Solubilities of L-Cystine, L-Tyrosine, L-Leucine, and Glycine in Aqueous Solutions at Various pHs and NaCl Concentrations

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Solubilities at 25 °C of four amino acids (L-cystine, L-tyrosine, L-leucine, and glycine) in aqueous solutions at various pHs and NaCl concentrations (0, 1, 3 mol dm^{-3}) are reported. The pH was varied from 0 to 13 using HCl or NaOH. The NaCl concentration strongly affects the solubilities of L-cystine, L-tyrosine, and L-leucine. In particular, the addition of NaCl increases the L-cystine solubility but reduces the solubility of L-tyrosine and L-leucine. The NaCl concentration has only a slight effect on the solubility of glycine. Experimental solubilities are correlated with the use of a chemical equilibrium model, good agreement in the pH range 1−12 was found. The analysis confirms the dependence reported in the literature between the solubility of amino acids and the salt concentration. For the amino acids examined, the parameters to calculate their solubilities in solutions with NaCl are also given.

Introduction

The recovery of amino acids by hydrolysis of keratinic material is of increasing importance due to progress in separation technologies. Typically, the hydrolysis is carried out in either strong acid or basic solutions. Moreover, some amino acids are recovered as precipitates by neutralization of the solution, and because the neutralization process leads to solutions with high salt contents, it is of interest to investigate the salt effect on the solubility of amino acids.

In recent years, the study of the equilibrium of amino acids has received renewed attention because of industrial interest in these products. These studies have also been motivated by the lack of data and by the fact that the only complete experimental solubilities date back to many years ago (Greenstein and Winitz, 1961; Dalton and Schmidt, 1933, 1935). Additional solubility data have been reported by various researchers, i.e. Needham *et al*. (1971), Orella and Kirwan (1989), Zumstein and Rousseau (1989), and Gatewood and Rousseau (1994). All the references deal with single amino acids, while some data on mixtures were published by Chen *et al*. (1989) and Jin and Chao (1992).

Our objective is to measure the solubility of amino acids in water at various pHs and NaCl concentrations. These results will be useful in the development of solution models to describe the thermodynamic properties of amino acid solutions. In particular, this work focuses on the solubility of L-cystine, L-tyrosine, L-leucine, and glycine.

Experimental Section

Solutions without salt are prepared by adding commercial HCl (Carlo Erba RPE 1 mol dm^{-3}) or NaOH (Carlo Erba RPE 0.1 mol dm^{-3}) to bidistilled water so as to attain a fixed pH. An excess of amino acid (L-cystine and L-tyrosine, Aldrich Chemie 99%; L-leucine and glycine, Jansen >99%) was then added to the solutions and the flask was maintained in a thermostatic bath at (25 ± 0.1) °C. The amino acid solutions were continuously shaken with a magnetic drive agitator to establish equilibrium.

Mixtures formed by the acid or basic solutions and the added amino acid were put in flasks and stirred for sufficient time (48 h) to reach equilibrium between the solid amino acid and the liquid solution.

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The stirring time of 48 h was determined so as to achieve equilibrium conditions. The possibility of reaching the equilibrium condition at 25 °C by starting from different temperatures was checked by approaching the target of 25 °C with two different solutions of amino acids: the first was equilibrated at 30 °C and the second at 20 °C; then the two solutions were brought to 25 °C and left to equilibrate at this temperature. No significant differences were revealed.

The hydration of the solid phase was checked in the following way: a weighed quantity of each of the amino acids examined was kept in contact with HPLC water for 48 h; the mixture thus obtained was filtered, and the solid, after being left to dry at room temperature in vacuo (<0.5 Torr), was weighed. The mass of the amino acids and the amount dissolved coincide with the original quantities that Aldrich Chemie and Jansen certify as anhydrous.

After equilibrium was established, a syringe was used to take a sample of about 10 cm^3. This sample was filtered in a thermostated filter, and the sample for the analysis was taken from the clear liquid.

L-Cystine and L-tyrosine exhibited very long dissolution times (about 24 h), especially when using a low amount of solid material. Due to these difficulties, we operated with amounts of solids at least 2 times greater than those required to achieve equilibrium. The experimental solubilities as a function of pH, obtained in the solutions without salt, are reported in Table 1. The pH values, measured with a pH meter (Metrohm 691 precision ±0.01 units), are not the equilibrium values, but those of the initial solutions. This is very significant, especially in pH regions close to neutral. In fact, in these zones pHs are very different from those of the equilibrium solutions, but they represent the acid or base concentrations.

The solubility of the amino acids in salt solutions was studied using the same procedure. The only difference was that the original solutions were prepared by adding the amount of anhydrous NaCl (Carlo Erba RPE 99.5 mol %) required to reach the selected molar concentrations. The measured solubilities, at different salt concentrations, are shown in Tables 2 and 3.

Each solubility was calculated by averaging the values obtained from three series of samples taken from three different flasks. The maximum deviations in the nine samples were $\pm 1 \times 10^{-5}$ mol dm^{-3} for L-cystine and

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Table 1. Experimental Solubilities *s* at 25 °C (Solutions without NaCl)

L-cystine		L-tyrosine		L-leucine		glycine	
pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}
1.00	0.017 01	1.00	0.035 75	0.00	0.9237	0.00	3.504
1.41	0.004 09	1.43	0.013 48	0.27	0.7427	0.27	3.089
1.82	0.001 80	1.74	0.008 01	0.53	0.4938	0.53	2.759
2.29	0.000 90	1.95	0.006 13	0.73	0.3619	0.73	2.755
3.04	0.000 70	2.69	0.003 48	1.11	0.3145	1.11	2.747
3.42	0.000 70	3.48	0.002 83	2.00	0.2154	2.00	2.721
4.02	0.000 70	4.45	0.002 86	3.02	0.1745	3.02	2.713
5.08	0.000 71	5.39	0.002 70	3.50	0.1721	3.50	2.728
7.00	0.000 69	7.00	0.002 80	4.50	0.1689	4.50	2.667
8.61	0.000 70	9.12	0.002 71	7.00	0.1772	7.00	2.741
10.71	0.001 13	9.53	0.002 77	10.70	0.1741	8.98	2.712
11.29	0.002 10	11.05	0.003 60	11.76	0.2234	9.99	2.757
11.75	0.004 25	11.48	0.005 78	12.20	0.1916	11.51	2.656
12.13	0.008 79	12.00	0.007 80	12.54	0.2032	12.11	2.667
12.65	0.026 21	12.57	0.028 56	12.71	0.2136	12.49	2.805
12.96	0.027 83	12.92	0.053 48	13.00	0.3357	13.00	3.052
13.00	0.028 25	13.00	0.060 02				

Table 2. Experimental Solubilities *s* at 25 °C (Solutions with 1 mol·dm^{-3} of NaCl)

L-cystine		L-tyrosine		L-leucine		glycine	
pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}
1.00	0.023 65	0.88	0.036 35	0.00	0.6053	0.00	3.580
1.41	0.006 17	1.37	0.013 41	0.30	0.5824	0.33	3.203
1.93	0.002 12	1.88	0.006 09	1.03	0.3739	1.06	2.896
2.47	0.001 16	2.44	0.003 67	1.97	0.1595	1.48	2.780
2.96	0.000 95	2.96	0.002 97	3.11	0.1372	2.51	2.732
4.16	0.000 85	3.06	0.002 90	4.05	0.1308	3.50	2.724
5.50	0.000 84	3.19	0.002 83	5.07	0.1305	4.10	2.729
5.78	0.000 84	4.65	0.002 71	5.40	0.122	5.15	2.711
7.00	0.000 85	7.00	0.002 76	7.00	0.1289	7.00	2.720
8.79	0.000 83	9.53	0.002 76	8.04	0.1308	8.04	2.741
9.28	0.000 87	10.42	0.003 03	8.98	0.1173	8.98	2.723
11.11	0.001 34	10.92	0.003 35	10.07	0.1008	10.06	2.693
11.89	0.003 30	11.68	0.004 76	11.03	0.1181	11.03	2.708
12.52	0.007 83	12.31	0.009 52	12.02	0.1838	12.02	2.745
12.91	0.019 67	12.80	0.022 46	13.00	0.2412	13.00	3.045
13.00	0.033 92	13.00	0.059 12				

Table 3. Experimental Solubilities *s* at 25 °C (Solutions with 3 mol·dm^{-3} of NaCl

L-cystine		L-tyrosine		L-leucine		glycine	
pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}	pH	*s*/ mol·dm^{-3}
0.67	0.039 67	0.95	0.025 89	0.00	0.4514	0.00	3.313
0.77	0.025 00	1.49	0.006 22	0.31	0.1029	0.31	2.916
1.18	0.007 83	2.05	0.003 84	1.03	0.1189	1.03	2.679
1.68	0.003 08	2.56	0.002 17	2.07	0.0914	2.07	2.636
2.62	0.001 28	2.73	0.002 08	3.12	0.0700	3.12	2.619
2.91	0.001 13	2.89	0.002 00	4.18	0.0804	4.18	2.617
3.97	0.001 10	3.17	0.001 91	4.88	0.0891	4.88	2.599
5.00	0.001 10	4.39	0.001 82	6.11	0.0750	6.11	2.595
7.00	0.001 10	7.00	0.001 97	7.00	0.0741	7.00	2.651
8.61	0.001 09	9.45	0.001 97	7.87	0.0834	7.87	2.604
10.61	0.001 61	10.52	0.002 21	9.12	0.0763	9.12	2.619
11.50	0.002 77	10.82	0.002 34	10.07	0.0921	10.07	2.656
12.02	0.003 96	11.02	0.002 64	11.04	0.0914	11.04	2.649
12.69	0.008 08	11.65	0.003 81	11.98	0.0837	11.98	2.720
12.91	0.022 00	12.33	0.008 46	13.00	0.1832	13.00	3.083
13.00	0.042 12	13.00	0.046 74				

L-tyrosine, $\pm 2.5 \times 10^{-4}$ mol dm^{-3} for L-leucine, and $\pm 5 \times 10^{-4}$ mol dm^{-3} for glycine.

Chemical analyses were carried out using two different procedures. The solutions containing L-cystine and L-tyrosine were analyzed spectrophotometrically (Shimatzu UV 160A). Due to their low solubilities, it was sufficient to dilute the sample, taken from the solution at equilibrium, in a 1:2 ratio so as to avoid saturation of the absorbance signal.

Preliminary spectrophotometric analyses were carried out to assess the reliability of the method. In particular, the major problem was represented by the interference of the absorption spectra of HCl and NaOH with that of L-cystine. Indeed, hydrochloric acid and sodium hydroxide exhibit high-intensity absorbance in the region where a maximum (≈210 nm) for L-cystine is attained. In contrast, L-tyrosine has a maximum at about 270 nm where both the HCl and NaOH absorbance is negligible. For this reason it was very important, particularly for the L-cystine analyses, to have, as a blank, solutions with acid or base concentrations the same as those of the samples because small differences lead to faulty measurements.

Calibration curves were made for L-cystine at 215 nm and for L-tyrosine at 270 nm. Absorption data at various L-cystine and L-tyrosine concentrations were correlated by a linear regression; the extinction coefficients are 6.6778 (dm^3 g^{-1}) for L-cystine (regression coefficient 0.998) and 6.2641 (dm^3 g^{-1}) for L-tyrosine (regression coefficient 0.998).

The solutions containing L-leucine and glycine were analyzed by determining the mass of the residue obtained by evaporating known volumes of samples. Due to their solubility, the small volumes (1−5 cm^3) considered contain at equilibrium detectable masses of solid amino acids. This method, which was extensively used by Jin and Cao (1992) and Needham *et al*. (1971), was first tested by determining the mass of the dissolved L-leucine and glycine from dried samples obtained from solutions with known amounts of dissolved amino acids. Quantitative detection was carried out from the mass of the solid obtained after the liquid of the sample was evaporated at (50 to 60) °C at atmospheric pressure. This maximum temperature was fixed to avoid a rapid evaporation, with possible losses of amino acids, and any thermal decomposition. The solid residue masses were determined, and when present, the mass of added NaCl was taken into account. A series of preliminary measurements was made to verify the reliability of the method.

Discussion of the Results

The solubilities were compared with those predicted by a simple model which takes into account only the chemical equilibria with the activities considered equal to the concentrations. For a divalent amino acid (A) in aqueous solution the following equilibria have to be considered:

$$A_{(s)} = A^0_{(aq)} \qquad K_1 \tag{1}$$

$$A^0_{(aq)} = A^{\pm}_{(aq)} \qquad K_2 \tag{2}$$

$$H_2O_{(l)} = OH^-_{(aq)} + H^+_{(aq)} \qquad K_3 \tag{3}$$

$$A^+_{(aq)} = A^{\pm}_{(aq)} + H^+_{(aq)} \qquad K_4 \tag{4}$$

$$A^{\pm}_{(aq)} = A^-_{(aq)} + H^+_{(aq)} \qquad K_5 \tag{5}$$

where $A_{(s)}$ and $A^0_{(aq)}$ are the amino acids in the solid and aqeous phases, respectively, $A^{\pm}$ represents the zwitterion, and A^+, A^{2+}, A^-, and A^{2-} are the charged forms of the amino acids.

In the case of L-tyrosine (trivalent) and L-cystine (tetravalent), the equilibrium equations are similar but it is necessary to consider one (eq 6) or two (eqs 6 and 7) more equations.

$$A^-_{(aq)} = A^{2-}_{(aq)} + H^+_{(aq)} \qquad K_6 \tag{6}$$

$$A^{2+}_{(aq)} = A^+_{(aq)} + H^+_{(aq)} \qquad K_7 \tag{7}$$

Table 4. Solubility Constants K_8 (mol·dm^{-3}) for Zwitterions

L-cystine	L-tyrosine	L-leucine	glycine
6.92×10^{-4}	2.74×10^{-3}	1.72×10^{-1}	2.44

Table 5. Chemical Equilibrium Constants

components	K_4/mol·dm^{-3}	K_5/mol·dm^{-3}	K_6/mol·dm^{-3}	K_7/mol·dm^{-3}
L-cystine	1.62×10^{-1}	1.55×10^{-2}	5.75×10^{-9}	3.24×10^{-9}
L-tyrosine	6.31×10^{-3}	7.76×10^{-10}	8.51×10^{-11}	
L-leucine	2.29×10^{-3}	5.02×10^{-10}		
glycine	2.18×10^{-3}	5.02×10^{-10}		

Greenstein and Winitz (1961) reported values of K_D (ratio of zwitterion concentrations to concentrations uncharged forms of amino acids) for amino acids in the range 10^5 to 10^6. Thus, reactions 1 (characterized by the thermodynamic solubility constant K_1) and 2 can be combined as follows (Pinho *et al.*, 1994):

$$A_{(s)} = A^{\pm}_{(aq)} \qquad K_8 \tag{8}$$

Thus, for pratical purposes

$$K_1 = K_8 = a_{\pm} \tag{9}$$

The equilibrium constants (K_i) for the reactions 3−7 are given as follows:

$$K_3 = a_{H^+}a_{OH^-} \tag{10}$$

$$K_4 = a_{A^{\pm}}a_{H^+}/a_{A^+} \tag{11}$$

$$K_5 = a_{A^-}a_{H^+}/a_{A^{\pm}} \tag{12}$$

$$K_6 = a_{A^{2-}}a_{H^+}/a_{A^-} \tag{13}$$

$$K_7 = a_{A^+}a_{H^+}/a_{A^{2+}} \tag{14}$$

where

$$a_i = \gamma_i m_i \tag{15}$$

γ_i is the molal activity coefficient, m_i and a_i are respectively the molality and activity coefficient of species i.

If the activity coefficient of the zwitterion (γ_z) can be assumed equal to unity, K_1 corresponds to the solubility of the zwitterion species. Thus, in the isoelectric range, where amino acid concentrations are low, we can assume that $\gamma_z = 1$ and evaluate K_s by measuring the solubility in this range. The assumption that $\gamma_z = 1$ seems reasonable for L-cystine, L-tyrosine, and L-leucine since their solubilities are low (mole fractions $<10^{-3}$). This assumption becomes less accurate for glycine because of its relatively high solubility (mole fraction about 5×10^{-2}).

The values of the solubility constants used in the model are given in Table 4. For L-cystine and L-tyrosine these parameters were evaluated from the solubility data in the

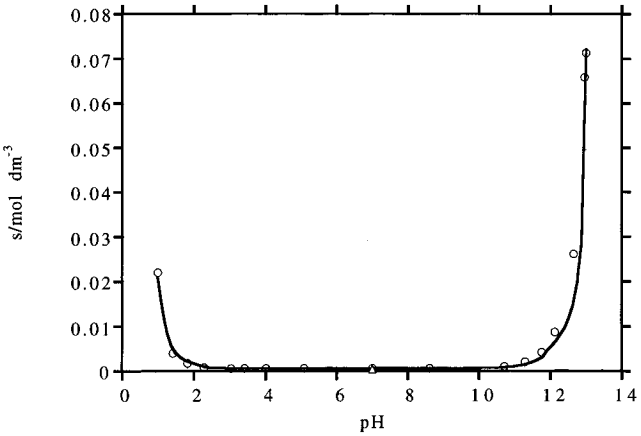

Figure 1. Comparison between experimental and calculated solubilities at 25 °C for L-cystine in aqueous solutions at various pHs (experimental data: (○) this work; (△) Dalton and Schmidt, 1933; (—) calculated).

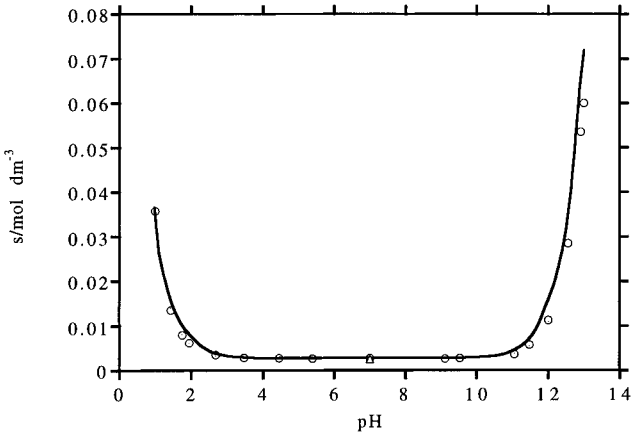

Figure 2. Comparison between experimental and calculated solubilities at 25 °C for L-tyrosine in aqueous solutions at various pHs (experimental data: (○) this work, (△) Dalton and Schmidt, 1933; (—) calculated).

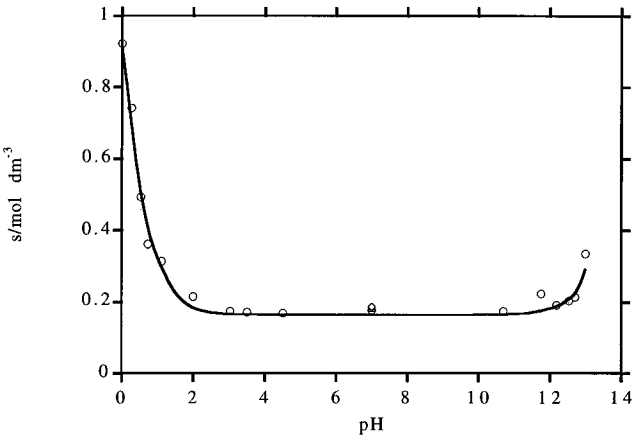

Figure 3. Comparison between experimental and calculated solubilities at 25 °C for L-leucine in aqueous solutions at various pHs (experimental data: (○) this work; (△) Dalton and Schmidt, 1933; (◇) Gatewood and Rousseau, 1994; (—) calculated).

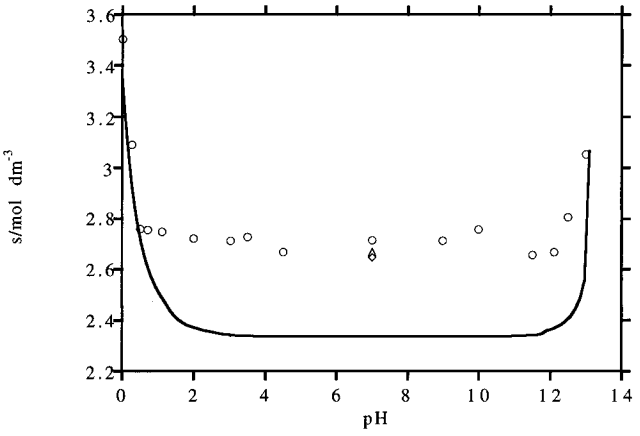

Figure 4. Comparison between experimental and calculated solubilities at 25 °C for glycine in aqueous solutions at various pHs (experimental data: (○) this work; (△) Dalton and Schmidt, 1933; (◇) Orella and Kirwan, 1989; (—) calculated).

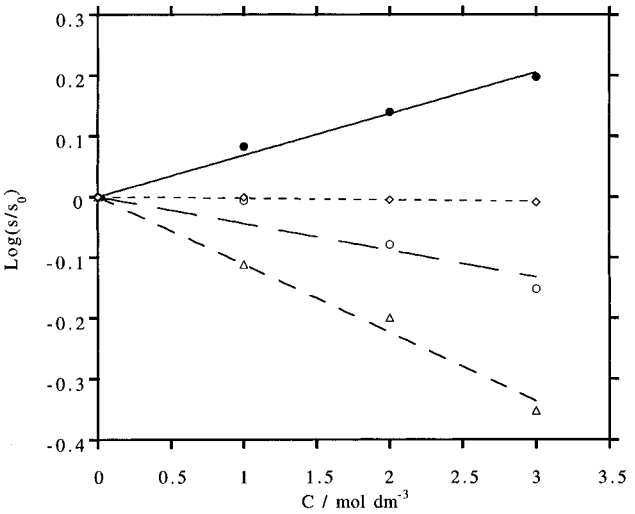

Figure 5. Amino acid solubilities as a function of NaCl concentration: L-cystine (calc (—), exp (●)), L-tyrosine (calc (— —), exp (○)), L-leucine (calc (— — —), exp (△), glycine (calc (- - -), exp (◇)).

Table 6. Salting Constants K_{so} (mol·dm^{-3})

L-cystine	L-tyrosine	L-leucine	glycine
0.068	−0.044	−0.112	−0.002

isoelectric range obtained in this work, while the values for L-leucine and glycine were calculated using a relation introduced by Pinho *et al.* (1994)

The equilibrium constants for reactions 4−7 of the amino acids were taken from the literature (Greenstein and Winitz, 1961) and are shown in Table 5.

In Figures 1−4 the solubilities obtained from the model are compared with our experimental values, and literature data are also shown. The agreement is quite good for three of the amino acids studied, while calculated solubilities for glycine differ from the experimental values, in the isoelectric region, by about 20%. This high difference may be due to the value of the solubility constant which was obtained by using an equation with great sensitivity to the parameters (Pinho *et al.*, 1994). Also the assumption that $\gamma_z = 1$ may not be sufficiently accurate and could explain these high differences. In fact the activity coefficient reported in the literature (Fasman, 1976) for solutions of glycine containing 3.0 mol dm^{-3} of amino acid is 0.742.

The effect of NaCl is shown in Figure 5, which is a plot of solubility in neutral solutions as a function of the NaCl concentration (C). It appears to be a linear dependence between the logarithm of the ratio of the solubility with (s) and without (s_0) NaCl and the salt concentration that allows us to calculate the salting out or salting in constants, K_{so}, by a linear regression. The values so obtained are reported in Table 6. The straight line confirms the dependence usually given in the literature (Greenstein and Winitz, 1961):

$$\log\left(\frac{s}{s_0}\right) = K_{so}C \qquad (16)$$

The solubility of L-cystine increases with salt concentration (C), glycine is little affected, while the solubility of L-tyrosine and L-leucine decreases with increased NaCl concentration.

Conclusions

We have presented experimental results on the solubilities of L-cystine, L-tyrosine, L-leucine, and glycine which are in good agreement with results previously reported in the literature. As the pH of the solution is lowered, the chemical equilibria, as shown in eqs 3−7, move toward the left and amino acid solubility increases because of the stabilization of the cation species. For the less soluble amino acids (L-cystine and L-tyrosine) and for L-leucine, the use of a simple model that only accounts for the chemical equilibria and which assumes the activities equal to the concentrations appears to be adequate to represent the solubilities in the pH range from 1 to 12. For more soluble amino acids, or for higher acid or base concentrations, the model is not adequate.

The addition of sodium chloride has different effects on the solubility of the amino acids studied. The solubility of L-cystine increases by about 50% as the NaCl concentration is changed from (0 to 3) mol·dm^{-3}; with the same increase in NaCl concentration, the solubility of L-tyrosine decreases by about 40% and that of L-leucine by more than 50%. The solubility of glycine, in the pH region 0−13, is only slightly influenced by changes in the salt concentration.

The linear dependence of the logarithm of the ratio of the solubilities of the amino acids with and without salt confirms the relationship (16) and allows us to calculate the effect of the salt on the amino acid concentration at saturation conditions.

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