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Phase Behavior of Amino Acids and Peptides in Aqueous Solutions Using the Modified Wilson Model

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Abstract: The modified Wilson model have been used to correlate the activity coefficients of amino acids and simple peptides in aqueous solutions. In the models studied in this reserch only the binary interaction parameters for amino acid-water molecules were taken into account. This treatment was based on this phenomenon that amino acid molecules are in their zweitter ionic in aqueous solutions. The results obtained from the model were compared with the experimental data of the activity coefficients in the literature. It is shown that this model can accurately correlate the experimental data for activity coefficients. The models were also used to correlate the solubility of amino acids in aqueous solutions. The results show that this model can accurately correlate the solubility experimental data over a wide range of temperature. The values for Δh, Δs and Δg of the solutions were calculated from the results of this model.

Key words: Phase Behavior, amino acid, peptide, modified Wilson model, aqueous solution, solubility

Introduction

Separation processes based on precipitation and crystallization has proven to be useful techniques for the concentration and separation of various biomolecules, such as amino acids (Belter *et al* .,1998). Amino acids are the basic units of proteins. Nass developed a model for the activity coefficient of many amino acids using the chemical reaction equilibrium constant of amino acids in aqueous solution (Nass, 1998). Gupta and Heidemann (1990) used the modified UNIFAC (Larsen *et al*., 1987) model to evaluate of the activity coefficients of amino acids in aqueous solutions and used some group parameters in predicting the solubility of antibiotic in water. Chen *et al.* (1989) modeled the activity coefficient of amino acids using the electrolyte NRTL model (Renon and Prausnitz, 1968; Chen *et al.,* 1986). Khoshkbarchi and Vera (1996,1998) developed a perturbed hard-sphere model for the activity coefficient of amino acids in aqueous solutions. Recently Pazuki *et al.* (2005) proposed a new model based on the perturbation theory to predict the activity coefficients and solubility of amino acids in pure water. In this research the modified Wilson model that predicted by Novak *et al.* (1987) were used to correlate the experimental activity coefficients data of amino acids and simple peptides. The model was also used to correlate the solubility of amino acid in aqueous solution over a wide temperature range. The values for Δh, Δs and Δg of the solutions were calculated from the results of this model.

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The Modified Wilson Model

Among several forms of the Wilson model modified for binary partially miscible mixtures, Novak *et al*. (1987) proposed the following form:

$$\frac{g^E}{RT} = -x_1 \ln(x_1 + x_2\Lambda_{12}) - x_2 \ln(x_2 + x_1\Lambda_{21}) + b_{12}x_1x_2 \quad (1)$$

where, b_{12} is the empirical parameter.The binary parameter is expressed in terms of molar energy parameter and the molar volumes of pure components and:

$$\Lambda_{ij} = \left(\frac{v_j}{v_i}\right)\exp\left(-\frac{a_{ij}}{T}\right) \quad (2)$$

A new ternary extended form of Eq. 1, which is not identical with the ternary modification proposed by Nagata (1995), has shown good applicability in the correlation of ternary liquid-liquid equilibrium. The activity coefficient for component (1) in binary mixture obtained from Gibbs energy of modified Wilson model as below:

$$\ln\gamma_1 = \frac{1}{RT}\left(\frac{\partial ng^E}{\partial n_1}\right)_{p,T,n_2} \quad (3)$$

Then activity coefficient of component 1 is given by:

$$\ln\gamma_1 = -\ln(x_1 + x_2\Lambda_{21}) + x_2\left[\frac{\Lambda_{12}}{x_1 + x_2\Lambda_{12}} - \frac{\Lambda_{21}}{x_1\Lambda_{21} + x_2}\right] + b_{12}x_2^2 \quad (4)$$

where, in Eq. 4 X_1 and X_2 are mole fraction of component 1 and 2, respectively.

Evaluation of the Parameters

The modified Wilson models were employed to correlate the experimental data of water-amino acids systems. For aqueous solutions of amino acids the data reported in Handbook of Biochemistry and Molecular Biology was used (Fasman, 1976). This model contains three adjustable parameters and for prediction the activity coefficient with this model we assumed. $\Lambda_{12} = \Lambda_{21}$. Therefore, this model contains two parameters Λ_{12} and b_{12}. Subscripts 1 and 2 refer to amino acid and water, respectively.

Parameters of amino acids and peptides were evaluated by fitting the experimental activity data. For all the cases, the following objective function was used:

$$OF = \sum(\gamma_i^{exp} - \gamma_i^{cal})^2 \quad (5)$$

Results and Discussion for the Activity Coefficient

The activity coefficients of amino acids and peptide should be converted from the molality scale to the mole fraction scale base using the following relation (Khoshkbarchi and Vera,1998):

$$\gamma^{(x)} = \gamma^{(m)}\left(1 + 0.001 M_w m\right) \tag{6}$$

Where, Mw is the molar mass of solvent and superscripts and, represent the molality and mole fraction scale, respectively.

For prediction of the activity coefficients of amino acids and peptides, considered the dissociation of amino acids in pure water is insignificant and the zwitterionic species are predominant, no term has been introduced in binary amino acid-water model to take into account the presence of ionic species in solution. The consideration of the terms that take into account the effect of ionic forms of amino acid molecules other than their zwitterionic form only makes the modeling more complex. Since the available experimental data for the activity coefficient of amino acids and peptides do not include the ionization of amino acids and peptides in water, consideration of the ionization term seems unnecessary at this point. The Fig. 1 and 2 shows that this model can accurately correlate the experimental activity coefficient data of amino acids and peptides in aqueous solutions. The binary interaction energy of model and the root mean square deviation of the correlation of the experimental data for amino acids and peptides are presented in Table 1. The root mean square deviations (rms) of this model are compared with those obtained by Chen *et al*. (1989), Gupta and Heidemann (1990), Pinho *et al*. (1994) and Khoshkbarchi and Vera, (1996,1998). These results show that the modified Wilson model a more accurate correlation than other models for the amino acids and peptides in aqueous solutions.

Solubility of Amino Acids in Water

When an amino acid dissolved in water, solid-liquid equilibrium of the amino acids is given by terms of fugacities:

$$f_i^L = f_i^s \tag{7}$$

By definition:

$$f_i^L = x_i \gamma_i f_i^0 \tag{8}$$

where, X_i is the mole fraction of the amino acid, γ_i is the unsymmetrical activity coefficient of amino acid based on mole fraction scale and f_i^0 is the fugacity of standard state corresponding to the activity coefficient approaching unity as the mole fraction of approaches zero. Thus:

$$x_i \gamma_i f_i^0 = f_i^s \tag{9}$$

The ratio f_i^s / f_i^0 is related to the standard Gibbs free energy change in the dissolving process with the following relation:

$$\Delta g = \Delta h - T\Delta s = -RT\ln\frac{f_i^s}{f_i^0} \tag{10}$$

Therefore:

$$\frac{f_i^s}{f_i^0} = \exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \tag{11}$$

Combining Eq. 9 and 11, one obtains:

$$x_i\gamma_i = \exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \tag{12}$$

Knowing the activity coefficient, Eq. 12, relates the solubility of amino acids to the temperature.

Results of Solubility of Amino Acids in Water

Equation 12 can predict the solubilities of amino acids in water at various temperatures by use of the adjustable parameters presented in Table 1.

Table 1: Parameters of the modified Wilson model for amino acids and peptides and the root mean square deviation

Amino acid	Λ_{12}	b_{12}	rms*100[a]	rms*100[b]	rms*100[c]	rms*100[d]	rms*100[e]	rms*100[f]
Alanine	0.4958	-1.2048	0.016	0.51	0.33	0.04	8.97	0.19
Glycine	0.1548	-2.7372	0.761	0.84	0.86	2.07	4.2	1.67
Proline	5.0879	5.5682	2.517	7.12	4.12	1.30	3.01	1.21
Valine	4.3727	4.8460	0.056	0.45	0.05	4.47	3.32	0.24
Threonine	0.4448	-1.3754	0.158	0.25	0.15	0.70	13.78	26.12
Serine	0.1069	-3.1445	0.232	1.19	1.16	2.80	12.15	16.43
Alanylalanine	3.5568	3.7818	0.403	0.40	0.43	2.04		0.52
Alanylglycine	0.1615	-2.7221	0.582	0.71	0.6	2.92		28.22
Glycilglycine	0.0779	-3.5128	0.925	2.81	0.6	2.44		28.22
Triglycine	0.0430	-4.1365	0.000		0.00	0.67		10.0

[a]This work; [b] Khoshkbarchi and Vera (1998); [c] Khoshkbarchi and Vera (1996); [d] Chen *et al.* (1989); [e] Gupta and Heidemann (1990); [f] Pinho *et al.* (1994).

Table 2: Regressed values of parameter for the modified Wilson model of several amino acids in aqueous solutions and the root mean square

Amino Acid	$\Delta h/R$	$\Delta s/R$	rms[a]	rms[b]	$\Delta h/R$[c]	$\Delta h/R$[b]	$\Delta h/R$[c]
L-Alanine	1041.7631	0.0608	0.058	0.2403	920.9	988.37	-0.2174
DL-Alanine	1313.2956	0.9676	0.128	0.2656	1107.2	1115.07	0.2127
Glycine	1181.7524	0.8392	0.085	0.3059	1711.1	1654.55	2.4184
L-Proline	509.0017	0.9327	0.116	0.1821	654.2	959.77	2.6235
L-Serine	2022.6269	3.5793	0.136	0.2224		2663.05	5.7438
DL-Serine	2204.2459	2.5735	0.015	0.1746	2717.7	2265.35	2.7173
L-Valine	529.3769	-2.8677	0.003	0.0641	452.9	498.01	-2.8980
DL-Valine	1536.3313	0.6852	0.044	0.0885	754.9	1264.96	-0.1305

[a] This Work, [b] Khoshkbarchi-Vera (1998), [c] Fasman (1976)

Table 3: Calculated and experimental values of, $\Delta g/RT_0$, $T_o = 298.15$

Amino Acid	$\Delta g/RT_0$[a]	$\Delta g/RT_0$[b]	$\Delta g/RT_0$[c]
L-Alanine	3.348	3.352	3.431
DL-Alanine	3.437	3.527	
Glycine	3.124	3.131	3.165
L-Serine	3.204	3.188	3.168
DL-Serine	4.819	4.881	
L-Valine	4.643	4.555	4.833
DL-Valine	4.467	4.373	
L-Proline	0.774	0.595	0.271

[a]This Work, [b] Khoshkbarchi-Vera(1998), [c] Fasman (1974)

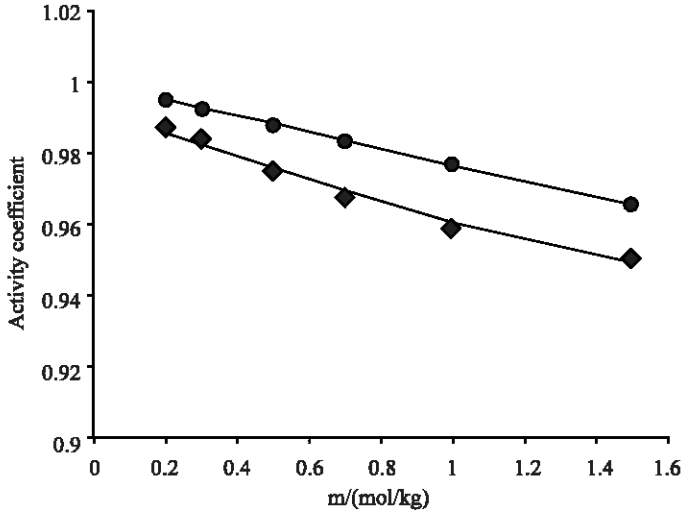

Fig. 1: Activity coefficient of the modified Wilson model for two amino acids plotted vs. molality: (–) result of the modified Wilson model; (◆) experimental data of Alanine; (●) experimental data of Theronine

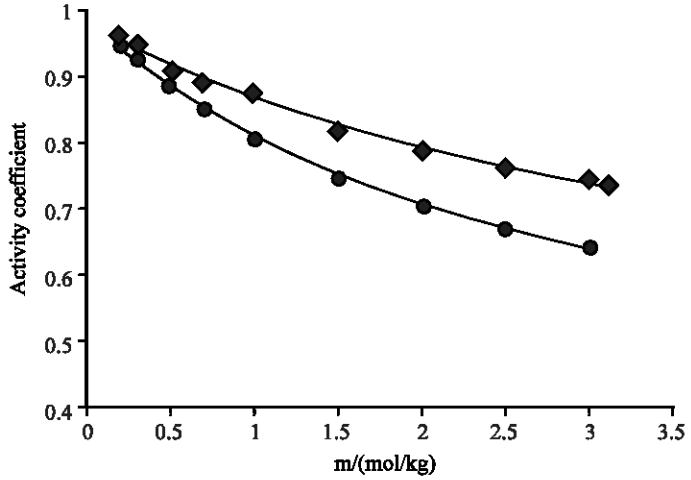

Fig. 2: Activity coefficient of the modified Wilson model for two amino acids plotted vs. molality: (–) result of the modified Wilson model; (◆) experimental data of Glycine; (●) experimental data of Serine

In Eq. 12 $\Delta s/R$ and $\Delta h/R$ are the standard entropy and enthalpy changes in aqueous solutions. These values are two adjustable parameters. In the calculation, the activity coefficients of optical isomers of amino acids are assumed to be equal. The results of calculation for the solubility of two amino acids with the modified Wilson model in aqueous solution are shown in Fig. 3. As shown in this figure this model can accurately predict the solubility of amino acids in aqueous solution over a wide

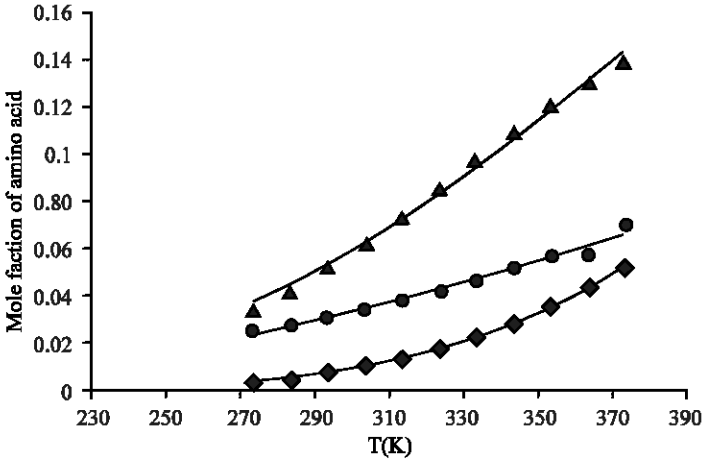

Fig. 3: Solubility of three amino acids for the modified Wilson model plotted vs. temperature: (–) result of the modified Wilson model; (◆) experimental data of DL-Serine; (▲) experimental data of Glycine; (●) experimental data of L-Alanine

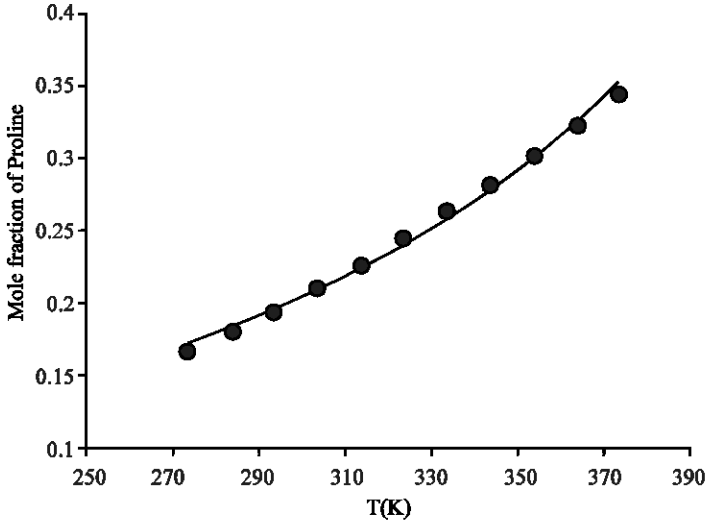

Fig. 4: Solubility of Proline plotted vs. temperature: (–) result of the modified Wilson model; (●) experimental data of Proline.

range of temperature. Figure 4 shows the correlation of Proline which is much more soluble in water than the other amino acids. As shown in this figure the accuracy of the modified Wilson model in correlating the experimental solubility data of Proline is as an amino acid in water. The value of $\Delta s/R$ and $\Delta h/R$ of Eq. 12 are presented in Table 2.

To make sure the capability of this model in predicting the other independent thermodynamic properties, it is interesting to compare the values for the dimensionless Gibbs free energy change of the solute. As seen in Table 3 the modified Wilson model accurately represents the values of $\Delta g/RT_0$ in comparison with the experimental data reported in the literature and the Khoshkbarchi-Vera model (1996).

Conclusions

The modified Wilson model has been applied for prediction of phase behavior of amino acids and peptides in pure water. The adjustable parameters of this model for amino acids and peptides were calculated by fitting the experimental activity coefficients of amino acids and peptides. Using these parameters for the activity coefficients, the solubilities of several amino acids in aqueous solution can be determined with the experimental values of standard enthalpy changes in solution. The predictions are acceptable a wide range of temperatures. The results of solubility of amino acids for Gibbs free energy change of the solute at temperature 298.15 K are in agreement with the experimental data available in the literature.

List of symbols

a: Molar energy parameter
b: Empirical parameter
f: Fugacity
g: Molar excess Gibbs free energy
m: Molality
M: Molar mass
OF: Objective function
rms: Root mean square
R: Universal constant of gas
T: Temperature
V: Molar volume of pure component
x: Mole fraction of liquid phase
Δg: Gibbs free energy change in the solution
Δh: Enthalpy energy change in the solution
Δs: Entropy energy change in the solution

Greek letters
γ: Activity coefficient
Λ: Binary interaction for Wilson model

Subscripts
i,j: Species
A: Amino acid
W: Water

Superscripts
exp: Experimental
cal: Calculation
°: Reference
L: Liquid
S: Solid
x: Mole fraction scale
m: Molality scale

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