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Solubilities of amino acids in water at various pH values under 298.15 K

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ABSTRACT

In this study the solubility of amino acids in water for various pH values at 298.15 K is investigated. A colorimetric method using a spectrophotometer was employed to measure solubility data for the five amino acids, DL-alanine, L-leucine, L-isoleucine, L-serine and DL-phenylalanine, in water. The pH effect, changing from 2 to 10 adjusted by the addition of HCl or NaOH, has a significant effect on the solubility data. This is due to the fact that amino acids appear in several forms in solution, depending on the pH, which are neutral zwitterions, cationic and anionic species. These are not only different forms in chemical equilibrium with each other, but also with hydrogen ions in an aqueous solution. To model the solubility of amino acids in water, these chemical equilibria were considered. An NRTL activity coefficient model was used to describe the non-idealities in the solution. The calculated results agree well with the experimental data.

1. Introduction

Amino acids, the major building blocks of proteins, are commonly used in the fields of food, medical and chemical industries. The main uses of amino acids are as flavor enhancers and nutritional supplement ingredients. Also, they are the key components in the synthesis of peptide drugs and biodegradable polymers. The amino acid can be obtained from the hydrolysis of protein-containing materials or from a fermentation broth. These processes are carried out in the presence of a strong acid or base, and the amino acids are recovered by precipitation by the neutralization of the solution. Therefore, the pH effects on the solubility of amino acids in water are imperative for a rational design of downstream processes in biotechnology.

Upon the dissolution of solid amino acids in water, the amino and carboxyl groups of the amino acid are ionized and almost completely convert to a zwitterion as

$$NH_2RCOOH\ (s) \overset{K}{\rightleftharpoons} NH_3{}^+RCOO^-\ (aq) \quad (1)$$

where K is the solubility constant of the zwitterion, which depends on temperature.

Furthermore, the solubility of amino acids in water is also governed by the pH. When acid is added to aqueous solution of amino acids, the reversible dissociation reaction shifts from right to left by

$$NH_3{}^+RCOOH \overset{K_1}{\rightleftharpoons} H^+ + NH_3{}^+RCOO^- \quad (2)$$

The zwitterion accepts a proton to form a cation due to the repression of ionization of its carboxyl group.

In addition, in the presence of a base, the zwitterion can donate a proton to form an anion by repressing of the ionization of the amino group. Thus, the dissociation reaction occurs forwards by

$$NH_3{}^+RCOO^- \overset{K_2}{\rightleftharpoons} H^+ + NH_2RCOO^- \quad (3)$$

K_1 and K_2 are the equilibrium constants of the two dissociation reactions.

In literature, some studies on the solubility of amino acids in water have been carried out. Dalton and Schmidt [1] calculated solubilities of certain amino acids in the temperature range of 0–100 °C. Tabulated solubility data of amino acids in water can also be found in several handbooks [2,3]. Carta and Tola [4] measured solubilities of four amino acids in aqueous solutions at various pH values and NaCl concentrations at 298.15 K. A continuation of this work was carried out by Carta [5]. These solubility data [4,5] were given on a molarity scale. Pradhan and Vera [6] determined the effect of acids (HCl and HNO_3) and bases (NaOH and KOH) on the solubility of DL-alanine at 298.15 K in a pH range of 2–10. Brown and Rousseau [7] reported the solubilities of L-isoleucine, L-leucine and L-valine in aqueous solutions containing sodium hydroxide. Orella and Kirwan [8] measured and correlated the solubility behavior of amino acids in mixed aqueous aliphatic alcohol solutions. These

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solubility data are valuable in the development of thermodynamic models for aqueous amino acid solutions. However, the number of experimental data on effect of temperature, pH, solvent and salt on the solubility of amino acids in water reported in literature is still limited.

Gupta and Heidemann [9] correlated some activity coefficient data of amino acids in water with a modified UNIFAC model [10] by introducing two new group contributions of glycine and proline. They developed an approach which employed a solid–liquid equilibrium relation and a modified UNIFAC model to describe the influences of both temperature and pH on the solubility of amino acids in water. However, the model prediction was not satisfactory for systems of amino acids containing methyl and methylene groups in the side chain. Pinho et al. [11] proposed a model to represent the solubility behavior of both amino acids and peptides in water by using the UNIFAC equation with a Debye-Hückel term to describe the activity coefficients in an aqueous amino acid solution. The calculated results showed satisfactory agreement with the experimental data. Chen et al. [12] developed a generalized thermodynamic framework to represent the solubility behavior of amino acids in water. Their calculated results were satisfactory in representing and predicting the solubilities of amino acids in an aqueous solution as function of temperature, ionic strength, solvent and pH. Kuramochi et al. [13] measured the solubilities of two amino acids in water at 298.15 K. They assigned six new UNIFAC groups to calculate the activity coefficients of amino acids in an aqueous solution, and proposed a model to predict the simultaneous solubilities of two amino acids in aqueous solutions. Xu et al. [14] applied a modified Wilson model for polymer aqueous solution to correlate the activity coefficients of amino acids in aqueous solutions. By assuming that all of the amino acid molecules in pure water are in zwitterionic form, the model was used to calculate the solubility of amino acids in water. Fuchs et al. [15] modeled the solubility data of glycine, DL-alanine and DL-methionine in aqueous and alcohol solutions by using PC-SAFT equation of state. The acid–base equilibria of the amino acids were taken into account to describe the influence of pH on the solubility. The model predicted the solubility of amino acids at different pH values in good agreement with the experimental data. Ji and Feng [16] applied SAFT equation of state to determine the solubility of amino acid in water and an aqueous solution containing another amino acid with good precision.

In this study, the solubilities of some amino acids in water in a pH range from 2 to 10 were measured at 298.15 K. For the L-leucine and L-isoleucine systems, the revealed solubility data [7] are restricted to the base aqueous solution. To our knowledge, the pH effects on solubilities of L-serine and DL-phenylalanine in an aqueous solution are not available in literature. Therefore, the amino acids investigated in this study are DL-alanine, L-leucine, L-isoleucine, L-serine and DL-phenylalanine. Further, the solid–liquid equilibrium relation combined with the NRTL activity coefficient model [17] is used to correlate the solubilities of amino acids in water at various pH values.

2. Experimental

2.1. Chemicals

The chemicals used were deionized water, sodium hydroxide (Riedel deHaen, 98 wt%), hydrochloric acid (Riedel deHaen, 37 wt%), ninhydrin (Riedel deHaen, 99 wt%), DL-alanine (Sigma–Aldrich, 99 wt%), L-leucine (Sigma–Aldrich, 98 wt%), L-isoleucine (Sigma–Aldrich, 98 wt%), L-serine (Sigma–Aldrich, 99 wt%) and DL-phenylalanine (Acros, 99 wt%). All chemicals were of analytical reagent grade and were used without further purification.

2.2. Equipment and procedures

The experimental apparatus consisted of an equilibrium cell provided with a circulating water thermostat, a magnetic stirrer, and a platinum probe (Hart Scientific Co., U.S.A.). The top side of the equilibrium tube was sealed with a septum to allow sampling. Deionized water and hydrochloric acid were mixed to make acidic solutions of the designated pH values. Similarly, basic solutions were prepared by adding sodium hydroxide to deionized water to reach the target pH values. The solid amino acid was then added in a small excess with respect to saturation. The liquid mixtures were shaken vigorously with a vortex mixer for 20 min to make sure that the amino acid dissolved completely before the solution is charged into the equilibrium cell. To ensure phase equilibrium, the mixture was stirred with a magnetic stirrer for 20 h and then allowed to settle for at least 4 h. In the equilibrium period, the temperature was kept at 298.15 K thermostatically and controlled with an accuracy of ±0.05 K. The final pH values of the equilibrium solutions were recorded by a pH meter (Thermo Orion 420Aplus, ±0.01 unit). The calibration of the pH meter was performed by using buffer solutions with pH 4.01, 7.00 and 10.01, respectively. A preheated syringe was used to avoid precipitation during sampling and the solubilities of amino acid in water were determined by ninhydrin assay with a colorimetric method.

2.3. Ninhydrin assay

To determine the amino acid concentration of the samples, ninhydrin is added which reacts with a free α-amino group to produce a Ruhemann purple color for colorimetric determination of amino acids [18]. The ninhydrin reagent, consisting of 10 ml of sodium acetate buffer solution at pH 5.40, 0.8 g of ninhydrin, 0.12 g of hydrindantin and 30 ml of DMSO, was freshly prepared. Generally, the saturated amino acid solution had to be diluted 800–1200 times to a concentration within the ninhydrin assay range to obey Beer–Lambert law. For the assay, 1.0 ml of ninhydrin reagent was mixed with 1.0 ml of the diluted amino acid sample solution in a capped vial. The vial was shaken by hand and then removed to a water bath at 100 °C for 30 min to allow the reaction to complete. After cooling to room temperature, 10 ml of an ethanol + water mixture with equal volumes was added to the vial. The resulting sample was then introduced to a cuvette and the absorbance was measured on the UV–vis spectrophotometer (Jasco V550) at a wavelength of 570 nm with a photometric accuracy of ±0.002 absorbance. A blank sample was duplicated by repeating the above steps, but the amino acid was replaced with deionized water. Standard samples for five amino acids of DL-alanine, L-leucine, L-isoleucine, L-serine and DL-phenylalanine with molality ranging from 0 to 0.0005 m were prepared and the calibrations showed a linear response with $r^2 = 0.9996$ or higher. The standard deviation, taking triplicates of each sample, is less than 0.004 m. The measurement accuracy of the solubility of amino acid in water is estimated around within ±1%.

3. Theory

3.1. Chemical model

It is well known that amino acids in aqueous solution appear in several forms as neutral zwitterions, cationic and anionic amino acid. To model the chemical equilibria of hydrogen ions and all amino acid species in water, we consider the ionization reactions, shown in Eqs. (1)–(3), in symbols as

$$A_{(s)} \overset{K}{\rightleftharpoons} A\pm_{(aq)} \quad (4)$$

$$A^+ \overset{K_1}{\rightleftharpoons} H^+ + A\pm \quad (5)$$

$$A \pm \overset{K_2}{\rightleftharpoons} H^+ + A^- \tag{6}$$

The solubility constant based on mole fraction, K_x, can be stated as,

$$K_x = x_{A\pm}\gamma^*_{A\pm} \tag{7}$$

where $x_{A\pm}$ and $\gamma^*_{A\pm}$ are the mole fraction and activity coefficient of amino acid zwitterions, respectively. The superscript asterisk denotes the activity coefficient in the asymmetric convention. According the assumptions [15] that the presence of additional acid and base in the solution can be neglected and all amino acid species have the same activity coefficients, the equilibrium constants of the two dissociation reactions, Eqs. (5) and (6), based on molarities can be written as

$$K_{1c} = \frac{[H^+][A\pm]}{[A^+]} \tag{8}$$

$$K_{2c} = \frac{[H^+][A^-]}{[A\pm]} \tag{9}$$

By introducing molar volume of the mixture and in a constant density situation, these become

$$K_{1c} = [H^+]\frac{x_{A\pm}}{x_{A^+}} \tag{10}$$

$$K_{2c} = [H^+]\frac{x_{A^-}}{x_{A\pm}} \tag{11}$$

Then we can obtain the true mole fractions of amino acid zwitterion, cation and anion through Eqs. (7)–(11)

$$x_{A\pm} = \frac{K_x}{\gamma^*_{A\pm}} \tag{12}$$

$$x_{A^+} = x_{A\pm}\frac{[H^+]}{K_{1c}} \tag{13}$$

$$x_{A^-} = x_{A\pm}\frac{K_{2c}}{[H^+]} \tag{14}$$

According to the mass balance of all amino acid species, the apparent mole fraction of amino acid, x_A, is followed as

$$x_A = x_{A\pm} + x_{A^+} + x_{A^-} \tag{15}$$

Thus the pH effects on the solubility of amino acid in water can be expressed as

$$x_A = \frac{K_x}{\gamma^*_{A\pm}}\left[1 + \frac{[H^+]}{K_{1c}} + \frac{K_{2c}}{[H^+]}\right] \tag{16}$$

The unit of solubility constant K_x has to be identical to that of the solubility x_A. For the convenience in all calculation steps, the solubility unit adopts to be mole fraction. However, our reported solubility data of amino acid in water will be given in molality scale by the following conversion,

$$m_A = \frac{x_A}{1 - x_A}\frac{1000}{18.02}. \tag{17}$$

3.2. Isoelectric point

The isoelectric point pI is defined as the pH where the number of the amino acid cations equals that of amino acid anions. So the net charge of all species in the solution is zero. Basically, the isoelectric condition must be satisfied together with the expression of the solubility of amino acid in water. Thus, the solubility of amino acid in water at pH = pI is

$$x_A = \frac{K_x}{\gamma^*_{A\pm}}[1 + 10^{(-pI+pK_{1c})} + 10^{(-pK_{2c}+pI)}] \tag{18}$$

The values of pI, pK_{1c} and pK_{2c} obtained from literature [19] were listed in Table 1. According to the fact that the formation of both cations and anions of the amino acid are insignificant in the isoelectric solution, almost 100% of the amino acid exists in zwitterion form. Then

$$x_A = x_{A\pm}. \tag{19}$$

From Eq. (18), the second and third terms in square brackets, representing the pH effect on the solubility, will be omitted by comparing the order of magnitude. At the isoelectric point, the solubility constant K_x can be simplified to a conventional form as

$$K_x = x_{A\pm}\gamma^*_{A\pm}. \tag{20}$$

3.3. Activity coefficient model

For a water (W) + amino acid zwitterion (A±) system, the nonideal behavior of the liquid phase can be taken care of by using the NRTL activity coefficient equation. The interaction energy parameters in the NRTL model can be determined from the solubility data for various pH values at 298.15 K. The value of the nonrandom factor α in the NRTL model was fixed at 0.3 in this study. The energy parameters are determined by minimizing the following objective function:

$$F = \sum_{i=1}^{N}(x_{A,i} - x^{cal}_{A,i})^2 \tag{21}$$

where N is the number of data points. It is noted that the symmetric activity coefficient of amino acid zwitterion, $\gamma_{A\pm}$, calculated from NRTL model has to be normalized to an asymmetric scale one, $\gamma^*_{A\pm}$, by

$$\ln \gamma^*_{A\pm} = \ln \gamma_{A\pm} - \ln \gamma^\infty_{A\pm} \tag{22}$$

where $\gamma^\infty_{A\pm}$ is the activity coefficient of amino acid zwitterion in an infinite-diluted reference state.

4. Results and discussions

The solubility measurements of five amino acids in water were conducted at 298.15 K. The experimental data were listed in Table 2 and the solubilities of DL-alanine, L-leucine, L-isoleucine, L-serine and DL-phenylalanine in aqueous solutions of various pH are shown in Figs. 1–5, respectively. In addition, the solubility data obtained from literature [1,2,8] at the isoelectric point are also presented in Table 2. To confirm the experimental results, the solubility data of DL-alanine reported in literature [7] are also plotted in Fig. 1. Our experimental results show a good agreement with the literature data.

From Figs. 1–5, we can observe that the measured solubility is strongly affected by the variation of pH for the DL-alanine, L-leucine, L-isoleucine and L-serine. As can be expected, the solubility of these amino acids is the lowest at the isoelectric point, and the solubility increases as the pH deviates from pI. Since the isoelectric point is the pH at which zwitterions carry a zero net charge, amino acids exhibit their lowest solubility in water at the isoelectric point. The solubility of these five amino acids in water at the isoelectric point

Table 1
Values of pK_{1c}, pK_{2c}, pI and K_x of amino acids at 298.15 K.

	DL-Alanine	L-Leucine	L-Isoleucine	L-Serine	DL-Phenylalanine
pK_{1c}[a]	2.35	2.33	2.32	2.21	2.58
pK_{2c}[a]	9.87	9.74	9.76	9.15	9.24
pI[a]	6.11	6.04	6.04	5.68	5.91
K_x[b]	0.0353	0.0031	0.0047	0.0803	0.0022

[a] Obtained from literature [19]
[b] Evaluated by Eq. (20) in this work.

Table 2
Experimental solubility data and correlated results of amino acids in water at 298.15 K.

DL-Alanine		L-Leucine		L-Isoleucine		L-Serine		DL-Phenylalanine	
pH	m_A/m	pH	m_A/m	pH	m_A/m	pH	m_A/m	pH	m_A/m
2.78	2.666	0.91	2.711	1.09	2.526	1.95	10.94	1.53	0.351
2.84	2.434	1.19	1.750	1.40	1.698	2.10	8.188	1.80	0.277
2.89	2.371	1.35	1.392	1.54	1.293	2.22	6.963	1.89	0.262
3.06	2.234	1.53	1.037	2.06	0.601	2.41	5.984	2.29	0.168
3.25	2.111	1.80	0.735	2.35	0.408	2.57	5.261	2.86	0.112
3.43	2.015	2.30	0.306	2.78	0.284	2.78	4.926	2.96	0.112
3.48	2.014	2.46	0.276	3.19	0.260	2.80	4.623	3.08	0.103
3.80	1.951	3.93	0.181	3.68	0.258	3.16	4.190	4.63	0.087
4.22	1.921	4.75	0.172	3.91	0.255	4.06	4.049	4.71	0.087
4.50	1.903	5.80	0.169	4.52	0.254	5.02	4.029	5.01	0.086
4.56	1.903	6.04[a]	0.168[b]	5.08	0.246	5.68[a]	4.013[b]	5.41	0.086
4.93	1.902	6.17	0.168	5.37	0.245	5.93	4.023	5.91[a]	0.085[c]
5.14	1.901	7.94	0.168	6.04[a]	0.244[d]	6.33	4.028	6.30	0.086
5.77	1.895	8.33	0.170	6.34	0.245	6.62	4.036	7.64	0.087
6.11[a]	1.877[c]	9.34	0.262	6.82	0.245	7.42	4.100	7.88	0.091
6.93	1.880	9.39	0.289	7.43	0.249	7.66	4.173	8.39	0.093
7.34	1.893	9.67	0.345	7.70	0.251	8.12	4.450	8.41	0.095
8.01	1.894	10.00	0.464	8.16	0.256	8.58	5.115	8.72	0.098
8.44	1.895	10.23	0.624	8.39	0.256	8.97	6.369	8.76	0.108
8.68	1.933	10.47	0.752	8.75	0.269	9.25	9.543	8.96	0.114
9.11	2.043	10.51	0.805	9.15	0.312			9.08	0.127
9.61	2.855	10.65	1.126	9.79	0.554			9.21	0.145
9.70	3.010	10.81	1.260	10.09	0.642			9.37	0.164
9.73	3.147	11.25	3.074	10.73	1.775			9.79	0.219
				11.02	2.762			10.21	0.329
D% = 1.29%		D% = 6.65%		D% = 3.40%		D% = 3.07%		D% = 2.08%	

[a] Isoelectric point.
[b] Reported by Lide [2].
[c] Reported by Dalton and Schmidt [1].
[d] Reported by Orella and Kirwan [8].

increases in the order of phenylalanine, leucine, isoleucine, alanine and serine.

It can also be seen that the polarity of the side chain also affects the dependence of solubility on the pH value of a solution. The various alpha amino acids differ in the type of side chain that is attached to their alpha carbon atom. Depending on the polarity of the side chain, amino acids vary in their hydrophilic or hydrophobic character, and consequently, their solubility in water. The side chain of alanine is a methyl group, which has a limited contribution to hydrophobicity. Leucine and isoleucine contain a branched propyl side chain with a methyl substitute at γ and β positions, respectively. They show similar solubilities in water at their isoelectric points. The hydroxyl group in serine's side chain allows this amino acid to dissolve better in aqueous solutions. Therefore, the solubility of serine is greater than that of the other four amino acids. On the other hand, the solubility of serine in an aqueous solution strongly depends on the pH of the solution, especially in extreme pH regions. Phenylalanine containing a bulky and non-polar benzyl group, exhibits the lowest solubility in water among the five amino acids under investigation. Both the cationic phenylalanine at a low pH and the anionic phenylalanine at a high pH retain their non-polar character. So the

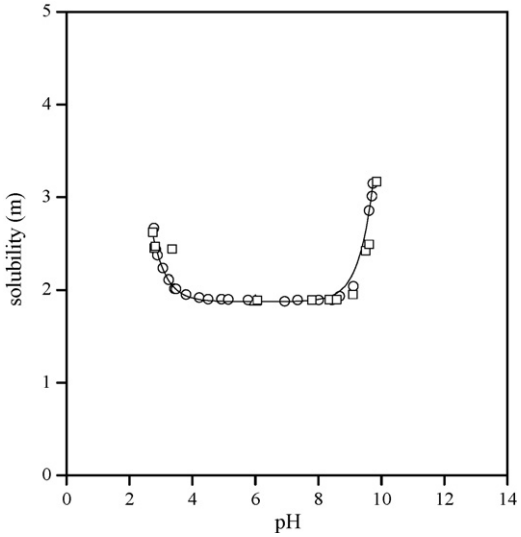

Fig. 1. The solubility of DL-alanine in water at 298.15 K. (○) Measured (this work); (△) isoelectric point; (□) Pradhan and Vera [6]; (–) NRTL model.

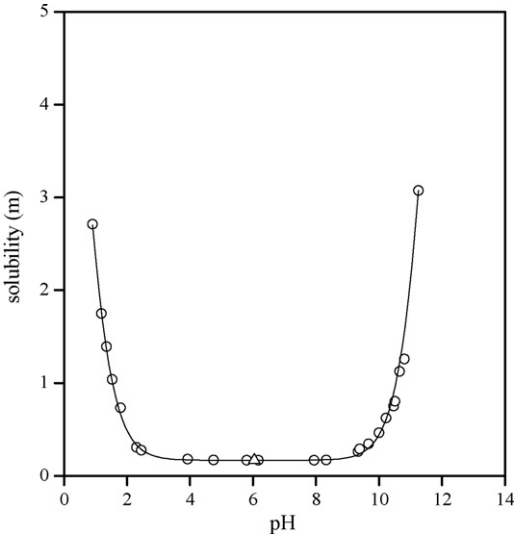

Fig. 2. The solubility of L-leucine in water at 298.15 K. (○) Measured (this work); (△) isoelectric point; (–) NRTL model.

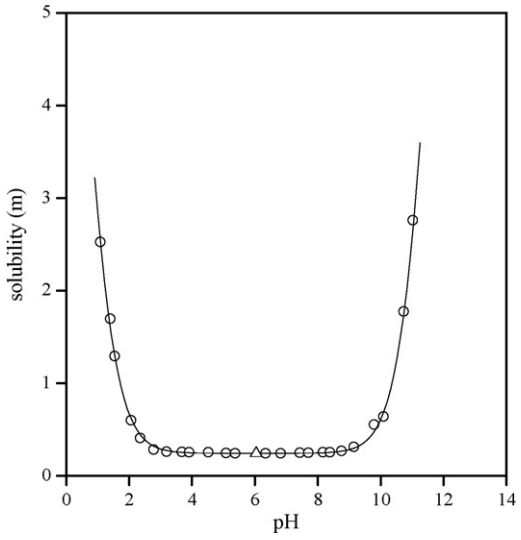

Fig. 3. The solubility of L-isoleucine in water at 298.15 K. (○) Measured (this work); (△) isoelectric point; (–) NRTL model.

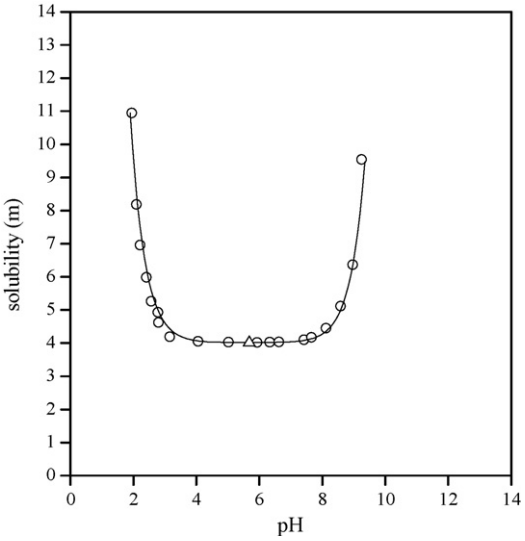

Fig. 4. The solubility of L-serine in water at 298.15 K. (○) Measured (this work); (△) isoelectric point; (–) NRTL model.

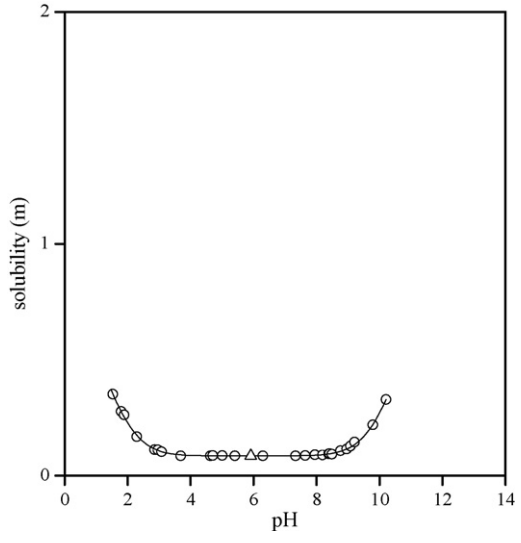

Fig. 5. The solubility of DL-phenylalanine in water at 298.15 K. (○) Measured (this work); (△) isoelectric point; (–) NRTL model.

Table 3
The correlated values of NRTL interaction energy parameters τ_{ij} (cal/mol) of the systems water (W) + amino acid zwitterion (A±).

	DL-Alanine	L-Leucine	L-Isoleucine	L-Serine	DL-Phenylalanine
$\tau_{WA\pm}$	−593.0	−892.2	−1003.6	−120.1	−2300.0
$\tau_{A\pm W}$	−224.7	−1146.4	−1171.6	−446.2	−3076.5

dependence of phenylalanine's solubility on pH value is relatively insignificant.

The solubility data were correlated by the NRTL activity coefficient model combined with a chemical model. Two matlab subroutines, fzero for the nonlinear zero finding by Eq. (16) and fminunc for the nonlinear regression by Eq. (21), were employed for the parameters estimation. The obtained values of the energy parameters are listed in Table 3. As mentioned above, at 298.15 K and at the isoelectric point, the solubility constant K_x can be determined from Eq. (20). For the five amino acids studied in this study, the values of the solubility constants, K_x, are also listed in Table 1. To illustrate our correlation results, the calculated solubility data are also plotted in Figs. 1–5. It can be seen that the calculated solubility curves match the experimental data very well and give fair representation over the whole pH range (pH 2–12). The average absolute relative deviations for the five amino acid aqueous systems are also given in Table 2. The deviations are quite acceptable, with an overall deviation being 3.32%.

5. Conclusion

In the present study, experimental data of the solubility of amino acids in water at various pH values were measured. The data were modeled using the NRTL model. The calculated results with two adjustable parameters were quite satisfactory.

List of symbols

A	amino acid
D	average absolute relative deviation
F	objective function
K	equilibrium constants
W	water
x	solubility in mole fraction
m	solubility in molality

Greek letter

γ	activity coefficient

Superscripts

±	zwitterion
+	cation
−	anion
∞	infinite dilute
cal	calculated value
*	asymmetric scale

subscripts

c	molarity basis
x	mole fraction basis

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