Solubility of Amino Acids in Mixed Solvent Systems

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Abstract

The solubilities of L-serine, L-threonine and L-isoleucine in the aqueous systems of ethanol, 1-propanol and 2-propanol were measured in the temperature range between 298.15 K and 333.15 K by means of a gravimetric method and a spectrophotometric technique based on a ninhydrin reaction. The solubility data from this work and from literature were used to explore the potentialities of the application of the excess solubility approach with the NRTL [1], modified NRTL [2], modified UNIQUAC [3, 4] equations and the model presented by Gude et al. (1996) [5, 6]. These four models give a global average relative deviation of 12.2 %, 12.0 %, 15.1 %, and 16.2 % for correlation and 16.3 %, 14.6 %, 27.3 %, and 22.0 % for prediction, respectively.

Introduction

Recently amino acids became the topic of many studies due to their biological and industrial importance. In 2004, our group started a systematic study on the solubility of several amino acids and diglycine in aqueous-alkanol solutions [7] that is now extended. The initial theoretical work [7] was focused on the application of the excess solubility approach with the NRTL model and the model presented by Gude et al. (1996) [5, 6]. In this work, the application of the excess solubility approach with the modified UNIQUAC model [3, 4] and the modified NRTL model [2] are also considered.

Experimental

Chemicals

In all experiments double-ionized water, supplied by Fresenius Kabi Pharma, was used. Ethanol and 1-propanol, 99.8 % purity, 2-propanol,

99.9 % purity were supplied by Merck. L-threonine, 99.0 % purity and L-isoleucine, 99.0 % purity were supplied by Fluka. All the chemicals were used as received without further purification.

Method

The analytical method was chosen to perform the measurements. The experimental procedure was already described in detail in previous publications [7, 8, 9]; it consists in the preparation of a saturated solution at constant temperature. Stirring is promoted during 48 h to reach the equilibrium conditions and, after, the solution is allowed to settle before sampling. Then, the solvent of the saturated solution is slowly evaporated and the remaining crystals are weighted. The process is repeated until a constant mass value is achieved. The composition of these solutions is measured differently depending on the alcohol mass fraction in the mixed solvent in amino acid free basis: when lower than 0.8 the analytical gravimetric method is applied; at higher alcohol concentrations, the spectrophotometric method based on a ninhydrin reaction [10] is used for quantitative determination of the extremely low solubilities of the amino acids.

For the amino acids, L-serine and L-threonine, both with a polar side chain, it can be observed that the relative solubility reduces severely with the increase of the alcohol concentration on the solvent mixture; however the effect is less pronounced for L-threonine (Figures 1 and 2). For L-isoleucine, with a hydrophobic side chain (-sec-butyl), the relative reduction in solubility, especially for small mole fractions of alcohol in the solvent mixture, is less significant. The temperature dependence of the relative solubility of L-serine and L-threonine is weak, but it affects considerably the relative solubility of L-isoleucine. A closer observation of Figure 3 shows that the relative solubility of L-isoleucine at low 1-propanol mole fractions (333.15 K) is superior to 1 indicating that at this temperature and alcohol composition, the solubility is higher than the solubility in water at the same temperature. This kind of behavior was already observed for the phenylalanine [11, 12].

When possible, the experimental data obtained in this work is compared with solubility data already available in the literature [12]. The agreement is very good (Figures 2 and 3).

Modeling

The excess solubility approach as presented by Ferreira et al. (2004) [7] was used with different thermodynamic models (NRTL [1], modified NRTL [2],

modified UNIQUAC [3, 4] and the one proposed by Gude et al. (1996) [5, 6]) to describe the solubilities of the amino acids glycine, DL-alanine, L-serine, L-threonine and L-isoleucine in aqueous aliphatic alcohol solutions at different temperatures. For each system, the parameters were estimated minimizing the following objective function (FOBJ):

$$FOBJ = \sum \left[\frac{\left(x_{aa,mix} / x_{aa,w} \right)_k^{calc} - \left(x_{aa,mix} / x_{aa,w} \right)_k^{exp}}{\left(x_{aa,mix} / x_{aa,w} \right)_k^{exp}} \right]^2 \quad \textbf{(1)}$$

where $x_{aa,mix}$ and $x_{aa,w}$ are the saturated solute mole fractions in the mixed solvent and in pure water, respectively, $x_{aa,mix}/x_{aa,w}$ is the relative solubility, and the superscripts *exp* and *calc* are the experimental and calculated relative solubility, respectively. The database used for the correlation includes the solubility data in aqueous alcohol solutions obtained in this work and solubility data available in the literature [7, 11-14].

The major difference between the models concerns the number of parameters needed to be calculated: the model proposed by Gude et al. (1996) requires a single amino acid specific parameter; the NRTL, the modified NRTL and the modified UNIQUAC equations require the same number of estimated parameters (for an amino acid for which solubility data is available in *n* aqueous-alkanol systems the number of parameters to be determined is *n* + 1) [7].

Results and Discussion

In general, the use of the modified NRTL equation combined with the excess solubility approach gives the best quantitative fit of the solubility data with a global average relative deviation of 12.0 % for correlation and 14.6 % for prediction. The NRTL, modified UNIQUAC and the model presented by Gude et al. (1996) give global average relative deviations of 12.2 %, 15.1 %, and 16.2 % for correlation and 16.3 %, 27.3 %, and 22.0 % for prediction, respectively. A comparison of the NRTL, modified NRTL and modified UNIQUAC is given in Figures 1 and 2 for the solubility of the amino acids in different alkanol aqueous systems at 298.15 K. As can be observed, the results are fairly good for the three models. In Figure 3, the influence of the temperature on the relative solubility of L-isoleucine in the aqueous 1-propanol systems is shown. The model proposed by Gude et al. (1996) gives a good description of the solubility data at 298.15 K for the water rich region. For the higher temperature studied (333.15 K) the NRTL model is the most successful and gives a very good quantitative description of the equilibrium data for the water rich region while the other models fail.

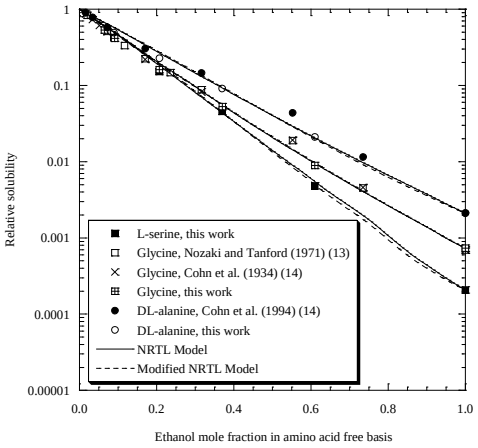

Figure 1. Relative solubilities of amino acids in water/ethanol solutions at 298.15 K.

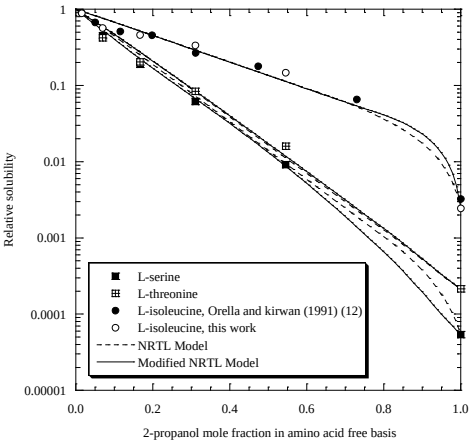

Figure 2. Relative solubilities of amino acids in water/2-propanol solutions at 298.15 K.

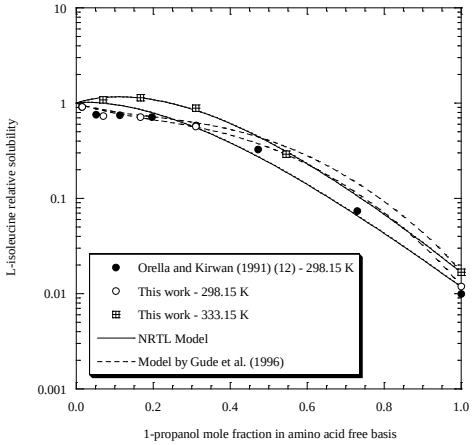

Figure 3. Relative solubility of L-isoleucine in water/1-propanol solutions at different temperatures.

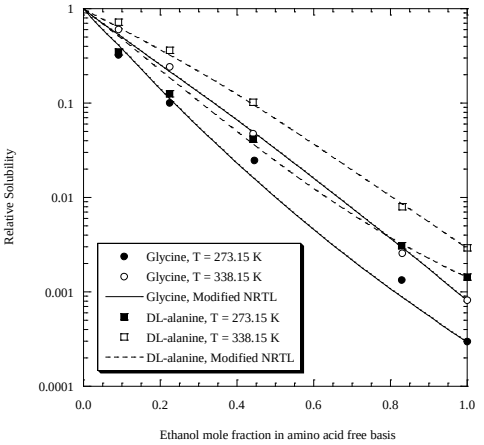

Figure 4. Modified NRTL predictions of the relative solubility of glycine and DL-alanine in water/ethanol solutions [15].

A very important feature of any model is its predictive ability. The predictions for glycine and DL-alanine at 273.15 K and 338.15 K obtained with the modified NRTL model can be seen from Figure 4**.** Clearly, the fact that the lowest temperature included in the correlation is 298.15 K indicates that some caution must be taken when predicting solubilities at temperatures outside the range covered during the fitting process.

Conclusions

Solubilities of different amino acids in aqueous alkanol mixtures were studied at temperatures ranging from 298.15 K to 333.15 K.

The excess solubility approach combined with the modified NRTL model can satisfactorily correlate and predict the amino acids solubilities in the different aqueous alcohol systems at the temperature range studied in this work. However, the modeling results show that conventional thermodynamic models present serious difficulties to account accurately for the hydrophobic effects that are present in these highly complex systems.

Acknowledgement

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