- Man is unable to think beyond his mental capacity, he can only recognize and copy that, which nature presents.

- Man is the only creature who forever offends against the biological principles. Such wanton acts will result in a boomerang effect, which in the long term will induce him to behave in a more biologically friendly manner. Biological processes are long term reactions.

However, this means that man like all other biological organisms is subordinate to basic requirements e.g. metabolism, reproduction, adaptation, mutation and the survival urge. Man is conscious of these without being able to escape from them. Hence, it follows that man is not the centre of the biological system. He must accept that he cannot simply re-model nature in accordance with his own individual ideas. Nature will retaliate.

*Lifetime of man is characterized by the bio-rhythms between the phases of the activity and regeneration.* During the phase of regeneration the massive need for free energy must be provided by means of metabolism. This free energy is particularly used up by the brain.

Man depends on all other biological species, but they in turn are not depended on man. The biological system is a symbiotic system.

At present the species man is characterized by increasing the expectancy and decreasing fertility. This development is gradually leading to a reduction in mankind's biological ability to survive. It remains to be seen, if our species compensates this weakness of survival through self-regulation in the next few generations.

The question is, if the mortality rate of the aged increases abruptly will there be a corresponding a wish of the younger generation to produce offspring again.

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L093

# EXPERIMENTAL MEASUREMENT AND MODELING OF THE SOLUBILITY OF AMINO ACIDS IN AQUEOUS SOLUTIONS CONTAINING ELECTROLYTES

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## Summary

The solubility of glycine, DL-alanine, and L-isoleucine was measured in the temperature range between 25 and 60 ºC for the aqueous systems of KCl, $Na_2SO_4$ and $(NH_4)_2SO_4$. The experimental data are compared with published values[1,2] being possible to find out big discrepancies. The solubility and activity coefficients data[3-6] were used to explore the potentialities of the Pitzer-Simonson-Clegg equation[7] for the thermodynamic description of the ternary systems water-KCl-glycine and water-KCl-DL-alanine at 25 ºC. This model, with five adjustable parameters, gives a global root mean square deviation of 0.36% for the correlation of the activity coefficients and 0.81% for the solubilities.

## Introduction

Physical properties such as solubility and activity coefficients are fundamental to the design and scale-up of chemical processes for separation, concentration and purification of biochemicals. The presence of electrolytes in solution may change significantly the solubilities of biochemicals, which has been used for salt-induced separation of proteins. Although amino acids are among the simplest biochemicals, they have many similarities with more complex biochemicals and are the building blocks of proteins. Therefore, in the last decade increasing attention has been given to the study of the electrolyte effect on the properties of aqueous solutions of amino acids.

## Experimental

**Chemicals.** In all experiments double-ionized water was used. Glycine, 99.7% purity and DL-alanine, 99% purity were supplied by Merck and L-isoleucine, 99% purity was obtained from Fluka. Potassium chloride and ammonium sulfate, 99.5% purity and sodium sulfate, 99% purity were supplied by Merck. All chemicals were used as received.

**Method.** The analytical gravimetric method[8] was the experimental technique chosen to perform the measurements. This method consists on the preparation of a saturated solution at constant temperature. The jacked glass cell is charged with the electrolyte solution, and amino acid in small excess to the amount required for saturation and the solution is continuously mixed during 48 h to reach the equilibrium. The stirring is then stopped during 7 h to settle the undissolved amino acid particles before sampling. After the slow evaporation of the solvent, samples are weighted and this process repeated until a constant mass value is achieved. Each experimental point is an average of at least three different measurements with high reproducibility[8].

The concentration and the nature of both the cation and the anion of the electrolyte present in solution have, as expected, a relevant influence on the solubility that leads to either a salting-in or salting-out effect. The experimental data obtained in this work and those previously reported for water-$Na_2SO_4$-amino acid[2] and for water-KCl-amino acid[1] are compared in Figures 1 and 2(a, b), respectively. The results show a big discrepancy for the water-KCl-amino acid systems and a very good agreement for the water-$Na_2SO_4$-amino acid systems.



**Figure 1.** Comparison of the measured solubility data with values reported in the literature[2] at 25 ºC.

## Model Development

The solubility data from this work and activity coefficient data compiled from the open literature[3-6] were used to explore the capabilities of the Pitzer-Simonson-Clegg equation for the thermodynamic description of these complex systems. In the mole-fraction-based model, the excess Gibbs energy is assumed to consist of short-range forces and long-range forces expressed using a Debye-Hückel term, according to eqs. (13) and (15) presented by Hu and Guo[7]. The expressions for the activity coefficient of water and the mean ionic activity coefficient of a 1:1 electrolyte in aqueous non-electrolyte solutions correspond to eqs. (22) and (23) of Hu and Guo[7]. The corresponding expression for the activity coefficient ($f_n$) of the amino acid is:

$$\ln f_n = 2A_x I_x^{3/2}/\left(1+\rho I_x^{1/2}\right) - I_x^2 B_{MX}\exp\left(-\alpha I_x^{1/2}\right) + \left[-x_1 W_{1,MX} + (1-x_n)W_{n,MX}\right]x_I + x_I^2 *$$
$$\left[-2x_1 U_{1,MX} + (1-2x_n)U_{n,MX}\right] + x_I^2\left[-3x_1^2 V_{1,MX} + x_n(2-3x_n)V_{n,MX}\right] + x_1 *$$
$$\left[(1-x_n)w_{1,n} + u_{1,n}\left[2(1-x_n)(x_1-x_n)-x_1\right]\right] + 2x_1 I_x(1-2x_n)Y_{1,n,MX}^{(0)} + x_1\left(x_I^3/4\right)(1-4x_n)Y_{1,n,MX}^{(1)} \tag{1}$$

where $x_1$ and $x_n$ are, respectively, the mole fraction of water and amino acid, $x_I$ the total mole fraction of ions, $W_{1,MX}$, $U_{1,MX}$ and $V_{1,MX}$ the model parameters to describe the interactions between water and the electrolyte, $W_{n,MX}$, $U_{n,MX}$, $V_{n,MX}$, $Y_{1,n,MX}^{(0)}$ and $Y_{1,n,MX}^{(1)}$ the model parameters which describe the interactions between the amino acid and electrolyte, $w_{1,n}$ and $u_{1,n}$ the coefficients for the description of water-amino acid interactions; $I_x$ the mole fraction ionic strength, $B_{MX}$ a specific parameter of the electrolyte; $A_x$ the Debye-Hückel parameter ($A_x = 2.9094$)[7]; $\rho$ the "closest approach" distance between the ions ($\rho = 14.0292$)[7] and $\alpha$ standard value ($\alpha = 13.0$)[7]. The model parameters $W_{1,MX}$, $U_{1,MX}$, $V_{1,MX}$ and $B_{MX}$ used for the binary system KCl-$H_2O$ were the ones listed in Table 1 of Hu and Guo[7]. For the binary systems glycine-$H_2O$ and DL-alanine-$H_2O$, the model parameters $w_{1,n}$ and $u_{1,n}$ were determined by fitting eq. (26) of Hu and Guo[7] to published osmotic coefficient data[9-12] (root mean square deviation – rmsd – 0.49%) or to water activity[13, 14] (rmsd equal to 0.18%) at 25 ºC.

The parameters left to be estimated, $W_{n,MX}$, $U_{n,MX}$, $V_{n,MX}$, $Y_{1,n,MX}^{(0)}$ and $Y_{1,n,MX}^{(1)}$ were calculated using the experimental data presented in this work and the ratio of the mean ionic activity coefficients of KCl in the presence of the amino acid to that in the absence of the amino acid at different electrolyte and amino acid molalities[3-6] at 25 ºC.

The solubility of an amino acid, $m_{AA}$, in an aqueous electrolyte solution is calculated by[1]:

$$m_{AA}\,\gamma_{AA} = m_{AA}^o\,\gamma_{AA}^o \tag{2}$$

being $m_{AA}^o$ the amino acid saturation molality in an aqueous solution, $\gamma_{AA}$ and $\gamma_{AA}^o$ the molality scale, unsymmetrically normalized, activity coefficients of the amino acid in the presence and in the absence of electrolyte, respectively.

## Results and Discussion

For each system the parameters were estimated minimizing the following objective function (Fob):
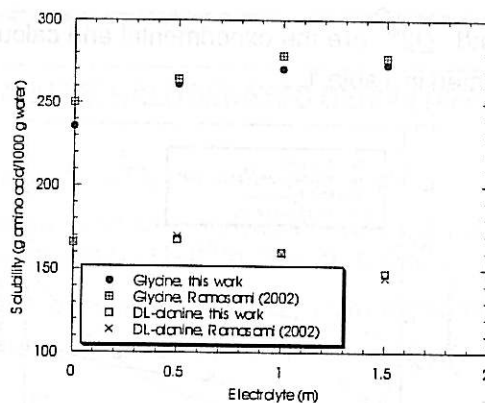
$$Fob = \sum_k \left(Q_k^{exp} - Q_k^{calc}\right)^2 \tag{3}$$

where $Q_k^{exp}$ and $Q_k^{calc}$ are the experimental and calculated quantity, respectively. The estimated parameters are presented in Table 1.
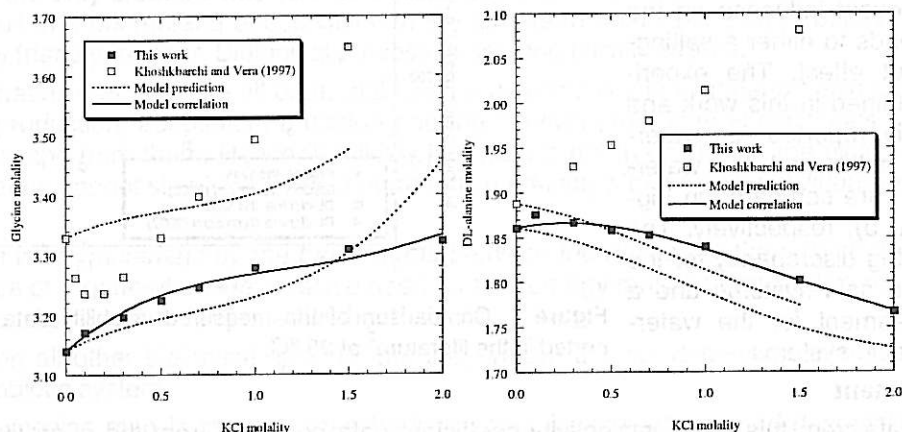


**Figure 2.** Comparison between model correlation, model prediction and experimental data: (a) water-KCl-glycine; (b) water-KCl-DL-alanine.

As can be seen from Figure 2(a, b), the model can accurately correlate the experimental solubility data of glycine (Figure 2a) and DL-alanine (Figure 2b) from this work over the whole range of KCl concentration with a rmsd of 1.03% and 0.49%, respectively.

A very important trend of any model is its predictive ability. To evaluate this feature only the ratio of the mean ionic activity coefficients of KCl in the presence of the amino acid to that in the absence of the amino acid at different electrolyte and amino acid molalities[3-6] at 25 °C were used in the correlation. The result of the model prediction for the data measured in this work and for data published by Khoshkbarchi and Vera[1] is also shown in Figures 2a and 2b. The model prediction is in good agreement with the solubility data measured in this work. The rmsd found were 5.86% for the system water-KCl-glycine and 3.76% for the system water-KCl-DL-alanine. For the data published by Khoshkbarchi and Vera[1] it is possible to find out big discrepancies, especially for the system with DL-alanine (rmsd 18.23%) where the solubility trend is inversely predicted. Somehow this fact proves the higher consistency of the measured data in this work.

**Table 1.** Model parameters for KCl aqueous solutions with glycine or DL-alanine.

| | $w_{1,n}$ | $u_{1,n}$ | $W_{n,MX}$ | $U_{n,MX}$ | $V_{n,MX}$ | $Y_{1,n,MX}^{(0)}$ | $Y_{1,n,MX}^{(1)}$ |
|---|---|---|---|---|---|---|---|
| Glycine | -37.9239 | 14.1923 | -20.7524 | 82.9325 | -278.0353 | 2.3572 | -2897.9120 |
| DL-alanine | -17.8261 | 5.8363 | 16.7072 | 89.3468 | -1003.3007 | -28.5140 | -2449.2244 |

## Conclusions

The Pitzer-Simonson-Clegg equations provide a good representation of activity coefficients[3-6] and amino acid solubilities when applied to the ternary systems DL-alanine or glycine-water-KCl at 25 °C, with a global rmsd of 0.41% for correlation. The prediction of the solubilities of DL-alanine or glycine, based only on the activity coefficients data[3-6], with rmsd of 4.92% and 13.72% was performed for data measured in this work and for data from Khoshkbarchi and Vera[1], respectively. Results will be further extended to mixtures including unsymmetrical electrolytes and other amino acids.

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