Finally, a comparison of the distribution coefficients presented in this work and those found in literature for the four studied DNP-amino acids on different ATPS was performed.



## 1. INTRODUCTION

Proteins are built with amino acids, which are of utmost importance in our body. There are essential amino acids that our bodies cannot synthesize and therefore have to be included in the diet through food or food supplements.<sup>1</sup>

Industrial production of these amino acids is carried out through a wide range of technologies that include microbial fermentation, chemical synthesis, protein hydrolysis, and the biotransformation of precursors with cells or enzymes.<sup>2</sup> In all cases, the amino acids are obtained at low concentrations and mixed with other compounds. The recovery process for the amino acids has three stages: (i) separation of insoluble compounds (sedimentation, centrifugation, and filtration), (ii) isolation of the amino acids (extraction, ultrafiltration, etc.), and (iii) purification using crystallization or precipitation by modification of pH. The conventional processes used in the purification of amino acids require techniques as crystallization, adsorption, ionic exchanged resins, or organic solvent extraction, this last one being the most used.<sup>2–6</sup> This last stage of purification is a complex process whose cost can reach up to 50% of the total cost of amino acids.

Over the last few years, the application of aqueous two-phase systems (ATPS) has been widely studied in the field of separation, purification, and recovery of different biomolecules as a healthier and environmentally friendly alternative to conventional processes.<sup>7–18</sup> For these reasons, the partitioning of four *N*-(2,4-dinitrophenyl)-amino acids (DNP-amino acid) using ATPS is presented in this work. The selected ATPS combine a new biodegradable and nontoxic solvent, the ionic liquid (IL) based on choline as cation and alanine as anion

(cholinium alaninate, [Ch][Ala]) and two inorganic salts,  $K_3PO_4$  and  $K_2HPO_4$ . The ILs composed of choline and amino acids (CAAILs) have gained an increased attention as a suitable alternative to replace the conventional ionic liquids (formed by imidazolium and pyrrolidinium cations) as CAAILs are obtained from natural compounds and, consequently, are not toxic and have low environmental impact.<sup>19,20</sup>

Therefore, the partitioning of four dinitrophenyl amino acids (DNP-L-leucine, DNP-L-valine, DNP-L-alanine, and DNP-glycine) in the ATPS {[Ch][Ala] (1) +  $K_3PO_4$  or  $K_2HPO_4$  (2) + water (3)} was studied at  $T = 298.15$  K and  $p = 101.3$  kPa and is presented in this work. With these systems, it is possible to evaluate the effect of the salt in the partition coefficient values of the four DNP-amino acids. Further, the experimental data were compared with those found in literature for the same studied DNP-amino acids on other ATPS.<sup>21–32</sup> To our knowledge, no data for the partitioning of these four amino acids on the ATPS involving [Ch][Ala] are available in the literature. It is worth mentioning that the experimental data for the binodal curve and the tie-lines of the studied ATPS were reported in a previous work.<sup>33</sup> Additionally, these experimental ATPS data were correlated for the first time using the nonrandom two-liquid (NRTL) thermodynamic model.<sup>34</sup>

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Table 1. Suppliers and Purities of Chemicals Used

| compound                                     | supplier                      | purity (wt %) <sup>a</sup> | purification method          | analysis method <sup>b</sup>      |
|----------------------------------------------|-------------------------------|----------------------------|------------------------------|-----------------------------------|
| choline hydroxide, 45 wt %, aqueous solution | Acros Organics                | ≥47 <sup>b</sup>           |                              | titration total base <sup>b</sup> |
| L-alanine                                    | Scharlau                      | ≥99 <sup>b</sup>           |                              | TLC <sup>c,b</sup>                |
| cholinium alaninate                          | synthesized in our laboratory | ≥93                        | vacuum desiccation           | NMR <sup>d</sup>                  |
| potassium phosphate tribasic                 | Sigma-Aldrich                 | ≥98 <sup>b</sup>           | drying with moderate heating | titration with HCl <sup>b</sup>   |
| dipotassium hydrogen phosphate anhydrous     | Scharlau                      | ≥98 <sup>b</sup>           | drying with moderate heating | titration with HCl <sup>b</sup>   |
| N-(2,4-dinitrophenyl)-L-alanine              | Tokyo Chemical Industry       | ≥98 <sup>b</sup>           |                              | HPLC <sup>e,b</sup>               |
| N-(2,4-dinitrophenyl)-L-valine               | Tokyo Chemical Industry       | ≥98 <sup>b</sup>           |                              | HPLC <sup>e,b</sup>               |
| N-(2,4-dinitrophenyl)-glycine                | Tokyo Chemical Industry       | ≥98 <sup>b</sup>           |                              | HPLC <sup>e,b</sup>               |
| N-(2,4-dinitrophenyl)-L-leucine              | Tokyo Chemical Industry       | ≥99 <sup>b</sup>           |                              | HPLC <sup>e,b</sup>               |

<sup>a</sup>wt %: weight percent. <sup>b</sup>Given by the suppliers. <sup>c</sup>Thin-layer chromatography. <sup>d</sup>Nuclear magnetic resonance. <sup>e</sup>High performance liquid chromatography.

Table 2. Feed Compositions and Experimental Phase Equilibrium Compositions (in Weight Percentage) and Their Corresponding Tie-Line Length (TLL) and Slope (STL) for the Systems {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + Water (3)} at T = 298.15 K and p = 101.3 kPa

| TL                                                                | feed                 |                        | top phase           |                       | bottom phase        |                       | STL   | TLL    |
|-------------------------------------------------------------------|----------------------|------------------------|---------------------|-----------------------|---------------------|-----------------------|-------|--------|
|                                                                   | [IL] <sub>feed</sub> | [salt] <sub>feed</sub> | [IL] <sub>top</sub> | [salt] <sub>top</sub> | [IL] <sub>bot</sub> | [salt] <sub>bot</sub> |       |        |
| {[Ch][Ala] (1) + K <sub>3</sub> PO <sub>4</sub> (2) + water (3)}  |                      |                        |                     |                       |                     |                       |       |        |
| TL1                                                               | 19.8                 | 36.3                   | 64.3                | 2.4                   | 0.4                 | 52.0                  | 1.288 | 80.906 |
| TL2                                                               | 25.1                 | 29.5                   | 61.2                | 3.0                   | 1.1                 | 48.3                  | 1.329 | 75.352 |
| TL3 <sup>b</sup>                                                  | 29.1                 | 24.1                   | 58.1                | 3.4                   | 1.9                 | 45.1                  | 1.349 | 70.013 |
| TL4                                                               | 33.0                 | 20.1                   | 55.9                | 3.8                   | 2.8                 | 43.1                  | 1.355 | 66.095 |
| TL5 <sup>b</sup>                                                  | 36.8                 | 15.2                   | 52.5                | 4.6                   | 5.1                 | 38.9                  | 1.384 | 58.479 |
| TL6                                                               | 40.2                 | 10.9                   | 49.2                | 5.6                   | 7.3                 | 35.1                  | 1.418 | 51.264 |
| {[Ch][Ala] (1) + K <sub>2</sub> PHO <sub>4</sub> (2) + water (3)} |                      |                        |                     |                       |                     |                       |       |        |
| TL1                                                               | 29.9                 | 34.2                   | 62.0                | 3.9                   | 3.5                 | 61.6                  | 1.013 | 82.180 |
| TL2                                                               | 31.9                 | 30.7                   | 59.0                | 4.3                   | 4.0                 | 59.7                  | 0.993 | 78.038 |
| TL3 <sup>b</sup>                                                  | 33.0                 | 27.5                   | 55.9                | 5.0                   | 4.2                 | 57.6                  | 0.981 | 73.770 |
| TL4                                                               | 36.1                 | 23.0                   | 54.1                | 5.9                   | 5.3                 | 55.8                  | 0.979 | 69.756 |
| TL5 <sup>b</sup>                                                  | 38.5                 | 18.0                   | 49.9                | 7.3                   | 6.0                 | 53.1                  | 0.960 | 63.428 |
| TL6                                                               | 40.0                 | 14.0                   | 46.5                | 8.8                   | 7.2                 | 50.9                  | 0.933 | 57.644 |

<sup>a</sup>Standard uncertainties, *u*, are *u*(*T*) = 0.2 K, *u*(*p*) = 10 kPa, *u*(*w*, weight percentage) = 10<sup>−1</sup>. <sup>b</sup>Experimental tie-line determined in this work

## 2. EXPERIMENTAL SECTION

**2.1. Chemicals.** Choline hydroxide, 45 wt % (weight percentage) aqueous solution, purchased from ACROS Organics, and the amino acid L-alanine, supplied by Scharlau, were used for the synthesis of the ionic liquid cholinium alaninate, named [Ch][Ala]. Inorganic salts, potassium phosphate tribasic (K<sub>3</sub>PO<sub>4</sub>), and dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) were provided by Sigma-Aldrich and Scharlau, respectively. Solid chemicals were used after drying with moderate heating to reduce their water content. Stock solutions were prepared with double distilled water procured by Panreac, and their corresponding compositions were obtained gravimetrically by lyophilization using a freeze-dryer Scan Vac, CoolSafe 55-4.

The dinitrophenyl amino acids selected were: N-(2,4-dinitrophenyl)-L-alanine (DNP-Ala), N-(2,4-dinitrophenyl)-L-valine (DNP-Val), N-(2,4-dinitrophenyl)-glycine (DNP-Gly), and N-(2,4-dinitrophenyl)-L-leucine (DNP-Leu), obtained from Tokyo Chemical Industry (TCI). These chemicals were used without any further purification due their low thermal stability. Furthermore, due to the low solubility of the DNP-amino acids in water, stock solutions of composition of 0.2 in weight percentage were prepared by weighing with double distilled water and were kept protected from light at room temperature. All solutions used in this work were prepared by weighing using an Adam Equipment balance model AAA250L

with an uncertainty in the measurement of  $\pm 2 \times 10^{-3}$  g. The purities and the suppliers of all chemicals used are presented in Table 1.

**2.2. Synthesis of [Ch][Ala].** The ionic liquid [Ch][Ala] was synthesized from choline hydroxide and the amino acid via simple neutralization reaction according to a procedure reported in a previous work.<sup>33</sup> The obtained CAAIL was dried under vacuum at *T* = 323.15 K for 48 h and stored under moisture-free conditions until its use; its water content (<180 ppm) was measured with a Mettler Toledo C20 Coulometric Karl Fisher Titrator with an uncertainty in the measurement of  $\pm 5$  . The chemical structure of the synthesized [Ch][Ala] was confirmed by <sup>1</sup>H-RMN (available as Supporting Information, Figure S1).

**2.3. Experimental Procedure.** Aqueous two-phase systems were prepared by weighing the appropriate amounts of each component of the ternary systems {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} at *T* = 298.15 K and *p* = 101.3 kPa. For the experimental measurements of the two new tie-lines (TLs) presented in this work, immiscible ternary mixtures of known composition were placed into glass equilibrium cells properly sealed with rubber covers. To ensure an adequate contact between all components, the mixtures were vigorously stirred for at least 4 h, and then they were allowed to settle overnight for the complete separation of the phases at *T* = 298.15 K in a PolyScience thermostatic bath

with digital temperature controller. A digital thermometer ASL model F200 with an uncertainty in the measurement of  $\pm 0.01$  K was used to control the temperature during the experiments. Compositions of the TL were determined using a UV vis spectrophotometer (Thermo Scientific Varioskan Flash) at 270 nm to calculate the concentration of [Ch][Ala] in both phases. A calibration curve of the absorbance versus the ionic liquid concentration was previously established. Water content was determined gravimetrically after freeze-drying using a lyophilizer, and the salt composition was calculated by mass balance. Feed compositions of the tie-lines reported in our previous work<sup>33</sup> were used for the partitioning studies, and the two new TLs presented in this work are shown in Table 2.

For the study of the partitioning of the four DNP-amino acids, different stock solutions of [Ch][Ala] (0.85 wt %),  $K_3PO_4$  (0.48 wt %),  $K_2HPO_4$  (0.61 wt %), and the four DNP-amino acids (0.2 wt %) with double distilled water were prepared by weighing. The components of the ternary mixtures were added using an automatic pipet (Multipipette XStream Eppendorf) in Eppendorf tubes. Each tie-line was replicated six times to evaluate the influence of the DNP-amino acid composition in the same tie-line. For this, different amounts of DNP-amino acid from 0 to 100 mL of the corresponding stock solution were added. To keep the tie-line composition constant, an adequate amount of water (from 100 to 0 mg) was pipetted to each sample. Samples were vigorously mixed in a vortex for 2 min, and phase separation was achieved by centrifugation (Minispin, Eppendorf) at  $13.4 \times 10^3$  rpm for 15 min. To ensure the complete separation of both phases and an adequate distribution of mixture components, all samples were settled for 4 h at  $T = 298.15$  K and  $p = 101.3$  kPa. After this, samples of each phase were carefully withdrawn and conveniently diluted, and their absorbance at a wavelength of 362 nm was measured using a UV vis spectrophotometer. The possible interferences due to salt and ionic liquid were minimized by diluting the top and bottom phases with water: the selected dilution factors (DF) were 60 and 30 for the top and bottom phase, respectively. Further, samples were analyzed against blanks (samples with the same composition but without amino acids). Furthermore, the ionic liquid [Ch][Ala] does not present value of absorbance at the wavelength selected to analyze the amino acids (362 nm). Partition coefficients ( $K$ ) were calculated using following equation:

$$K = \frac{\text{Abs}(\text{top phase})DF_{\text{top}}}{\text{Abs}(\text{bottom phase})DF_{\text{bottom}}} \quad (1)$$

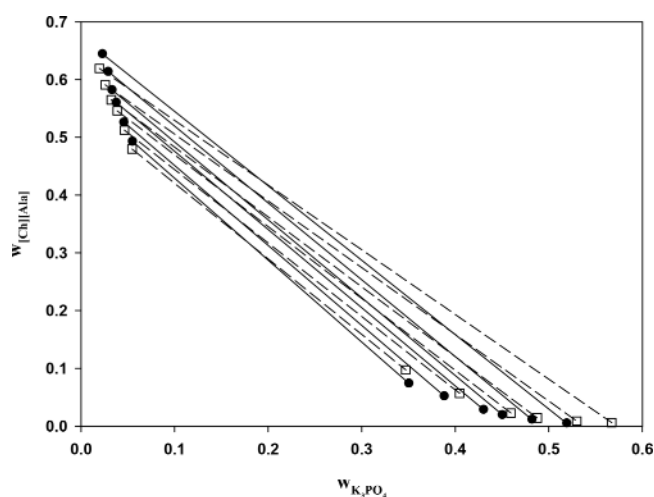
where Abs and DF represent the absorbance and the dilution factor of the indicated phase, respectively. Therefore,  $K$  values were obtained as the slope of absorbance in the top phase versus absorbance in the bottom phase (they were corrected with the respective dilution factors). The adopted procedure is commonly used in this type of study.<sup>21,23,24</sup>

### 3. RESULTS AND DISCUSSION

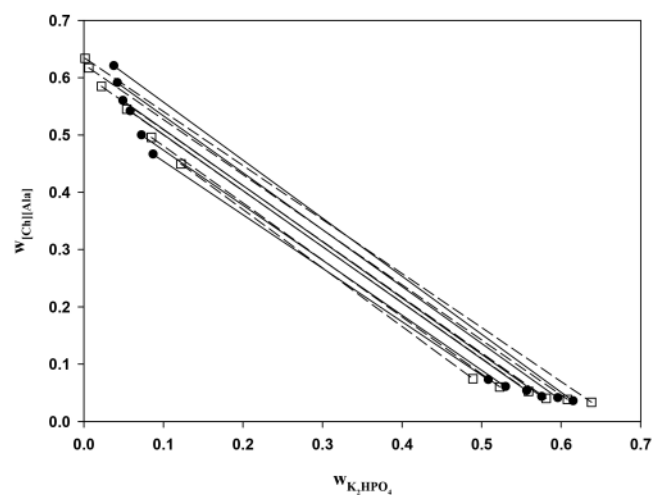
Binodal curves and tie-lines were reported in a previous work;<sup>33</sup> feed compositions and compositions of these tie-lines are shown in Table 2. Moreover, in this Table, the compositions of two new experimental tie-lines are also presented, as one objective of this work is the modeling of the experimental data with the NRTL equation, which was not done previously.<sup>33</sup>

**Table 3.** NRTL Parameters  $g_{ij}$ ,  $g_{ji}$ , and  $i_j$  and Root-Mean-Square Deviation of the Composition,  $\sigma_x$ , Obtained from LLE Data Correlation Using NRTL for the ATPS at  $= 298.15$  K and  $p = 101.3$  kPa

| $i$                                                               | $j$ | $\Delta g_{ij}$ (J·mol <sup>-1</sup> ) | $\Delta g_{ji}$ (J·mol <sup>-1</sup> ) | $i_j$ | $\sigma x$ |
|-------------------------------------------------------------------|-----|----------------------------------------|----------------------------------------|-------|------------|
| {water (1) + [Ch][Ala] (2) + K <sub>3</sub> PO <sub>4</sub> (3)}  |     |                                        |                                        |       |            |
| 1                                                                 | 2   | 19 942                                 | 2612.2                                 | 0.6   | 0.69       |
| 1                                                                 | 3   | 542.22                                 | 412.82                                 | 0.6   |            |
| 2                                                                 | 3   | 25 421                                 | 8457.7                                 | 0.6   |            |
| {water (1) + [Ch][Ala] (2) + K <sub>2</sub> HPO <sub>4</sub> (3)} |     |                                        |                                        |       |            |
| 1                                                                 | 2   | 39 999                                 | 41 254                                 | 0.6   | 0.41       |
| 1                                                                 | 3   | 1577.5                                 | 741.21                                 | 0.6   |            |
| 2                                                                 | 3   | 9653.8                                 | 9898.5                                 | 0.35  |            |



**Figure 1.** Experimental and calculated LLE data for the {[Ch][Ala] (1) +  $K_3PO_4$  (2) + water (3)} ATPS. Solid circles and solid lines represent experimental tie-lines, and empty squares and dashed lines represent calculated values using the NRTL model.

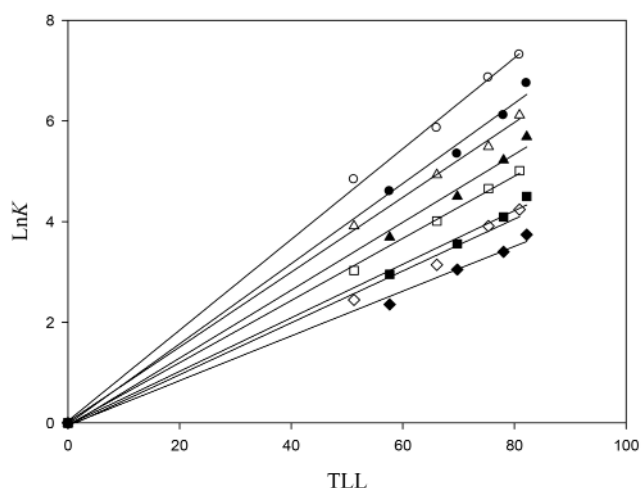
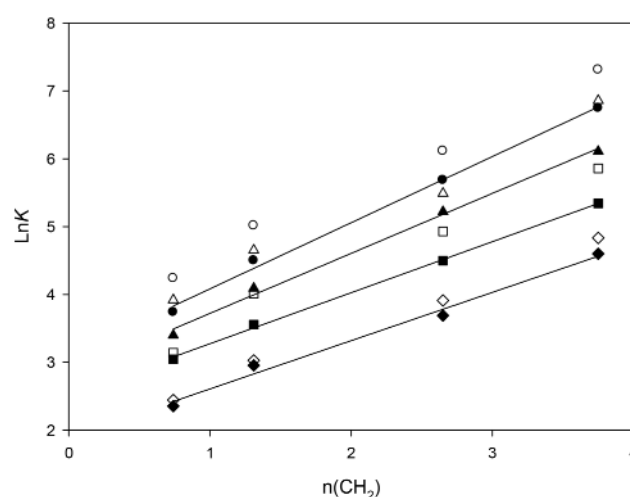


**Figure 2.** Experimental and calculated LLE data for the {[Ch][Ala] (1) +  $K_2HPO_4$  (2) + water (3)} ATPS. Solid circles and solid lines represent experimental tie-lines, and empty squares and dashed lines represent calculated values using the NRTL model.

Previous to the experiments for the measurement of the phase diagrams, preliminary studies of the formation of ATPS involving [Ch][Ala] with different organic and inorganic salts were carried out. These tests showed that the new

**Table 4.** Partition Coefficients Obtained for Four DNP-Amino Acids for the Systems {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + Water (3)} at  $T = 298.15$  K and  $p = 101.3$  kPa<sup>a</sup>

| number of TL | [IL] <sub>feed</sub> | [salt] <sub>feed</sub> | TLL                                                                 | K             |              |               |             |
|--------------|----------------------|------------------------|---------------------------------------------------------------------|---------------|--------------|---------------|-------------|
|              |                      |                        |                                                                     | DNP-L-leucine | DNP-L-valine | DNP-L-alanine | DNP-glycine |
|              |                      |                        | {[Ch][Ala] (1) + K <sub>3</sub> PO <sub>4</sub> + (2) + water (3)}  |               |              |               |             |
| TL 1         | 19.764               | 36.334                 | 80.906                                                              | 1500          | 453          | 151           | 69          |
| TL 2         | 25.021               | 29.529                 | 75.348                                                              | 952           | 242          | 105           | 50          |
| TL 3         | 32.960               | 20.064                 | 66.095                                                              | 350           | 138          | 55            | 23          |
| TL 4         | 40.200               | 10.870                 | 51.264                                                              | 126           | 50           | 21            | 11          |
|              |                      |                        | {[Ch][Ala] (1) + K <sub>2</sub> HPO <sub>4</sub> + (2) + water (3)} |               |              |               |             |
| TL 1         | 29.860               | 34.197                 | 82.179                                                              | 852           | 295          | 90            | 42          |
| TL 2         | 31.927               | 30.668                 | 78.036                                                              | 451           | 185          | 60            | 30          |
| TL 3         | 36.098               | 23.006                 | 69.754                                                              | 209           | 90           | 35            | 21          |
| TL 4         | 40.002               | 14.046                 | 57.642                                                              | 99            | 40           | 19            | 10          |

<sup>a</sup>Standard uncertainties,  $u$ , are  $u(T) = 0.2$  K,  $u(p) = 10$  kPa,  $u(K) = 1$ .**Figure 3.** Logarithm of the partition coefficients of the DNP-amino acids versus tie-line length for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} systems at  $T = 298.15$  K and  $p = 101.3$  kPa. Symbols: (○) DNP-L-leucine, (△) DNP-L-valine, (□) DNP-L-alanine, and (◇) DNP-glycine for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} and (●) DNP-L-leucine, (▲) DNP-L-valine, (■) DNP-L-alanine, and (◆) DNP-glycine for the {[Ch][Ala] (1) + K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} and (○) fits from eq 4.**Figure 4.** Logarithm of partition coefficients of the DNP-amino acids versus of the average number of methylene groups for the four tie-lines of the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} systems at  $T = 298.15$  K and  $p = 101.3$  kPa. Number of tie-lines: (●) TL1, (▲) TL2, (■) TL3, and (◆) TL4 for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} system; (○) TL1, (△) TL2, (□) TL3, and (◇) TL4 for the {[Ch][Ala] (1) + K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} system; and (○) fits from eq 5 for (●) TL1, (▲) TL2, (■) TL3, and (◆) TL4 for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} system.

biodegradable ionic liquid [Ch][Ala] is not able to form ATPS with the inorganic salts potassium carbonate, sodium carbonate, or potassium bicarbonate or the organic salts sodium citrate, potassium citrate, sodium tartrate, or potassium sodium tartrate. For this reason, the ATPS {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} and {[Ch][Ala] (1) + K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} were chosen for this work. The results obtained devising the preliminary studies of the ATPS formation are in agreement with previous publications from literature,<sup>35,36</sup> where it was shown that cholinium-based ILs are not able to undergo phase splitting with acid salts.

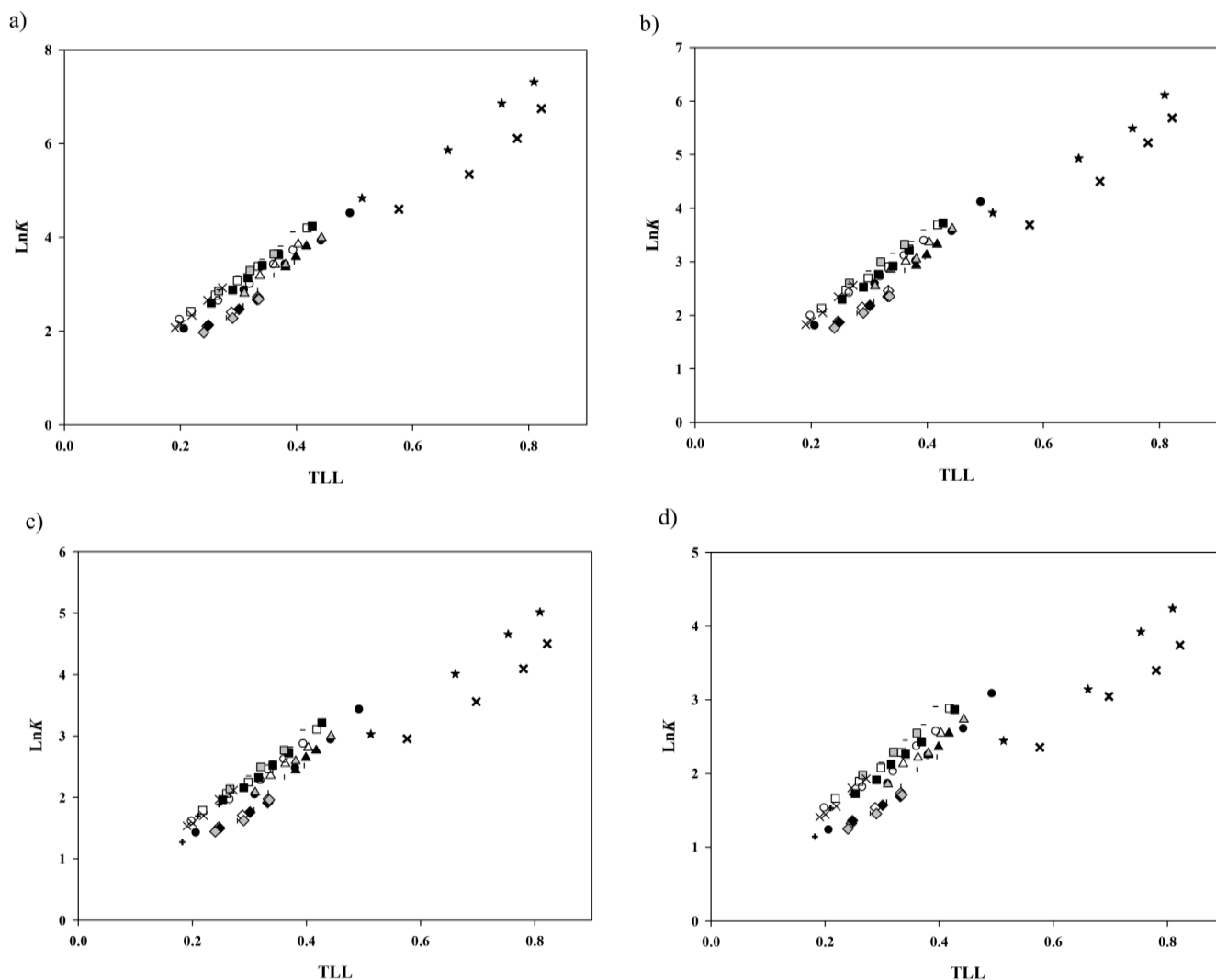
**3.1. NRTL Correlation.** The experimental LLE data obtained in this work were combined with the data reported in the previous work<sup>33</sup> and were represented using the NRTL thermodynamic model. Even though this model was not initially developed to represent systems involving electrolytes, it has provided satisfactory results for ternary ATPS containing ILs.<sup>37</sup>

The root-mean-square deviation of the composition,  $\sigma x$ , was calculated to assess the accuracy of the correlation:

$$\sigma x = \sqrt{100 \left( \frac{\sum_i^m \sum_j^{n-1} (x_{ij}^{I, \text{exp}} - x_{ij}^{I, \text{cal}})^2 + (x_{ij}^{II, \text{exp}} - x_{ij}^{II, \text{cal}})^2}{2mn} \right)} \quad (2)$$

where  $m$  is the number of tie-lines and  $n$  is the number of components in the mixture. The superscripts I and II refer to the two phases in equilibrium,  $x_i^{\text{exp}}$  and  $x_i^{\text{cal}}$  are the experimental and NRTL calculated mole fractions of component  $i$  in both phases, respectively.

The binary interactions parameters  $\Delta g_{ij}$  and  $\tau_{ij}$  together with  $\sigma x$  obtained in the correlation of the two ATPS are summarized in Table 3. As it can be observed from this Table, the calculated deviations indicate that the NRTL model describes satisfactorily the experimental data. The good performance of this thermodynamic model can be visually confirmed in Figures 1 and 2, where the experimental tie-lines and those calculated using the NRTL equation are plotted for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} and {[Ch][Ala]



**Figure 5.** Logarithm of the distribution coefficients of the DNP-amino acids: (a) DNP-L-leucine, (b) DNP-L-valine, (c) DNP-L-alanine, and (d) DNP-glycine versus tie-line length for the ATPS: (○) {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> (2) + water (3)} (this work), (×) {[Ch][Ala] (1) + K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} (this work), (□) {PEG 4000 (1) + Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>21</sup> (■) {PEG 6000 (1) + Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>21</sup> (▣) {PEG 8000 (1) + Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>23</sup> (◇) {PEG 8000 (1) + Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)} at  $T = 296.15$  K,<sup>22</sup> (●) {PEG 8000 (1) + K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)} at  $T = 296.15$  K,<sup>22</sup> (△) {PEG 4000 (1) + K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>23</sup> (▲) {PEG 6000 (1) + K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>23</sup> (▴) {PEG 8000 (1) + K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (2) + water (3)},<sup>23</sup> (◇) {PEG 4000 (1) + KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub> (2) + water (3)},<sup>23</sup> (◇) {PEG 6000 (1) + KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub> (2) + water (3)},<sup>23</sup> (◇) {PEG 8000 (1) + KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub> (2) + water (3)},<sup>23</sup> (◇) {PEG 8000 (1) + Na<sub>2</sub>SO<sub>4</sub> (2) + water (3)},<sup>24</sup> (◇) {PEG 8000 (1) + Mg<sub>2</sub>SO<sub>4</sub> (2) + water (3)},<sup>24</sup> (×) {PEG 8000 (1) + Na<sub>2</sub>SO<sub>4</sub> (2) + water (3)} at  $T = 296.15$  K,<sup>25</sup> (+) {PEG 8000 (1) + Na<sub>2</sub>SO<sub>4</sub> (2) + water (3)}.<sup>26</sup> Note: All represented values were determined at  $T = 298.15$  K and  $p = 101.3$  kPa, except those indicated.

(1) + K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} at  $T = 298.15$  K and  $p = 101.3$  kPa, respectively. From these figures, it is possible to observe that the compositions calculated with NRTL show the same tendency as the experimental tie-lines. For both systems, in the bottom phase (rich in salt), an increase of TLL causes a decrease in salt concentration and an increase in IL concentration. For the top phase (IL-rich phase), an increase in TLL shows an increase in salt concentration and a decrease in IL concentration.

It should be referred that the tie-lines (experimental and calculated) in Figures 1 and 2 are plotted in weight fraction. This was done taking into account that the experimental data were measured and presented in weight fraction.

**3.2. Partitioning.** The partition coefficients of the four DNP-amino acids obtained using eq 1 for the {[Ch][Ala] (1) + K<sub>3</sub>PO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> (2) + water (3)} ATPS at  $T = 298.15$  K

and  $p = 101.3$  kPa are presented in Table 4. Moreover, Figure 3 represents the logarithm of the partition coefficients for the four DNP-amino acids as a function of the TLL.

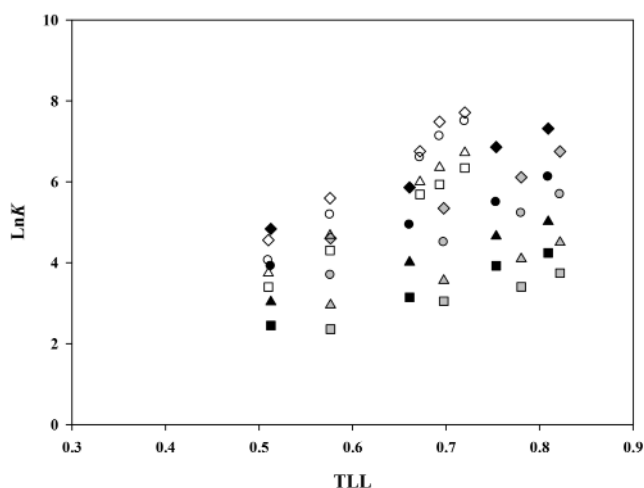
The TLL was calculated using the following equation:

$$\text{TLL} = [([\text{IL}]_{\text{top}} - [\text{IL}]_{\text{bot}})^2 + ([\text{salt}]_{\text{top}} - [\text{salt}]_{\text{bot}})^2]^{1/2} \quad (3)$$

where the  $[\text{IL}]_{\text{top}}$  and  $[\text{IL}]_{\text{bot}}$  are the weight fraction of the ionic liquid on the top and bottom phases, respectively, and  $[\text{salt}]_{\text{top}}$  and  $[\text{salt}]_{\text{bot}}$  are the weight fraction of the salt on the top and bottom phases, respectively.

In Table 4 and Figure 3, the following results can be observed:

An increase of  $\ln K$  when increasing the values of TLL. These results are expected because an increase of TLL means an increase of the concentration of ionic liquid on



**Figure 6.** Logarithm of the partition coefficients of the DNP-amino acids: ( $\diamond$ ) DNP-leucine, ( $\circ$ ) DNP-valine, ( $\triangle$ ) DNP-alanine, and ( $\square$ ) DNP-glycine for the ATPS: (black) {[Ch][Ala] (1) +  $\text{K}_3\text{PO}_4$  (2) + water (3)} (this work), (gray) {[Ch][Ala] (1) +  $\text{K}_2\text{HPO}_4$  (2) + water (3)} (this work), (empty) {C<sub>6</sub>Mim DCA (1) +  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  (2) + water (3)}.<sup>32</sup>

the top phase. This result is the same for the four DNP-amino acids in the two studied ATPS.

Higher values of the  $K$  (and  $\ln K$ ) for the DNP-amino acids for the ATPS containing  $\text{K}_3\text{PO}_4$  were obtained.

The partition coefficient values of DNP-amino acids for the {[Ch][Ala] (1) +  $\text{K}_3\text{PO}_4$  or  $\text{K}_2\text{HPO}_4$  (2) + water (3)} ATPS follow the trend:  $K_{\text{leucine}} > K_{\text{valine}} > K_{\text{alanine}} > K_{\text{glycine}}$ . This tendency is in agreement with previously studied ATPS.<sup>21–32</sup>

Further, in Figure 3, the lineal fit of  $\ln K$  versus TLL is plotted, showing that the results are in accordance with the commonly used expression:

$$\ln K = \alpha \text{TLL} \quad (4)$$

where  $\alpha$  is a constant value which depends on the ATPS composition. The values of  $\alpha$  together with the  $R^2$  of the four DNP-amino acids on the two studied ATPS are presented in Table S1 (available in the Supporting Information).

Figure 4 displays the relationships obtained for  $\ln K$  and the number of equivalent methylene groups in the aliphatic side chain of DNP-amino acids. From this figure, the linearity between the value of  $\ln K$  and the number of methylene groups can be observed. For ease of reading, as the lines for the two ATPS overlap, only the lines for {[Ch][Ala] (1) +  $\text{K}_3\text{PO}_4$  (2) + water (3)} system is displayed.

This can be explained using the following equation.

$$\ln K = C + E n(\text{CH}_2) \quad (5)$$

where  $n(\text{CH}_2)$  is the equivalent number of methylene units of a solute and  $C$  and  $E$  are constants, which represent the total contribution of the nonalkyl part of the DNP-amino acid structure and the contribution of  $\text{CH}_2$  to the partition coefficient, respectively. In Table S2 (Supporting Information), the values of  $C$ ,  $E$ , and  $R^2$  are shown.

**3.3. Comparison with Literature.** A comparison of the partition coefficients presented in this work and those found in literature for the same studied DNP-amino acids on different ATPS was carried out.<sup>21–32</sup> It should be noted that only those data that allow the representation of distribution coefficients

versus tie-line length were considered in Figures 5 and 6. Nevertheless, all data found in literature were taken into account to elucidate the conclusions reached.

First, the comparison between the partition coefficients presented in this work and those available for the partitioning of the same DNP-amino acids on conventional ATPS,<sup>21–30</sup> which are formed by polymer and salt, is displayed in Figure 5. As can be observed, the TLL values presented in this work (values between 0.5 and 0.8) are greater than those reported in the literature.<sup>21–26</sup> Then, the conclusions were drawn considering that the partition coefficient increases linearly with the TLL.

From Figure 5, it can be concluded that the distribution coefficients obtained for DNP-L-leucine and DNP-L-valine on the ATPS {[Ch][Ala] (1) +  $\text{K}_3\text{PO}_4$  (2) + water (3)} show intermediate values with regard to those found in literature.<sup>21–26</sup> Furthermore, it can be observed that the highest values of the distribution coefficients were obtained for the ATPS involving polyethylene glycol (PEG) with an average molecular weight of 8000.<sup>22–26</sup> Moreover, the ATPS involving the same polymer (PEG8000) and organic<sup>23</sup> or inorganic salts<sup>24–26</sup> at the same temperature lead to similar  $K$  values for all the studied DNP-amino acids.

Additionally, a comparison of the distribution coefficients obtained in this work with those presented by other authors for ATPS containing ionic liquids<sup>31,32</sup> was performed. The logarithm of the partition coefficients versus the tie-line length for the separation of the studied amino acids on the ATPS studied in this work and those previously reported for the ATPS {C<sub>6</sub>Mim DCA (1) +  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  (2) + water (3)}<sup>32</sup> is shown in Figure 6. Comparing all results found in literature for the separation of the four DNP-amino acids on the ATPS with ionic liquids, it can be inferred that the highest distribution coefficients are those reported for the ATPS involving the conventional ionic liquid C<sub>6</sub>Mim DCA.<sup>32</sup>

Finally, it should be addressed that the trend for the partition coefficients of the studied DNP-amino acids was  $K_{\text{leucine}} > K_{\text{valine}} > K_{\text{alanine}} > K_{\text{glycine}}$  for all the aqueous two-phase systems.<sup>21–32</sup>

## 4. CONCLUSIONS

In this work, partition coefficients ( $K$ ) of *N*-(2,4-dinitrophenyl)-L-leucine, *N*-(2,4-dinitrophenyl)-L-valine, *N*-(2,4-dinitrophenyl)-L-alanine, and *N*-(2,4-dinitrophenyl)-glycine in {[Ch]-[Ala] (1) +  $\text{K}_3\text{PO}_4$  or  $\text{K}_2\text{HPO}_4$  (2) + water (3)} ATPS were determined at  $T = 298.15 \text{ K}$  and  $p = 101.3 \text{ kPa}$ .

The experimental LLE data of these ATPS were reported in our previous work,<sup>33</sup> and the new two tie-lines experimentally determined in this work were satisfactorily described by the NRTL thermodynamic model.

The results obtained for the partitioning of the DNP-amino acids showed that an increase in the value of the tie-line length leads to an increase of the  $K$  values. Further, the highest distribution coefficients were obtained for the amino acid DNP-L-leucine on the ATPS involving the inorganic salt  $\text{K}_3\text{PO}_4$  with the order found for the  $K$  values:  $K_{\text{leucine}} > K_{\text{valine}} > K_{\text{alanine}} > K_{\text{glycine}}$ . Finally, the comparison among the results presented in this work and those found in literature for the partitioning of the studied DNP-amino acids on different ATPS showed that the studied systems present intermediate values of  $K$  for the DNP-L-leucine and DNP-L-valine in conventional ATPS while ATPS involving traditional ionic liquid exhibit higher partition coefficients.

## ASSOCIATED CONTENT

## Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jced.9b00052.

Figure S1,  $^1\text{H}$  NMR of cholinium alaninate,  $[\text{Ch}][\text{Ala}]$ , with  $\text{D}_2\text{O}$ ; Table S1, parameter,  $\beta$ , and  $R^2$  obtained from eq 4; Table S2, parameters  $C$ ,  $E$ , and  $R^2$  obtained from eq 5 (PDF)

## AUTHOR INFORMATION

## Corresponding Author

Tel.: +351 22508 1653; Fax: +351 22508 1674; E-mail: eamacedo@fe.up.pt.

## Notes

The authors declare no competing financial interest.

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