

# **Salt Effects on the Solubility of Aromatic and Dicarboxylic Amino Acids in Water**

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## Abstract

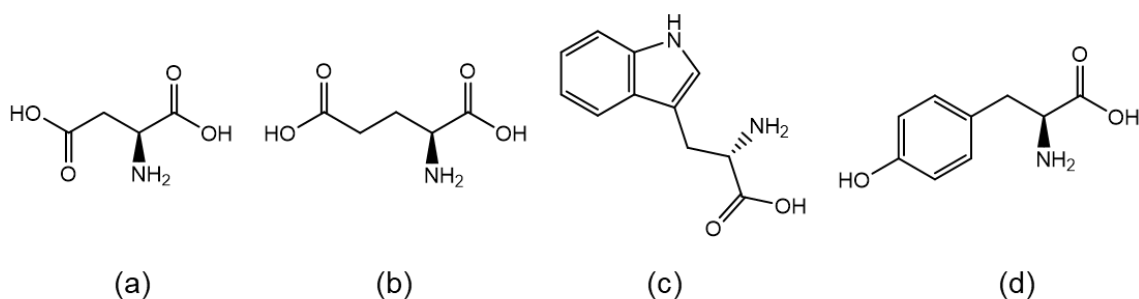
The salt effect on the solubility of the amino acids L-aspartic acid, L-glutamic acid, L-tryptophan, and L-tyrosine, seldomly found in the literature, was studied at 298.2 K, in aqueous solutions of KCl and  $(\text{NH}_4)_2\text{SO}_4$ , for salt concentrations up to  $2.0 \text{ mol}\cdot\text{kg}^{-1}$ . In this concentration range, both salts are salting-in agents for glutamic acid and aspartic acid, with a stronger effect induced by  $(\text{NH}_4)_2\text{SO}_4$ . Regarding the two aromatic amino acids, a slight increase in the solubility was obtained at low salt concentrations, followed by a stronger salting-out effect, more pronounced by  $(\text{NH}_4)_2\text{SO}_4$  than by KCl. The relative solubility data obtained in this work were compared to literature data for other amino acids in the same electrolyte solutions to establish a relative solubility ranking connected to their structure.

Finally, the solubility data were modeled using the electrolyte Perturbed-Chain Statistical Association Theory (ePC-SAFT). The modeling requires parameters for the amino acids and ions as well as melting properties of the amino acids. All these parameters and properties were obtained from previous works. To quantitatively describe the solubility of amino acids upon salt addition, binary interaction parameters ( $k_{ij}$ ) between any amino acid and anions were determined, while between any amino acid and the cations were fixed to  $k_{ij} = 0.08$ . The  $k_{ij}$  parameters between amino acid and the inorganic anions show very similar values for amino acids of the same chemical class (e.g.  $k_{ij}$  between anion and amino acid with apolar side chains), which may be used to systematically reduce the number of adjustable parameters in future work.

**Keywords:** solubility, amino acids, electrolytes, ePC-SAFT, salt effect

## 1. Introduction

The knowledge of the thermodynamic properties of amino acids (AA), peptides, and proteins in aqueous saline solutions is of utmost importance from a biological perspective and in many food and pharmaceutical applications [1,2]. The study of the aqueous solubility of the lower molecular weight amino acids in electrolyte solutions can provide important insights regarding the molecular-level interactions associated with the ion effects affecting the behavior of these biomolecules [3]. An extensive literature review on the solubility of amino acids in electrolyte solutions was published recently [4], and the most studied biomolecules were glycine, alanine, valine, and serine, showing the need to extend these studies to other amino acids. In this work, the L isomers of four amino acids seldomly studied were selected having side chains of different polarity: hydrophobic ones with an aromatic ring in tyrosine or tryptophan; and polar acidic ones in aspartic acid and glutamic acid (Figure 1).



**Figure 1.** Structural formulas of a) L-aspartic acid; b) L-glutamic acid; c) L-tryptophan; d) L-tyrosine.

These amino acids are common building blocks of several relevant proteins, as reviewed in Drauz *et al.*[1]: aspartic acid in edestin, hemoglobin, and barley globulin; glutamic acid in gliadin, zein, wheat gliadin, and maize zein; tyrosine in fibrin, silk fibroin, and papain; and tryptophan in fibrin and egg lysozyme. However, for these compounds, only a few solubility data in aqueous electrolyte solutions can be found in the literature, as summarized in Table S1. Most data refer to the solubility in NaCl solutions for L-aspartic acid [4–6], L-glutamic acid [6] and L-tyrosine [7–9], and KCl solutions for L-glutamic acid [10] and L-aspartic acid [4].

Regarding the electrolytes, potassium chloride (KCl) and ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>)

were selected, following previous studies with these salts and other more commonly studied amino acids such as glycine, DL-alanine and L-alanine, L-isoleucine, L-threonine, DL-serine and L-serine, L-valine, and L-proline [11–21]. These ions are included in the Hofmeister series [22] and are expected to induce different aqueous solubility effects.

Thus, the main aim was to study the solubility of the L isomers of aspartic acid (Asp), glutamic acid (Glu), tryptophan (Trp), and tyrosine (Tyr), at 298.2 K, in aqueous solutions of KCl and  $(\text{NH}_4)_2\text{SO}_4$ , for salt concentrations up to  $2.0 \text{ mol}\cdot\text{kg}^{-1}$ . Additionally, the pH of the saturated solutions was measured, and the solid phase of the amino acids was characterized by X-Ray Diffraction (XRD).

Complementarily, the thermodynamic modeling of these complex mixtures is essential to cover all the salt/amino acid combinations, temperature and salt concentration conditions while reducing the experimental data needed. Recently, the melting properties of twenty amino acids were measured by Fast Scanning Calorimetry, and the Perturbed-Chain Statistical Association Theory (PC-SAFT) was successfully applied to the prediction of the solubility of 15 amino acids in water (including the ones studied in this work) in a wide temperature range [23]. Thus, to account for the presence of the salts, the electrolyte Perturbed-Chain Statistical Association Theory (ePC-SAFT) [24,25] was used in this work to describe the solid-liquid equilibria of the ternary mixtures water + amino acid + salt. The model has been already applied to a wide range of mixtures of amino acids and peptides, water, organic solvents, and electrolytes, being a practical tool for designing processes involving complex multicomponent systems [18,26,27].

## **2. Experimental**

### **2.1. Chemicals**

The source and purity of the chemicals used are given in Table 1. The salts were dried in the oven at 343.2 K and, after, cooled in the desiccator with silica gel. The amino acids were used as received from suppliers. Ultrapure water was used in the solubility experiments (resistivity of  $18.2 \text{ M}\Omega\cdot\text{cm}$ , no particles with size  $\geq 0.22 \mu\text{m}$ , and total organic carbon  $< 5 \text{ ppb}$ ).

**Table 1.** Source and mass fraction purity of the compounds.

Chemical name	CAS registry number	Source	Mass fraction purity <sup>a</sup>
L-aspartic acid (L-Asp)	56-84-8	Alfa Aesar	0.98
L-glutamic acid (L-Glu)	56-86-0	Alfa Aesar	0.990
L-tryptophan (L-Trp)	73-22-3	Affymetrix	0.985
L-tyrosine (L-Tyr)	60-18-4	Merck	0.99
Ammonium sulfate	7783-20-2	Panreac	0.99
Potassium chloride	7447-40-7	Panreac	0.99

<sup>a</sup> The minimum mass fraction purity was reported in the certificate of analysis from the manufacturer.

## 2.2. Solid phase studies

The solid phases of the amino acids, as received from suppliers and filtrated from the saturated ternary solutions containing a  $2.0 \text{ mol}\cdot\text{kg}^{-1}$  salt concentration, were analyzed by powder and single crystal X-Ray diffraction. Powder XRD data were collected on a X'Pert MPD Philips diffractometer, using Cu-K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ), with a curved graphite monochromator, a set incident area of  $10 \text{ mm}^2$ , and a flat plate sample holder, in a Bragg–Brentano para-focusing optics configuration. Intensity data were collected by the step counting method (step  $0.02^\circ$  and time 5 s) in the range  $5^\circ < 2\theta < 50^\circ$ .

The cell parameters of suitable crystals of the solutes provided from suppliers as well the solid samples obtained after filtration, were determined on a Bruker D8 Quest photon 100 CMOS, with monochromated Mo-K $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) and operating at 150 K (standard uncertainty  $u = 2 \text{ K}$ ). The selected crystals were placed at 40 mm from the detector and the spots were measured using different counting times (varying from 5 to 30 s).

## 2.3. Solubility experiments

The solubility was measured using the traditional shake flask method coupled to the gravimetric or UV spectroscopy methods to analyze the composition of the saturated solutions. First, aqueous solutions of KCl or  $(\text{NH}_4)_2\text{SO}_4$  were prepared at defined molalities. Then, for each salt molality, an estimated amount of amino acid in a slight excess relative to the expected saturation limit and 80 ml of the salt solution were added to one Erlenmeyer flask. An

analytical balance (Denver Instrument,  $\pm 0.0001$  g) was used for these measurements. Next, the ternary mixture was stirred with a magnetic stirrer inside a thermostatic bath at 298.2 K ( $\pm 0.1$  K) for 30 hours. Its speed was kept between 500-700 rpm. After, the saturated solution was left to rest in the bath for at least 12 hours. Four samples (approximately 2 cm<sup>3</sup>) were collected from the ternary system with preheated plastic syringes with syringe filters (0.45  $\mu$ m).

The concentration of L-tyrosine in the saturated ternary systems was measured by UV spectroscopy (Jasco V-730 spectrometer). To do so, calibration curves for this amino acid were obtained at 274 nm wavelength ( $R^2 = 0.9998$ ). For the remaining amino acids, the gravimetric method was applied. To evaporate all water, the samples were first placed in a hood and, after, dried in a drying stove at 343.2 K for one week. The drying process was maintained until the crystals were completely dried. After determining the weight of the glass vessels empty, with solution, and with dry sample, the amount of evaporated water and solid residue could be determined. Then, the mass of dissolved amino acid was calculated from the knowledge of the initial salt concentration [11].

The pH of solutions was measured using a pH meter (WTW InoLab pH Level 1) and pH-electrode (WTW SenTix 41). The calibration of the equipment was done with pH 4 and pH 7 buffers, at 298.2 K.

### 3. Thermodynamic modeling

The solid-liquid equilibrium relation between solid AA and the saturated liquid aqueous phase was applied according to Prausnitz [28]. Among others, this relation assumes pure solid AA phase, i.e., no mixed crystals, and allows determining the AA solubility  $x_i^L$  as:

$$\ln(x_i^L \cdot \gamma_i^L) = \frac{\Delta h_{0i}^{SL}}{R \cdot T_{0i}^{SL}} \left(1 - \frac{T_{0i}^{SL}}{T}\right) - \frac{1}{R \cdot T} \int_{T_{0i}^{SL}}^T \Delta c_{p0i}^{SL}(T) dT + \frac{1}{R} \int_{T_{0i}^{SL}}^T \frac{\Delta c_{p0i}^{SL}(T)}{T} dT \quad (1)$$

$$\Delta c_{p0i}^{SL}(T) = (a_{c_{p0i}^L} - a_{c_{p0i}^S}) \cdot T + (b_{c_{p0i}^L} - b_{c_{p0i}^S}) = \Delta a^{L-S} \cdot T + \Delta b^{L-S} \quad (2)$$

In Eq. (1),  $\gamma_i^L$  and  $R$  are the activity coefficient of AA in the saturated solution and the universal gas constant, respectively, and  $T$  is the absolute temperature of the system. Then,  $\Delta h_{0i}^{SL}$  is the melting enthalpy at the melting temperature ( $T_{0i}^{SL}$ ), and  $\Delta c_{p0i}^{SL}(T)$  is the difference between the heat capacities of the liquid state (L) and the solid state (S) of the pure AA. In Eq. (2),  $\Delta c_{p0i}^{SL}(T)$  was assumed to change linearly with temperature, and  $a_{c_{p0i}^L}$  ( $a_{c_{p0i}^S}$ ) and  $b_{c_{p0i}^L}$  ( $b_{c_{p0i}^S}$ ) are the slope and the intercept of the liquid (solid) heat capacities, respectively.

In a previous work [29], the melting properties of several AA were measured with Fast Scanning Calorimetry (FSC), applying heating rates up to 20 000 K·s<sup>-1</sup>. These experimentally determined melting properties are required as input for the thermodynamic framework PC-SAFT to predict the aqueous AA solubility using Eq. (1). The activity coefficients required in Eq. (1) were calculated using the revised ePC-SAFT, which combines the classical PC-SAFT [24] and the Debye-Hückel theory to account for the interactions among ions. The revised ePC-SAFT assumes the residual Helmholtz energy ( $a^{res}$ ) as the sum of independent contributions accounting for hard-chain ( $a^{hc}$ ), dispersion ( $a^{disp}$ ), association ( $a^{assoc}$ ), and Coulombic attraction ( $a^{Coulomb}$ ) energies according to Eq. (3):

$$a^{res} = a^{hc} + a^{disp} + a^{assoc} + a^{Coulomb} \quad (3)$$

In this work, polar interactions were not accounted for and the standard Berthelot-Lorentz combining rules were applied for segment diameter and dispersion energy, while the Wolbach and Sandler mixing rules [30] were applied for the association parameters. An advanced model for electrolyte solutions [31] is available, which was not used in the present work as the conditions here focus on concentrated aqueous electrolyte solutions, and a deep study of new binary parameters within the new advanced model is not yet available. The dielectric constant is required for ePC-SAFT. The temperature-dependent function presented by Cameretti *et al.* [25] was applied, representing the relative permittivity of pure water, assumed independent of the salt in the solution, in consistency with the modeling procedure when the ion parameters were fitted. The pure-component parameters of the AA and water are summarized in Table S2 in Supporting Information, while the ion parameters are given

in Table S3. These parameters were published in the literature and were obtained from previous works, including the melting properties. An exception are the values of  $\Delta h_{0i}^{SL}$ ,  $\Delta a^{L-S}$  and  $\Delta b^{L-S}$  of L-proline presented in Table S2, which were determined in this work based on FSC (fast scanning calorimeter) data from Do *et al.* [23], as the fine-tuned values were not available in that publication (relatively large experimental uncertainties). According to the procedure previously followed [23], these melting properties were fitted to the solubility data of L-proline in water using the FSC data [23] as starting values, and allowing the fine-tuned values only to vary within the FSC-determined uncertainty.

## 4. Results and discussion

### 4.1. Solid-phase studies

The solid phases of the amino acids, as received from suppliers and filtrated from the saturated ternary solutions containing a  $2.0 \text{ mol}\cdot\text{kg}^{-1}$  salt concentration, were analyzed by powder and single crystal X-ray diffraction. For all solutes, the solid phase recovered from the ternary mixtures kept the structure of the initial sample from the supplier.

Crystals of L-glutamic acid were indexed by single crystal X-ray diffraction with cell parameters ( $a = 5.183 \text{ \AA}$  ( $u = 0.013 \text{ \AA}$ ),  $b = 6.960 \text{ \AA}$  ( $u = 0.019 \text{ \AA}$ ) and  $c = 17.35 \text{ \AA}$  ( $u = 0.04 \text{ \AA}$ ); orthorhombic P; at 150 K ( $u = 2 \text{ K}$ )) comparable to the sample with CCDC deposition number 1587065 with  $Z = 4$  and density =  $1.603 \text{ g/cm}^3$  [32] (Figure S1). In the case of L-aspartic acid, the cell parameters of the crystals ( $a = 5.131 \text{ \AA}$  ( $u = 0.007 \text{ \AA}$ ),  $b = 6.932 \text{ \AA}$  ( $u = 0.010 \text{ \AA}$ );  $c = 7.564 \text{ \AA}$  ( $u = 0.010 \text{ \AA}$ );  $\beta = 100.52^\circ$  ( $u = 0.03^\circ$ )); Monoclinic P; at 150 K ( $u = 2 \text{ K}$ )) were comparable to the sample with CCDC deposition number 652520 with  $Z = 2$  and density =  $1.678 \text{ g/cm}^3$  [33] (Figure S2). The L-tryptophan and L-tyrosine samples were analyzed by powder X-ray diffraction showing all the same powder patterns as the samples with CCDC deposition numbers 986569 [34] and 1208550 [35], respectively (Figures S3 and S4). These structures are comparable to those reported by Do *et al.* [23], for which the melting properties are available.

### 4.2. Solubility data



The experimental solubility of the amino acids measured in this work is reported in Table 2 as a function of the salt concentration.

**Table 2.** Solubility of the amino acids  $S_{AA}$  (g of AA/kg of water; standard deviation<sup>a</sup> between brackets) in aqueous solutions of potassium chloride and ammonium sulfate at temperature  $T = 298.2$  K and pressure  $P = 0.1$  MPa.<sup>b</sup>

Salt	Salt molality/ (mol/kg of water)	$S_{AA}$ /(g of AA/kg of water)				
		L-aspartic acid	L-glutamic acid	L-tryptophan	L-tyrosine	
No salt	0.000	5.140 (0.031) <sup>c</sup>	8.568 (0.029)	12.639 (0.045)	0.427 (0.002)	
	0.300		9.760 (0.024)	12.782 (0.072)	0.434 (0.008)	
	0.700		10.404 (0.028)	12.952 (0.067)	0.412 (0.004)	
	KCl	1.000		10.716 (0.060)	12.509 (0.057)	0.394 (0.010)
		1.500		11.264 (0.139)	12.051 (0.032)	0.379 (0.009)
		2.000		11.677 (0.077)	11.134 (0.017)	0.345 (0.017)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.333	7.228 (0.028)	11.376 (0.038)	13.084 (0.069)	0.457 (0.012)	
	0.700	8.899 (0.053)	12.484 (0.050)	12.166 (0.057)	0.437 (0.015)	
	1.000	9.999 (0.056)	13.236 (0.004)	11.522 (0.196)	0.407 (0.013)	
	1.500	10.998 (0.135)	14.177 (0.114)	9.992 (0.096)	0.353 (0.008)	
	2.000	12.116 (0.350)	13.980 (0.332)	8.778 (0.123)	0.300 (0.004)	

<sup>a</sup> The standard deviation was calculated using the equation  $\sqrt{\sum(x - \bar{x})^2 / (n - 1)}$  (for each ternary mixture, 4 samples were collected from one saturated solution); <sup>b</sup> Standard uncertainties ( $u$ ) and relative standard uncertainties ( $u_r$ ):  $u(T) = 0.1$  K,  $u_r(p) = 0.05$ ,  $u(\text{salt molality}) = 0.001 \text{ mol}\cdot\text{kg}^{-1}$  and  $u_r(S_{AA}) = 0.05$ . <sup>c</sup>Data taken from the literature [4].

The solubility of the AA in all the aqueous solutions follows the order L-Trp > L-Glu > L-Asp > L-Tyr. For all amino acids, their solubilities in pure water at 298.2 K were already reported by several authors, as collected in Table S4. It should be mentioned that, in many cases, the solid phase structure was not registered. Nevertheless, the literature average and standard deviation were calculated for comparison purposes and are also reported in Table S4. As can be seen, the values measured in this work generally agree with the literature. For L-tryptophan a difference to the literature average close to 0.9 g per 1000 g of water was found. Nevertheless, the standard deviation of the seven values collected in the literature is also high (1.212 g of AA/1000 g of water). Regarding the aqueous saline solutions, only the solubility of L-Glu in aqueous KCl solutions was reported by other researchers [10] and are compared with our data in Figure S5. As can be seen, the salting-in effect obtained in this work differs considerably from the salting-out published by Abualreish and Noubigh [10].

For the sake of comparison, the impact of NaCl on the solubility of L-Glu measured by Bretti *et al.* [6] is also represented in Figure S5. As can be seen, both NaCl and KCl (our data) induce a similar salting-in effect, which follows the same behavior obtained from the analysis of the results of other amino acids with the same chloride salts [4], thus supporting the consistency of our results. The solubility of L-Trp and L-Tyr in aqueous solutions of NaCl presented in literature could not be compared with our results because of the concentration scale (molarity) or data available only for the DL mixture of isomers. Still, it was possible to notice that they also induce a salting-out effect [7,8,36,37] as observed here for KCl, at larger molalities ( $0.7 \text{ mol}\cdot\text{kg}^{-1}$  for L-Tyr and  $1.0 \text{ mol}\cdot\text{kg}^{-1}$  for L-Trp).

Finally, the pH of the saturated solutions was also measured at 298.2 K and it is shown in Table 3. In the measured pH range, all the amino acids are mainly present as zwitterions [38,39].

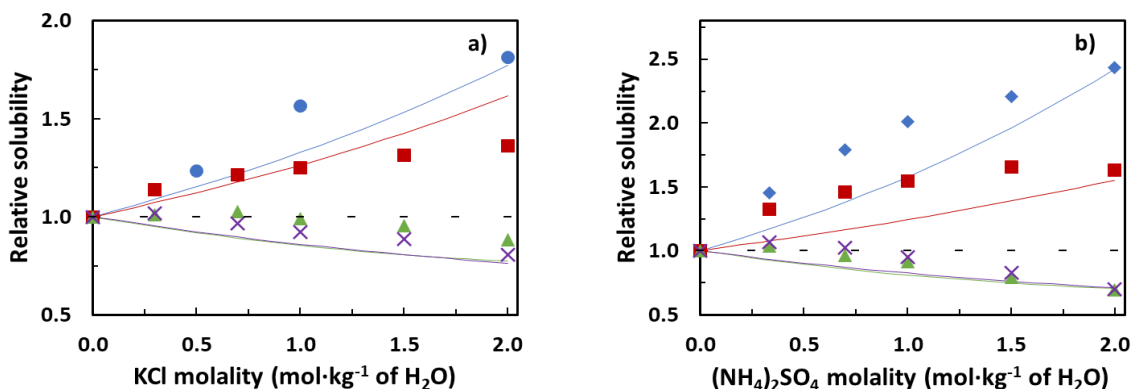
**Table 3.** pH range of saturated solutions of AA in aqueous  $(\text{NH}_4)_2\text{SO}_4$  or KCl solutions at different molalities, at 298.2 K.

AA	pH range in the electrolyte solution	
	$(\text{NH}_4)_2\text{SO}_4$	KCl
L-aspartic acid	2.77*-3.27	-
L-glutamic acid	3.22*-3.49	3.19-3.22*
L-tryptophan	5.37-5.89*	5.89*-5.98
L-tyrosine	5.19-5.66*	5.66*-5.92

\* Isoelectric point in pure water [40]. Temperature and pH standard uncertainties are  $u(T) = 0.1 \text{ K}$  and  $u(\text{pH}) = 0.05$ , respectively.

Figure 2 presents the relative solubility (ratio between the solubility of amino acid in aqueous salt solutions and their solubility in pure water) for the studied systems. The solubility of L-Asp in KCl aqueous solutions reported previously [4] is also included for comparison purposes. A salting-in effect is observed in both electrolyte solutions for L-Asp and L-Glu. For the more hydrophobic amino acids, L-Trp and L-Tyr, a very small salting-in effect at lower concentrations is observed, followed by a decrease in the relative solubility that shows salting-out at higher salt molalities. At higher molalities the ammonium sulfate is a stronger

agent both as salting-in (L-Asp and L-Glu) or salting-out (L-Trp and L-Tyr). Considering the four amino acids, the ranking of the relative solubility in KCl is L-Tyr < L-Trp < L- Glu < L-Asp. In the case of the solutions containing  $(\text{NH}_4)_2\text{SO}_4$ , the aromatic amino acids swap their position: L-Trp < L-Tyr < L- Glu < L-Asp.



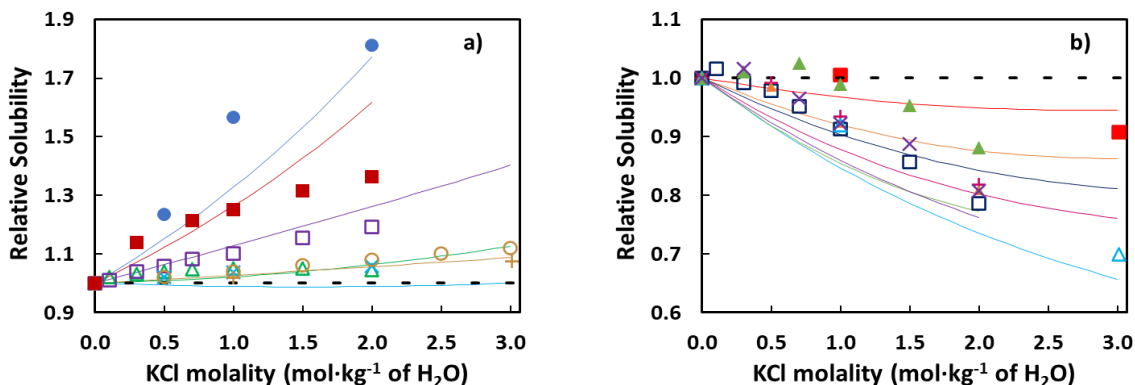
**Figure 2.** Relative solubility of the amino acids studied in this work in aqueous solutions of KCl (plot a) and  $(\text{NH}_4)_2\text{SO}_4$  (plot b), at 298.2 K. L-aspartic acid: ● [4] and ◆ (this work); L-glutamic acid: ■, (this work); L-tryptophan: ▲ (this work); L-tyrosine: × (this work). Lines were calculated with ePC-SAFT using the parameters from Tables S2, S3 and 4.

The molecular level interpretation of these data can be partly supported by previous results from molecular dynamics studies in which both the anion and cations effects were evaluated. Tome *et al.* [3] proposed a mechanism based on the type and strength of the interactions between the anions and the nonpolar moieties of the amino acids alanine, valine, isoleucine, and 2-aminodecanoic acid. The interactions between the high charge density sulfate anion and the apolar moieties of the amino acids were absent, as this anion showed a stronger interaction with the charged groups. In turn, the chloride anion should have a lower impact on the amino acid's aqueous solubility as it interacts weakly with both polar and apolar segments [3]. Later, Tome *et al.* [14] studied a series of salts that included the monovalent  $\text{NH}_4^+$  and  $\text{K}^+$ , and their influence on the solubility of alanine, valine, and isoleucine. The simulation results showed that no significant interactions occurred between these cations and the apolar parts of the studied amino acids, even if the interactions of the ammonium ion were somewhat stronger. A more significant association of the ammonium cation to the carboxylate group

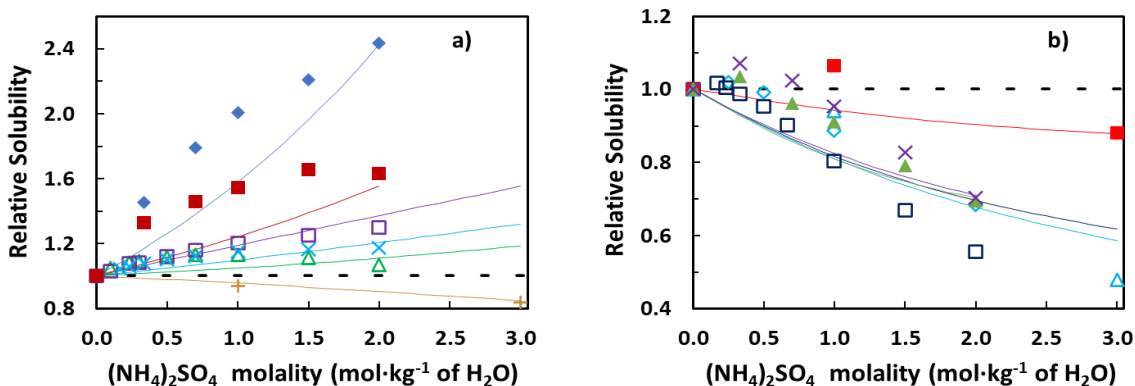
was found, compared to  $K^+$ , that may cause the formation of weak single charged positive complexes, which are more soluble in water. Those simulation results [3,14] were obtained for amino acids with apolar side chains such as isoleucine or valine and may assist the interpretation of the results obtained for L-Tyr and L-Trp, which have even larger apolar side chains. In the case of the anion effect, the sulfate ion should cause a higher salting-out effect than  $Cl^-$ , due to its lower interaction with the apolar regions of the amino acids, as confirmed by the higher magnitude of L-Tyr and L-Trp salting-out at higher molality if compared to potassium chloride salt. Therefore, those interactions seem to prevail over the more significant interactions predicted for the  $NH_4^+$  when compared to  $K^+$ .

Considering the polar acidic amino acids, both contain an additional polar COOH group with a much higher affinity to interact with the cations. In this case, the attractive interactions of the ions with the oppositely charged groups of the amino acids ( $NH_3^+$  and  $COO^-$ ) seem to prevail over the interactions with the more hindered and smaller nonpolar moieties available in L-Asp and L-Glu. And, as mentioned above, those interactions with the charged groups are stronger using  $NH_4^+$  than  $K^+$ , or  $SO_4^{2-}$  compared to  $Cl^-$ , supporting the stronger salting-in effect of ammonium sulfate. Comparing the AA, it is also possible to see that the salting-in is more pronounced for aspartic acid, probably due to the additional  $CH_2$  group in the structure of the more hydrophobic glutamic acid.

Figures 3 and 4 compare the solubility data measured in this work with literature data available for glycine (Gly) and other L-isomers of amino acids (L-alanine, L-Ala; L-isoleucine, L-Ile; L-serine, L-Ser; L-threonine, L-Thr; L-Proline, L-Pro; L-valine, L-Val; L-leucine, L-Leu; L-phenylalanine, L-Phe) in aqueous solutions of KCl and  $(NH_4)_2SO_4$ , respectively. The chemical structures of the AA are given in Figure S6. The data were selected considering the consistency analysis discussed in previous work [4].



**Figure 3.** Relative solubility, at 298.2 K, in aqueous solutions of KCl of:  
a) L-Asp - ● [4]; L-Glu - ■ (this work); L-Ser - □ [12]; L-Thr - △ [12]; L-Pro - ○ [20], + [18]; Gly - × [4];  
b) L-Trp - ▲ (this work); L-Tyr - × (this work); L-Ala - ■ [18]; L-Ile - □ [12]; L-Val - △ [18]; L-Leu - ▲ [4];  
L-Phe - + [4]. Lines were calculated with ePC-SAFT using the parameters from Tables S2, S3 and 4.



**Figure 4.** Relative solubility, at 298.2 K, in aqueous solutions of  $(\text{NH}_4)_2\text{SO}_4$  of:  
a) L-Asp - ◆ (this work); L-Glu - ■ (this work); L-Ser - □ [13]; L-Thr - △ [13]; L-Pro - + [18]; Gly - × [13];  
b) L-Trp - ▲ (this work); L-Tyr - × (this work); L-Ala - ■ [18]; L-Ile - □ [13]; L-Val - △ [18], ◇ [14]. Lines were calculated with ePC-SAFT using the parameters from Tables S2, S3 and 4.

As can be seen, the ranking of their relative solubility, evaluated at  $1.0 \text{ mol}\cdot\text{kg}^{-1}$  salt concentration, is similar in both salt solutions:

- L-Ile  $\approx$  L-Leu  $\approx$  L-Tyr  $\approx$  L-Val  $\approx$  L-Phe < L-Trp < L-Ala < L-Pro  $\approx$  Gly  $\approx$  L-Thr < L-Ser < L-Glu < L-Asp, in KCl solutions.
- L-Ile < L-Trp  $\approx$  L-Val  $\approx$  L-Pro  $\approx$  L-Tyr < L-Ala < L-Thr  $\approx$  Gly < L-Ser < L-Glu < L-Asp, in  $(\text{NH}_4)_2\text{SO}_4$  solutions.

The pattern identified above may now be generalized for a much larger number of AA. It shows that, in general,  $(\text{NH}_4)_2\text{SO}_4$  induces a more significant effect on their aqueous solubility than KCl, either it is salting-in on the more polar amino acids or salting-out on the less polar. Using as examples the AA in the extremes of the scale, at  $2.0 \text{ mol}\cdot\text{kg}^{-1}$  salt concentration, the relative solubilities of the most polar L-Asp are 2.4 and 1.8 in  $(\text{NH}_4)_2\text{SO}_4$  and KCl solutions, respectively. And, for the apolar L-Ile, the relative solubilities are 0.6 and 0.8 in  $(\text{NH}_4)_2\text{SO}_4$  and KCl solutions, respectively.

Upon adding the salts, a salting-in effect is also observed on the solubility of L-Ser and L-Thr. Both amino acids have a polar hydroxyl group in their side-chain, but L-Thr has an additional  $-\text{CH}_2-$  group compared to L-Ser which explains their relative solubility ranking. The simplest AA, glycine, also presents a salting-in effect [13]. The remaining amino acids have mostly apolar side-chains (aliphatic and aromatic), and salting-out effects are observed.

### 4.3. Thermodynamic modelling

The ePC-SAFT model allows the calculation of the solubility of amino acids in aqueous electrolyte solutions and might be applied in a predictive way, i.e., without using binary interaction parameters ( $k_{ij}$ ) between the inorganic ions and the amino acid.  $k_{ij}$  are used to correct the geometric mixing rule of the dispersive energy  $u_{ij}$  of a mixture of two components  $i$  and  $j$ , obtained from the Lorentz–Berthelot combining rules:

$$u_{ij} = \sqrt{u_i u_j} \cdot (1 - k_{ij}) \quad (4)$$

Predicting the amino acid solubility upon salt addition yields results that are quite satisfying for a few systems only (e.g., nonpolar AA in water +  $(\text{NH}_4)_2\text{SO}_4$ ), while higher deviations for most of the systems were observed (e.g., amino acids with polar substituents in water +  $(\text{NH}_4)_2\text{SO}_4$ ). Consequently, it was decided to estimate the  $k_{ij}$  parameters between ions and amino acids; as ePC-SAFT is an ion-based modeling approach, two  $k_{ij}$  parameters (anion - amino acid and cation - amino acid) might be adjusted to experimental solubility data.

To reduce the number of  $k_{ij}$  parameters, only one  $k_{ij}$  parameter was estimated for each ternary amino acid + water + salt system, using the experimental solubility data from this work and

the literature. Knowledge from previous work indicates that ePC-SAFT rather overestimates the solubility of amino acids in salt solutions [26]. Thus, the  $k_{ij}$  parameters between any combination of inorganic cation - amino acid was set to  $k_{ij} = 0.08$ , slightly reducing the interaction energy between inorganic cations and the AA. Therefore, only the  $k_{ij}$  parameters between  $\text{Cl}^-$  - amino acid and  $\text{SO}_4^{2-}$  - amino acid were adjusted to experimental solubility of amino acid in the respective aqueous electrolyte solution at 298.2 K and 1 bar, and are listed in Table 4.

**Table 4.** ePC-SAFT binary  $k_{ij}$  parameters between inorganic anions and amino acid estimated in this work<sup>a</sup>. Valid only with the parameters in Tables S2 and S3.

Amino acid	$k_{ij}$ Anion-AA	
	$\text{Cl}^-$	$\text{SO}_4^{2-}$
Glycine	-0.1	-0.2
L-Alanine	-0.1	0.2
L-Valine	0	0.3
L-Leucine	-0.1	0.3
L-Isoleucine	-0.1	0.3
L-Phenylalanine	-0.1	0.3
L-Serine	-0.4	-0.5
L-Threonine	-0.15	0
L-Aspartic acid	-0.4	-0.5
L-Glutamic acid	-0.4	0
L-Proline	-0.2	1.0
L-Tyrosine	0.1	0.6
L-Tryptophan	0	0.6

<sup>a</sup> For  $\text{K}^+$  and  $\text{NH}_4^+$ ,  $k_{\text{cation,AA}} = 0.08$ .

It becomes obvious from Table 4 that  $k_{ij}$  parameters between several ions and amino acids of the same chemical class are very similar. In this manner, it can be inferred that  $k_{ij}$  parameters follow some trends:

- #  $\text{SO}_4^{2-}$  and amino acids with apolar side chains (Ala, Val, Leu, Ile, and Phe): positive  $k_{ij}$  values were found, which are 0.2 or 0.3.

- # Cl<sup>-</sup> and amino acids with apolar side chains: slightly negative  $k_{ij}$  values equal to -0.1.
- # Cl<sup>-</sup> / SO<sub>4</sub><sup>2-</sup> and amino acids with polar/acidic side chains: negative  $k_{ij}$  values close to -0.4.

Exceptions were found for the amino acids L-Pro, L-Tyr and L-Trp. Nevertheless, apart from these exceptions, the results suggest that these  $k_{ij}$  parameters might be transferable to other systems as well, which will be investigated in future work.

The final correlations were already shown in Figures 2, 3, and 4. As can be seen, the model is able to capture the general salting-in and salting-out trends for the amino acids, though it only predicts the monotonic increase or decrease of the relative solubility, not being able to describe the maximum values observed for some systems.

Finally, to quantitatively evaluate the quality of the ePC-SAFT correlations, the average relative deviations (ARD) between calculated (“calc”) and experimental (“exp”) solubility values were obtained for each ternary system (water/electrolyte/amino acid), using the following expression:

$$ARD(\%) = 100 \cdot \frac{1}{NP} \sum_{k=1}^{NP} \left| \frac{S_k^{exp} - S_k^{calc}}{S_k^{exp}} \right| \quad (5)$$

In this equation, NP is the total number of data points for each ternary mixture, and  $S$  represents the solubility of the amino acid (g amino acid/ 1000 g water). The results are presented in Table S5. The global ARD for the solubility over all the systems studied in this work is 8% for KCl aqueous solutions and 10% for (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Higher deviations were obtained for the systems experimentally studied in this work, all higher than 14% in ammonium sulfate aqueous solutions, with the highest ARD of 22% and 17% being obtained for L-tryptophan in KCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions, respectively. The ARD values for all the other systems, obtained from the literature review, is less than 10.6%.

The results can be considered satisfactory as these systems are very difficult to represent not only due to the presence of the electrolyte, but also to the zwitterionic character of the AA. These difficulties have been shown in detail before [12], when correlating the solubility data



of AA in aqueous potassium chloride solutions at different temperatures using a complete correlation model such as Pitzer–Simonson–Clegg [41].

## 5. Conclusions

The solubility of L-aspartic acid, L-glutamic acid, L-tryptophan, and L-tyrosine was reported in aqueous KCl and  $(\text{NH}_4)_2\text{SO}_4$  solutions at 298.2 K, up to  $2.0 \text{ mol}\cdot\text{kg}^{-1}$  salt molality. The study of the crystal structures of all the original AA and the crystals obtained after the filtration of their saturated solutions in aqueous salt solutions showed, in all cases, precisely the same AA solid structures. At  $2.0 \text{ mol}\cdot\text{kg}^{-1}$  salt concentration, both electrolytes induced salting-in in the polar acidic L-Asp and L-Glu and salting-out in the more hydrophobic aromatic L-Tyr and L-Trp. Those effects are stronger in ammonium sulfate solutions than in potassium chloride. These are amino acids for which both experimental and modeling results are scarce in the literature, reinforcing the importance of this work by considering AA with aromatic or two carboxylic groups.

The experimental data were described using ePC-SAFT, an ion-specific modeling approach. All water, amino acids, and ions parameters were obtained from previous works. Nevertheless, a reduced number of ion-specific interaction parameter  $k_{ij}$  between each amino acid and ions have been estimated. The  $k_{ij}$  parameters between each AA and the cation was fixed to  $k_{ij} = 0.08$ , and only one  $k_{ij}$  value between the AA and the anion have been estimated, for each ternary system, to quantitatively describe the solubility of amino acids upon salt addition. The obtained  $k_{ij}$  parameters between the AA and the inorganic anions show very similar values for amino acids of the same chemical class (e.g.,  $k_{ij}$  between anion and amino acid with apolar side chain), maintaining the predictive character of the model, potentially allowing extrapolation to other systems.

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