Korean J. Chem. Eng., **27**(1), 267-277 (2010)
DOI: 10.1007/s11814-009-0351-z

Study on the activity coefficients and solubilities of amino acids in aqueous solutions with perturbed-chain statistical associating fluid theory

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(*Received 24 June 2009 • accepted 16 October 2009*)

Abstract−Perturbed-chain statistical associating fluid theory (PC-SAFT) was applied for modeling the thermodynamic properties of aqueous amino acid solutions. To account for the association phenomena of amino acids occurring in the aqueous solution, the zwitterionic forms of amino acids are assumed to be associating species with proton donor and acceptor sites. Also, in order to reduce the number of adjustable parameters of PC-SAFT equation of state (EoS) for amino acids from five to three, it is assumed that segment numbers of amino acids are linearly related with the molecular weight of amino acids, and the association volume parameters of amino acids can be set to a fixed value. Thus, 3-parameters of PC-SAFT EoS for amino acids were estimated by simultaneously fitting the activity coefficients of amino acid and densities data in the aqueous amino acid solutions. The PC-SAFT EoS with estimated 3-parameters of amino acid is found to well describe activity coefficients of amino acid and densities of the aqueous amino acid solutions. Also, this equation was used for predicting solubilities of amino acids as well as the water activities and osmotic coefficients in the aqueous amino acid solutions. The predicted values of these properties are in good agreement with the experimental data.

Key words: Amino Acids, Densities, Activity Coefficients, Solubilities, PC-SAFT EoS

INTRODUCTION

In the industrial synthesis processes of biological materials, many biochemicals such as amino acids and peptides are produced in a very complex aqueous media of which the concentration of products is dilute. Thus, the studies in biotechnology have drawn much attention to the development of efficient processes for the separation, concentration, and purification of bioproducts [1,2]. To design and scale-up bioseparation processes, it is essential to know the thermodynamic properties of bioproduct mixtures such as activity coefficients, solubilities of biochemicals and densities in the aqueous systems. Although amino acids have the simplest molecular structures among many biochemicals, they have many similarities with more complex biochemicals, and then are substances of importance for understanding the fundamental physical properties of biochemical systems. Therefore, the prediction of activity coefficients, solubilities of amino acids and densities in aqueous amino acid solutions have been the subject of many researches.

Until now, a number of studies have focused on the modeling of activity coefficients and solubilities of amino acids in aqueous solutions, and these studies have been mainly performed in two distinct approaches: the excess Gibbs free-energy (g^E) approach and residual Helmholtz free energy approach (or equation of state (EoS) approach). In the g^E approach, since Nass [3] modeled the activity coefficients of amino acids in aqueous solutions by introducing equilibrium constants of amino acid ionic species, based on the chemical theory, various types of the local composition method and group contribution method, such as modified Wilson [4], NRTL [5], electrolyte-NRTL [6], UNIQUAC [7], and UNIFAC [8-10] equations, have been applied for modeling the activity coefficients of amino acids in aqueous solutions. In some cases [6,7,9,10] of these models, the long range electrostatic interaction term was introduced to account for the activity coefficients of different ionic species formed from the ionization of amino acid molecules. Recently, Pazuki et al. [11] correlated the activity coefficients and solubilities of amino acids in aqueous solutions, by using the free-volume group contribution model. On the other hand, Khoshkbarchi and Vera [12,13] proposed the residual Helmholtz free energy model based on the perturbation theory for the system containing amino acids, to correlate activity coefficients of amino acids in aqueous solutions. In their model, the contribution of the reference system to the residual Helmholtz free energy was represented by the equation of state for hard-spheres proposed by Mansoori et al. [14], and in the perturbation term, the contribution of dispersion and dipole-dipole interactions were considered by using the first-order Barker-Henderson theory [15]. Liu et al. [16], assuming that amino acids and peptides are of chain-like molecules, incorporated the hard-sphere chain term into the model of Khoshkbarchi and Vera [12]. Mortazavi-Manesh et al. [17] also used, for the reference term, the equation of Ghotbi and Vera [18] instead of the equation of Mansoori et al. [14], in order to correlate activity coefficients of amino acids and simple peptides. In their work, the results of their model were compared with those of several other models [6,9,11,12]. Pazuki et al. [19] attempted to extend the perturbed hard-chain theory (PHCT) developed by Beret and Prausnitz [20] to the modeling of solubilities and activity coefficients of amino acids in aqueous solutions. Besides, Park, et al. [21] extended their own model known as hydrogen-bonding lattice fluid

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equation of state (NLF-HB) [22] to the correlation of activity coefficients in the amino acid/water mixtures. Recently, the statistical associating fluid theory (SAFT) [23,24] has been utilized for the modeling of thermodynamic properties in the biochemical mixtures. The extension of the SAFT to the amino acid solutions seems to be a significant attempt, as the SAFT has a merit that associations (hydrogen bond) of amino acids can be taken into account, based upon the explicit theoretical frameworks. Ji et al. [25] applied the SAFT EoS (the version of Hung and Radosz [26]) to the description of densities and water activities of aqueous amino acid solutions. Fuchs et al. [27] modeled the solubility of amino acid in water-alcohol mixtures at different pH values, by using the perturbed-chain SAFT (PC-SAFT) EoS [28]. More recently, Cameretti and Sadowski [29] applied the PC-SAFT EoS to the predictions of vapor pressures and densities of aqueous amino acid and polypeptide solutions. In the above works based upon the SAFT, the amino acids have been assumed as the associating species with the proton donor and acceptor sites, to account for the non-ideality due to the association of amino acids in the aqueous solution.

In view of the extensions of SAFT-family EoSs to the aqueous amino acid solutions carried out by Ji et al. [25], Fuchs et al. [27] and Cameretti and Sadowski [29] as stated above, these have been limited to the correlations of solubility, density and vapor pressure, but have not considered the activity coefficients of amino acids that are essentially needed for designs of separation process of amino acids. Therefore, the present work focuses on the modeling of the activity coefficients of amino acids as well as densities in the aqueous amino acid solutions by using the PC-SAFT EoS. Thus, parameters of PC-SAFT EoS are estimated from the simultaneous fitting of activity coefficients of amino acids and densities in aqueous amino acid solutions. Also, the solubilities of amino acids in water over the temperature range of 273.15-373.15 K are correlated. Furthermore, the influence of pH values on the solubilities of amino acids is predicted, and solubilities of two amino acids in water, which have been rarely treated in other works, also are predicted. The predicted solubilities of two amino acids are compared with the experimental data available in the literature.

THERMODYNAMIC BACKGROUND

Most amino acids are widely recognized to exist largely as the zwitterionic species (NH_3^+-CHR-COO^-) in aqueous solutions at intermediate pH values. As the zwitterionic species have both a positively charged ammonium group (-NH_3^+) and a negatively charged carboxylate group (-COO^-), these maintain the electrical neutrality but have a strong dipole moment owing to their dipolar structure. In addition, both ammonium and carboxylate groups in a zwitterionic form of amino acid molecule are known to be able to solvate with water molecules and also associate between each other. The solvation (or hydration) and self-association phenomena of amino acid molecules play an important role in the phase behavior of aqueous amino acid solutions, and then much theoretical effort has been devoted to interpreting these phenomena [30]. Therefore, in this work, the zwitterions of amino acid molecules are assumed as associating species with proton donor and acceptor sites, and also neutral chain-like species composed of several spherical segments with equal size. Then, the Helmholtz free energy of the mixture including chain-like species can be obtained from the PC-SAFT EoS [28] expressed such as:

$$A^{res}/NkT = A^{hc}/NkT + A^{disp}/NkT + A^{assoc}/NkT \quad (1)$$

Here, the hard-sphere chain term, A^{hc}/NkT is given by

$$A^{hc}/NkT = A^{hs}/NkT + \sum_i x_i(1-m_i)\ln g_{ii}^{hs} \quad (2)$$

where the first term of right hand side, A^{hs}/NkT, is the hard-sphere term, the second term refers to the formation of chain, and g_{ii}^{hs} is the radial pair distribution for segments of component i in the hard sphere mixtures. The A^{hs}/NkT and g_{ii}^{hs} can be obtained from the expressions of Boublik [31] and Mansoori [14] such as:

$$A^{hs}/NkT = \frac{6}{\pi\rho}\left[\frac{\xi_2^3+3\xi_1\xi_2\xi_3-3\xi_1\xi_2^3}{\xi_3(1-\xi_3)^2} - \left(\xi_0 - \frac{\xi_2^3}{\xi_3^2}\right)\ln(1-\xi_3)\right] \quad (3)$$

$$g_{ij}^{hs} = \frac{1}{(1-\xi_3)} + \left(\frac{d_{ii}d_{jj}}{d_{ii}+d_{jj}}\right)\frac{3\xi_2}{(1-\xi_3)^2} + \left(\frac{d_{ii}d_{jj}}{d_{ii}+d_{jj}}\right)^2\frac{2\xi_2^2}{(1-\xi_3)^3} \quad (4)$$

where ξ_0, ξ_1, ξ_2 and ξ_3, are defined as:

$$\xi_n = \frac{\pi}{6}\rho\sum x_i m_i d_i^n \quad n \in 0,1,2,3 \quad (5)$$

The temperature-dependent diameter of segment, d_i is represented by

$$d_i = \sigma_i[1-0.12\exp(-3\varepsilon_i/kT)] \quad (6)$$

where ε_i and σ_i denote the dispersion interaction energy between segments of chemical component i, and the temperature-independent segment diameter of component i, respectively. Also, the dispersion Helmholtz free energy of chain molecules in the mixture, A^{disp}/NkT, of Eq. (1) is expressed as [28]:

$$A^{disp}/NkT = A_1^{disp}/NkT + A_2^{disp}/NkT \quad (7)$$

$$A_1^{disp}/NkT = -2\pi\rho I_1(\eta,\bar{m})\sum_i\sum_j x_i x_j m_i m_j\left(\frac{\varepsilon_{ij}}{kT}\right)\sigma_{ij}^3 \quad (8)$$

$$A_2^{disp}/NkT = -\pi\rho\bar{m}C_1 I_2(\eta,\bar{m})\sum_i\sum_j x_i x_j m_i m_j\left(\frac{\varepsilon_{ij}}{kT}\right)^2\sigma_{ij}^3 \quad (9)$$

Here, σ_{ii} and ε_{ii} refer to the segment parameters of pure component i, thus $\sigma_{ii}=\sigma_i$ and $\varepsilon_{ii}=\varepsilon_i$. Also, parameters between unlike segments of different components, σ_{ij} and ε_{ij}, are obtained by

$$\sigma_{ij} = \frac{1}{2}(\sigma_{ii}+\sigma_{jj}) \quad (10)$$

$$\varepsilon_{ij} = \sqrt{\varepsilon_{ii}\varepsilon_{jj}}(1-k_{ij}) \quad (11)$$

where k_{ij} is the interaction parameter between unlike segments of component i and j. $I_1(\eta, \bar{m})$, $I_2(\eta, \bar{m})$ and C_1 of Eqs. (8) and (9) are in detail explained in the original paper of PC-SAFT EoS [28], and thus are omitted in this work.

Meanwhile, for defining the association term A^{assoc}/NkT of Eq. (1), it is first of all required to assign the association sites of zwitterions of amino acid and solvent (water) molecules. A water molecule is assumed to have four associating sites, two of which represent lone electron pairs of oxygen atom and the others represent protons: i.e., 4C-type [26]. However, for the case of amino acids, it is

needed to get a clue which might lead to the specification of association scheme, i.e., number of association sites and type of association. In recent years, there have been many advances in the research on association behaviors of amino acids by using the molecular simulation method such as Monte Carlo (MC) or molecular dynamics (MD) techniques [32-35]. Tunon et al. [32] reported that 2.8 water molecules are bound to a nitrogen atom of zwitterionic form of glycine and 2.3 water molecules also are bound to the oxygen atoms of zwitterion. Chang et al. [33] reported that hydration numbers range from 3.2 to 3.4 for the ammonium H of zwitterion and from 5.7 to 5.9 for the carboxylate O over several amino acids like glycine, serine, threonine, tyrosine, etc. Besides, Rossi et al. [34] and Troitino et al. [35] elucidated the occurrence of self-association (hydrogen bond) between the ammonium H of zwitterion and the carboxylate O, through the molecular dynamic simulations. Apart from these molecular simulation studies, recently, Cameretti and Sadowski [29] assumed that each of NH_2 and COOH groups in amino acid has two proton donor sites and two acceptor sites, respectively. The molecular simulation methods provide the meaningful bases in explicitly interpreting association behaviors and structures of amino acids. However, in this work, for the sake of the modeling of equation of state available for engineering calculations, it is assumed that the ammonium group ($-NH_3^+$) and carboxylate group ($-COO^-$) of zwitterion of amino acid have two proton donor sites and two acceptor sites, respectively. This assumption can be noted to be similar to the work of Cameretti and Sadowski [29]. Hence, the Helmholtz free energy due to the solvation (hydration) and self-association of zwitterions of amino acids in aqueous solutions can be obtained from the SAFT model [23,24].

$$A^{assoc}/NkT=\sum_i x_i\left[\sum_{A_i}\left[\ln X^{A_i}-\frac{X^{A_i}}{2}\right]+\frac{1}{2}M_i\right] \tag{12}$$

$$X^{A_i}=\left[1+\rho\sum_i x_i\sum_{B_j}X^{B_j}\Delta^{A_iB_j}\right]^{-1} \tag{13}$$

$$\Delta^{A_iB_j}=g_{ij}^{hs}(d_{ij})\left[\exp\left(\frac{\varepsilon^{A_iB_j}}{kT}\right)-1\right]\sigma_{ij}^3\kappa^{A_iB_j} \tag{14}$$

where X^{A_i} is the mole fraction of component not bonded at site A, $\Delta^{A_iB_j}$ and is the association strength between site A on component i and site B on component j. The cross-association parameters, $\varepsilon^{A_iB_j}$/k and $\kappa^{A_iB_j}$, between the site A of component i and site B of component j are estimated by the combining rules proposed by Wolbach and Sandler [36].

$$\varepsilon^{A_iB_j}=\frac{\varepsilon^{A_iB_i}+\varepsilon^{A_jB_j}}{2}\quad\text{and}\quad\kappa^{A_iB_j}=\sqrt{\kappa^{A_iB_i}\kappa^{A_jB_j}}\left[\frac{\sqrt{\sigma_{ii}\sigma_{jj}}}{(\sigma_{ii}+\sigma_{jj})/2}\right]^3 \tag{15}$$

The equation of state is obtained, from the thermodynamic model of Helmholtz free energy in the aqueous solutions described above, such as:

$$P/\rho kT=\frac{1}{V}\left(\frac{\partial A^{res}/kT}{\partial\rho}\right)_{N,T}=P^{hc}/\rho kT+P^{disp}/\rho kT+P^{assoc}/\rho kT \tag{16}$$

Also, the residual chemical potential of component i is evaluated from the partial derivate of Helmholtz free energy with respect to the number of molecules of component i.

$$\mu_i^{res}(T,V)/kT=\frac{1}{V}\left(\frac{\partial A^{res}/kT}{\partial N_i}\right)_{V,T,N_{i\neq k}}=\mu_i^{hc}/kT+\mu_i^{disp}/kT+\mu_i^{assoc}/kT \tag{17}$$

In the above Eqs. (16) and (17), the hard-sphere chain term $P^{hc}/\rho kT$ and μ_i^{hc}/kT, dispersion term $P^{disp}/\rho kT$ and μ_i^{disp}/kT, and association term $P^{assoc}/\rho kT$ and μ_i^{assoc}/kT are explained in detail elsewhere [28], thus not described in this paper.

Since the activity coefficient of non-volatile material like amino acid has to satisfy the unsymmetric condition in which the activity coefficient become unity as the mole fraction approaches zero; therefore the activity coefficient of amino acid is defined with the reference state of infinite dilute concentration [37].

$$\gamma_A=\frac{V^l_{m_A\to0}}{V^l}\exp\left[\frac{\mu_A^{res}(T,V)-\mu_A^{res}(T,V)_{m_A\to0}}{kT}\right] \tag{18}$$

The γ_A of Eq. (18) is the mole fraction-based activity coefficient, and is converted into the activity coefficient in molality scale by the following relation:

$$\gamma_A^{(m)}=\gamma_A/(1+0.001M_sm_A) \tag{19}$$

where m_A is the molality of amino acid and M_s is the molecular weight of solvent (water). Also the activity coefficient of water is expressed as:

$$\gamma_w=\frac{V^{l,o}}{V^l}\exp\left[\frac{\mu_w^{res}(T,V)-\mu_w^{res,o}(T,V)}{kT}\right] \tag{20}$$

and the water activity and osmotic coefficient are given by

$$a_w=x_w\gamma_w \tag{21}$$

$$\phi=-\frac{1000}{m_AM_s}\ln a_w \tag{22}$$

RESULTS AND DISCUSSION

1. Estimation of Parameters for Amino Acids: Correlations of Densities and Activity Coefficients

In this work, as mentioned earlier, the zwitterions of amino acids dissolved in the aqueous solution are assumed to be the neutral chain-like molecules consisting of identical hard spheres, and also to be the associating species which have two proton donor and two acceptor sites. Thus parameters of PC-SAFT EoS for amino acids are five, namely, the number of segments m_i, the segment diameter σ_i, dispersion energy parameter ε_i/k, and additional two association parameters, $\varepsilon^{A_iB_i}$/k and $\kappa^{A_iB_i}$. In general, parameters of EoS are estimated from the fitting of experimental saturated liquid density and vapor pressure data. However, for non-volatile chemicals such as amino acids and polymers, these experimental data are not available. Therefore, different methods have been attempted. Ji et al. [25] estimated 5-parameters of the SAFT EoS for amino acids from the critical properties (volume, pressure and temperature) and normal boiling temperature obtained by a group contribution method [38], whereas Fuchs et al. [27] calculated 5-parameters of the PC-SAFT EoS from the fitting of vapor pressure and density data of aqueous amino acid solutions. Also, Cameretti and Sadowski [29] determined 5-parameters of PC-SAFT EoS as well as the two solubility relating parameters, i.e., the melting enthalpies and melting temperatures,

from the simultaneous fitting of densities, vapor pressures and solubilities data of aqueous amino acid solutions. In the present work, with a view to the precise description of activity coefficients of amino acids as well as densities in aqueous amino acid solutions, the parameters of amino acid were estimated by simultaneously fitting activity coefficients of amino acid and densities data in aqueous amino acid solutions at 298.15 K. Hence, the objective function is as:

$$F_{obj}=\sum_{i}^{NP}[(\rho_{exp}-\rho_{cal})/\rho_{exp}]_i^2+\sum_{i}^{NP}[(\gamma_{A,exp}-\gamma_{A,cal})/\gamma_{A,exp}]_i^2 \quad (23)$$

In the regression procedures for estimating parameters of amino acids, the interaction parameter between amino acid and water molecule, k_{ij} of Eq. (11), was estimated together with five parameters of amino acid. The estimated 5-parameters for 12 amino acids chosen in this work are presented in Table 1. In the above procedures for estimating parameters of amino acids, pure water parameters needed to calculate activity coefficients of amino acids and densities in the aqueous solution, were used as the parameters obtained in the our previous work [39] in which aqueous electrolyte solutions were modeled based upon the PC-SAFT EoS incorporated with the primitive mean spherical approximation: refer to Table 1. As shown in Table 1, the correlated densities and activity coefficients of amino acids are well agreeable with the experimental data, with root mean square deviations (RMSDs) for densities and activity coefficients of amino acids which are less than about 0.3 and 0.8%, respectively. Among the estimated 5-parameters, the values of association volume $\kappa^{A_iB_i}$ parameter are in the narrow ranges from about 0.024 to about 0.036. Also, the estimated segment numbers m_i yield a tendency to be linear with the molecular weight, as shown in Fig. 1. The linearity of m_i against the molecular weight is given by

$$m_i=0.07552M_{w,i}-1.95459 \quad (24)$$

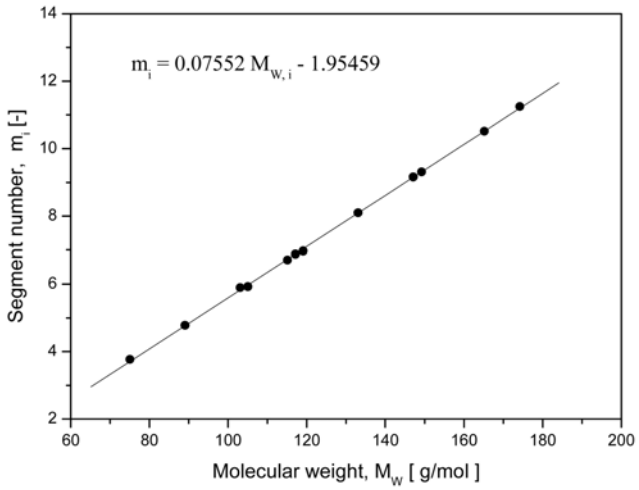

Fig. 1. The linear relation between the number of segments and molecular weight for all amino acids considered in the present work.

In order to reduce the number of parameters of amino acid, $\kappa^{A_iB_i}$ is assumed as a fixed value equal to 0.032, and also m_i is assumed to be suitable with the relation of Eq. (24). Under these conditions, 3 parameters of amino acids, σ_i, ε_i/k, and $\varepsilon^{A_iB_i}$/k, were estimated through the regression similar to the earlier procedure. The results are listed in Table 2. This table shows that the correlated densities and activity coefficients of amino acids are in good agreement with the experimental data, with average RMSDs of 0.050 and 0.211%, respectively. Also, in Table 2, the correlating results for activity coefficients of amino acids are compared with those carried out by Mortazavi-Manesh et al. [17], Pazuki et al. [19] and Pazuki et al. [11]. As can be seen from this comparison, the present model produces the ac-

Table 1. Estimated 5-parameters of amino acids and the correlating results of densities and activity coefficients of amino acids data at 298.15 K

Amino acids	M_w [g/mol]	Parameters of amino acid					k_{12}[a]	RMSD (%)[b]		Data source	
		m_i [-]	σ_i [Å]	ε_i/k [K]	$\varepsilon^{A_iB_i}$/k [K]	$\kappa^{A_iB_i}$ [-]		ρ	$\gamma_A^{(m)}$	ρ	γ_A
Glycine	75.07	3.7656	2.5780	181.42	3067.27	0.031965	−0.1340	0.126	0.157	[45]	[42]
DL-Alanine	89.09	4.7793	2.7034	311.99	3110.39	0.031929	−0.1032	0.293	0.317	[45]	[42]
L-Serine	105.09	5.9258	2.5067	363.90	3193.53	0.031082	−0.1110	0.028	0.315	[46]	[41]
DL-Proline	115.13	6.7117	2.6562	333.35	2146.37	0.023503	−0.1235	0.154	0.676	[45]	[41]
DL-Valine	117.15	6.8779	2.6773	324.86	3168.41	0.028659	−0.1223	0.050	0.242	[46]	[41]
DL-Threonine	119.12	6.9747	2.6955	333.91	3139.71	0.034062	−0.1083	0.143	0.121	[47]	[42]
DL-Methionine	149.21	9.3112	2.6300	336.52	3212.46	0.035245	−0.1175	0.044	0.827	[48]	[41]
Arginine	174.20	11.2470	2.5618	368.45	3270.91	0.036289	−0.1349	0.030	0.434	[45]	[52]
α-Amino-butyric acid	103.12	5.8963	2.7333	305.77	3437.60	0.030586	−0.1138	0.109	0.064	[46]	[42]
L-Aspartic acid	133.10	8.0965	2.6645	353.62	3156.69	0.033270	−0.1174	0.012	0.001	[49]	[41]
L-Glutamic acid	147.13	9.1582	2.4729	361.16	3288.48	0.030784	−0.1595	0.021	0.001	[50]	[41]
L-Phenylalanine	165.19	10.5149	2.6686	366.46	3265.31	0.031365	−0.1215	0.025	0.043	[51]	[41]
								V^l	P^{sat}	Reference	
Water (4C-type)		1.0175	3.0348	339.39	1538.43	0.031733	-	0.287	0.256	[39]	

[a] k_{12}: interaction parameter between water (1) and amino acid (2)

[b] $\text{RMSD}\ (\%)=100\times\sqrt{(1/N)\times\sum_{i}^{N}[(X_{exp}-X_{cal})/X_{exp}]_i^2}$, $X=\rho$, $\gamma_A^{(m)}$, V^l and P^{sat}

Table 2. Estimated 3-parameters of amino acids and the correlating results of densities and activity coefficients of amino acids data at 298.15 K, based on the conditions such as: m_i=0.07552$M_{w,i}$−1.95459 and κ^{A,B_i}=0.032

Amino acids	Parameters of amino acid			k_{12}	RMSD (%)[a]		RMSD (%)[b]	RMSD (%)[c]	RMSD (%)[d]
	σ_i [Å]	ε_i/k [K]	ε^{A,B_i}/k [K]		ρ	$\gamma_A^{(m)}$	$\gamma_A^{(m)}$	$\gamma_A^{(m)}$	$\gamma_A^{(m)}$
Glycine	2.5944	162.43	3147.38	−0.1527	0.126	0.162	0.66	0.083	0.66
DL-Alanine	2.6785	348.43	2989.28	−0.0988	0.015	0.065	0.03	0.004	0.07
L-Serine	2.5086	365.16	3200.30	−0.1114	0.041	0.319	0.24	0.089	1.53
DL-Proline	2.6534	334.97	2156.26	−0.1235	0.155	0.680	1.38	0.157	-
DL-Valine	2.6790	266.24	3121.53	−0.1466	0.032	0.046	1.06	0.013	0.06
DL-Threonine	2.5809	357.85	3586.19	−0.1219	0.033	0.139	0.09	0.013	0.25
DL-Methionine	2.5690	281.85	3023.14	−0.1464	0.015	0.641	-	-	0.00
Arginine	2.5614	368.38	3280.27	−0.1348	0.048	0.425	-	-	-
α-Amino-butyric acid	2.7334	305.61	3445.25	−0.1134	0.091	0.045	-	-	-
L-Aspartic acid	2.7005	327.87	3364.97	−0.1186	0.008	0.001	-	-	-
L-Glutamic acid	2.4726	362.32	3296.95	−0.1593	0.021	0.001	-	-	-
L-Phenylalanine	2.6291	347.30	3649.00	−0.1325	0.015	0.003	-	-	-
Overall average					0.050	0.211	0.58	0.060	0.43

[a]: this work; [b]: Mortazavi-Manesh et al. [17]; [c]: Pazuki et al. [19]; [d]: Pazuki et al. [11]

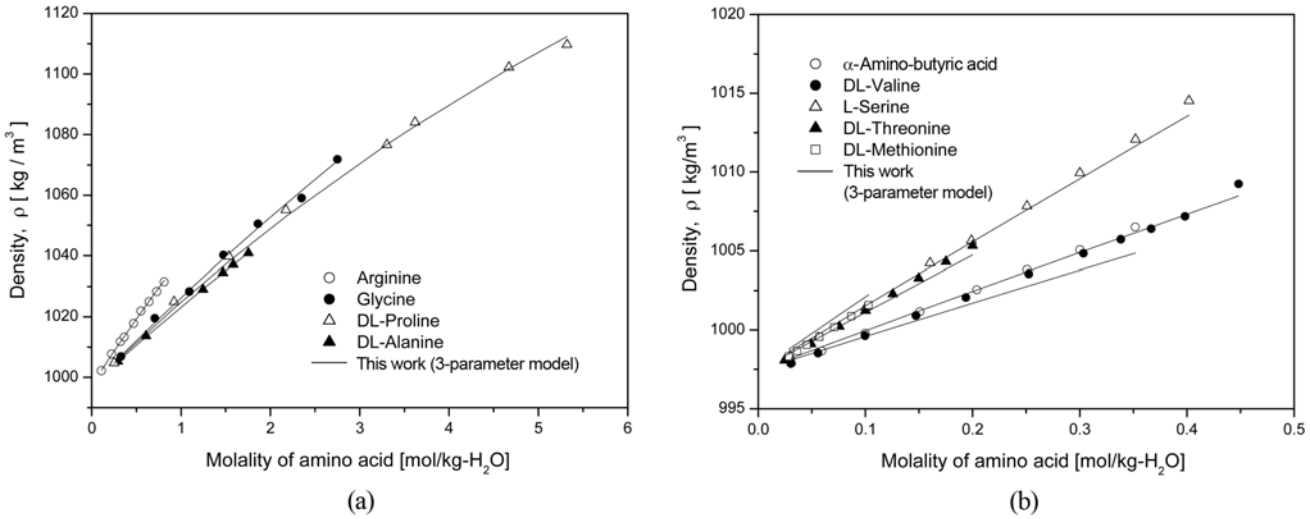

Fig. 2. (a) Correlated and experimental densities of aqueous amino acids solutions at 298.15 K: arginine, glycine, DL-proline and DL-alanine. (b) Correlated and experimental densities of aqueous amino acids solutions at 298.15 K: α-amino-butyric acid, DL-valine, L-serine, DL-threonine and DL-methionine.

curate correlations comparable to other models. While the correlating results of densities cannot be compared with those of different models, as RMSDs of densities for amino acid solutions considered in the present work have not been sufficiently reported in literatures. For clarity, the correlated densities of amino acid aqueous solutions and correlated activity coefficients of amino acids are compared with experimental data in Fig. 2 and 3, respectively. In these figures, the comparisons of L-Aspartic acid, L-Glutamic acid and L-Phenylalanine aqueous solutions in which both of RMSDs of densities and activity coefficients are very small as shown in Table 2 were omitted. As shown in these figures, it is found that the correlations of densities and activity coefficients of amino acids, for all aqueous solutions of amino acids considered in this work, agree well with the experimental data over the whole molality ranges of amino acid. Furthermore, to test whether the estimated 3-parameters of amino acids are useful or not for describing other thermodynamic properties of amino acid solutions, the water activities and osmotic coefficients in the amino acid solutions at 298.15 K were calculated from Eq. (21) and Eq. (22), respectively, using the estimated 3-parameters of amino acid. The predicted water activities and osmotic coefficients are compared with experimental data in Figs. 4 and 5, respectively. In these figures, the predicted values of water activities and osmotic coefficients show good agreement with the experimental data.

2. Solubilities of Amino Acids in the Aqueous Solutions

When amino acids dissolve in the aqueous solution, amino acid molecules are known to dissociate into several ionic species according to the values of pH in aqueous solution. The different ionic species

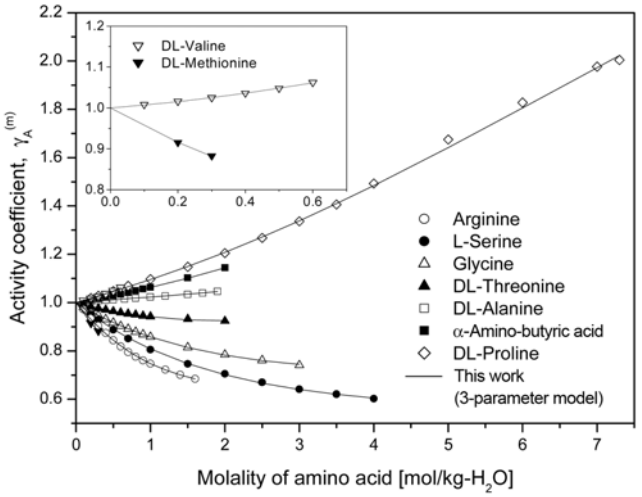

Fig. 3. Correlated and experimental activity coefficients of amino acid in the aqueous solutions at 298.15 K.

of amino acids are formed by the following reactions:

$$NH_2CHRCOOH \rightleftharpoons NH_3^+CHRCOO^- \qquad K_D \qquad (25)$$

$$NH_3^+CHRCOOH \rightleftharpoons H^+ + NH_3^+CHRCOO^- \qquad K_1 \qquad (26)$$

$$NH_3^+CHRCOO^- \rightleftharpoons H^+ + NH_2CHRCOO^- \qquad K_2 \qquad (27)$$

where K_D, K_1, and K_2 are the equilibrium constants, defined as:

$$K_D = \frac{[NH_3^+CHRCOO^-]}{[NH_2CHRCOOH]} \qquad (28)$$

$$K_1 = \frac{[H^+][NH_3^+CHRCOO^-]}{[NH_3^+CHRCOOH]} \qquad (29)$$

$$K_2 = \frac{[H^+][NH_2CHRCOO^-]}{[NH_3^+CHRCOO^-]} \qquad (30)$$

and the brackets indicate molality units. Almost amino acid molecules dissolved in the aqueous solution are known to convert to the zwitterion according to Eq. (25), since K_D has a very large value in the range of 10^5-10^6 [40]. In the aqueous solution, when a proton donor or acceptor agent is present, the zwitterions convert to a positively charged ion by gaining a proton such as Eq. (26), or to a negatively charged ion by losing a proton according to Eq. (27). Pinho et al. [9] have reported that the zwitterions are predominant in isoelectric solutions at which the isoelectric point is defined by pI= $(pK_1+pK_2)/2$, but for the value of pH much smaller than pI, positively charged ions become predominant, while negatively charged ions are dominant at values of pH much higher than pI. Therefore, the solubilities of amino acids are influenced with the value of pH in the aqueous solution.

The relation for solubilities of amino acids can be determined from the solid-liquid equilibrium for the amino acids. Khoshkbarchi and Vera [12] have proposed the relation for solubilities of amino acids, based upon the solid-liquid equilibrium relations for amino acids performed by Gupta [8], as the following equation:

$$x_A^s \gamma_A = \exp[\Delta s/R - \Delta h/RT]\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right] \qquad (31)$$

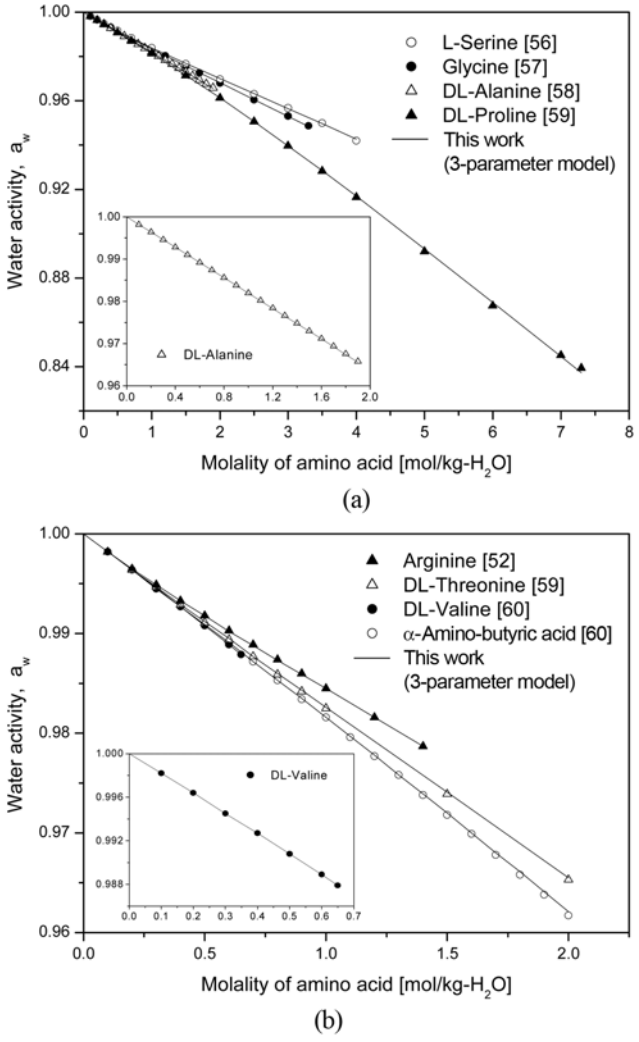

Fig. 4. (a) Predicted and experimental water activities of the aqueous amino acid solutions at 298.15 K: L-serine, glycine, DL-alanine and DL-proline. (b) Predicted and experimental water activities of the aqueous amino acid solutions at 298.15 K: Arginine, DL-threonine, DL-valine and α-amino-butyric acid.

where x_A^s and γ_A are the mole fraction and activity coefficient of amino acid in the saturated aqueous solution, respectively, and Δs and Δh are the change in the molar entropy and enthalpy of amino acid from the standard state of zwitterion to the solid state. Also, $[H^+]$ is the molality of hydrogen ion in the solution, and K_1 and K_2 refer to the equilibrium constant defined by Eqs. (29) and (30). In case of which amino acids dissolve in the neutral water, in other words, in the absence of a proton donor or acceptor, amounts of cationic and anionic amino acids formed by Eqs. (26) and (27) can be negligible. Then, Eq. (31) can be written as:

$$x_A^s \gamma_A = \exp[\Delta s/R - \Delta h/RT] \qquad (32)$$

In the above relation, provided that Δs and Δh are known, the solubility of amino acid based on the mole fraction, x_A^s, can be directly predicted from Eq. (32), as the activity coefficient of amino acid is determined from Eq. (18). However, since the reported values of Δs and Δh are not adequately available in the literature, these are

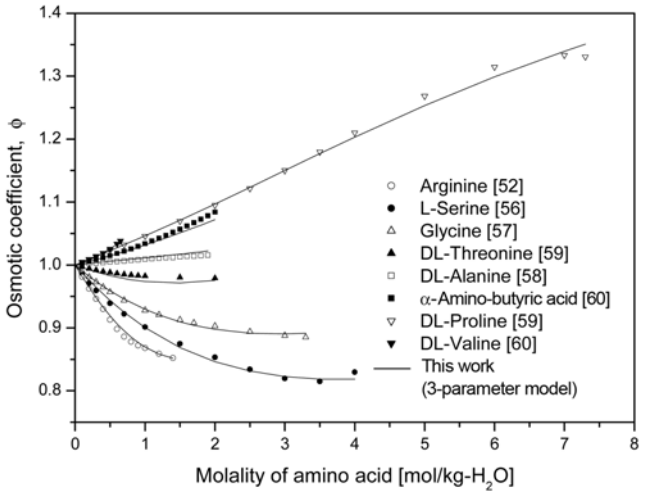

Fig. 5. Predicted and experimental osmotic coefficients of the aqueous amino acid solutions at 298.15 K.

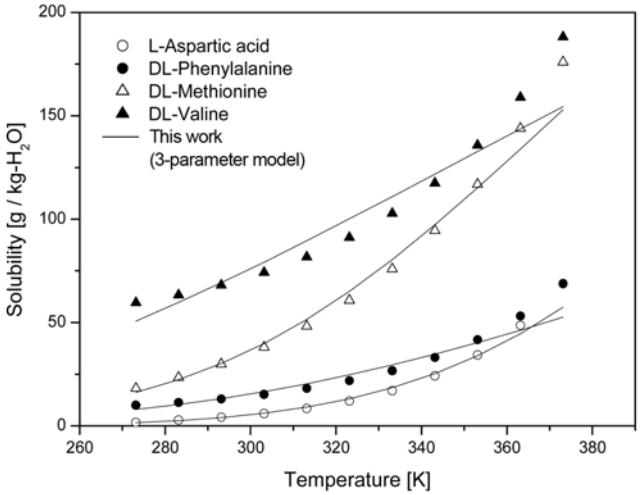

Fig. 6. Correlated and experimental solubilities of amino acid in the aqueous solutions.

often evaluated from the fitting of experimental solubilities data. In the present work, using the activity coefficient relations of amino acid with the estimated 3-parameters listed in Table 2, the experimental solubility data of amino acids in pure water at various temperatures ranged from 273.15 K to 373.15 K, collected in the CRC handbook [41], were regressed based upon the objective function such as:

$$F_{obj}=\sum_{i}^{NP}[(m^{s}_{A,exp}-m^{s}_{A,cal})/m^{s}_{A,exp}]^{2}_{i} \quad (33)$$

Here, m^{s}_{A} is the solubility of amino acid based on the molality. For the optical isomers of amino acid, i.e., L-type and DL-type, it is assumed that parameters of the present model, needed to calculate activity coefficients, are equal to each other. The values of Δs and Δh estimated through the above regression are listed in Table 3, and the comparisons of correlated solubilities of amino acids and experimental data also are presented in Table 3. The correlating results of other models carried out by several authors are also listed in Table 3. In this table, the solubilities of amino acids correlated from the present model with estimated 3-parameters exhibit a little better agreement with experimental data than the results of other models. In Fig. 6, the correlated solubilities with the large RMSD greater than about 6.0% are compared with the experimental data. The correlated solubilities of DL-valine, DL-phenylalanine, DL-methionine and L-aspartic acid are largely deviated with the experimental data at higher temperatures than 360 K. Meanwhile, to make sure of the soundness of estimated values of Δs and Δh, the Gibbs free energy changes of amino acid solutions at 298.15 K were calculated from the estimated values of Δs and Δh, by using the relation proposed by Khoshkbarchi and Vera [12]. They reported that the Gibbs free

Table 3. Estimated values of Δh/R and Δs/R, and correlating results for the solubilities of amino acids over the temperatures ranged from 273.15 K to 373.15 K

Amino acids	Δh/R	Δs/R	RMSD (%)[a]	RMSD (%)[b]	RMSD (%)[c]	RMSD (%)[d]
Glycine	1766.63	2.8108	1.49	6.27	2.1	8.4
L-Alanine	1345.23	1.1899	4.24	3.99	3.1	5.0
DL-Alanine	1639.32	2.1763	5.33	5.60	4.0	6.0
L-Serine	2660.21	5.7713	5.81	7.78	8.2	1.4
DL-Serine	2822.74	4.6538	3.34	3.75	5.5	7.1
L-Proline	1680.71	5.4060	0.65	3.81	4.4	1.9
L-Valine	480.51	−3.0488	1.69	1.67	9.1	0.8
DL-Valine	1371.08	0.1887	9.47	10.84	9.7	10.8
L-Methionine	1405.08	−0.4266	1.02	-	-	-
DL-Methionine	2364.38	2.3557	6.70	-	-	-
L-Aspartic acid	3755.95	5.2978	8.30	-	-	-
DL-Aspartic acid	3689.20	5.5304	1.44	-	-	-
L-Glutamic acid	3416.68	4.6627	5.71	-	-	2.1
DL-Phenylalanine	2050.80	0.4505	12.50	-	-	0.1
Overall average			4.84	5.46	5.76	4.36

[a]This work; [b]Mortazavi-Manesh et al. [17]; [c]Pazuki et al. [19]; [d]Pazuki et al. [11]

Table 4. Calculated and experimental values of $\Delta g/RT_o$, T_o=298.15 [K]

Amino acids	$\Delta g/RT_0$[a]	$\Delta g/RT_0$[b]	$\Delta g/RT_0$[c]	$\Delta g/RT_0$[d]	$\Delta g/RT_0$[e]
Glycine	3.114	3.110	3.754	3.119	3.165
L-Alanine	3.322	3.304	3.485	3.407	3.431
DL-Alanine	3.322	3.353	5.528	3.400	-
L-Serine	3.151	3.152	3.970	4.857	3.168
DL-Serine	4.814	4.901	4.906	4.838	-
L-Proline	0.231	–0.091	2.861	0.855	0.271
L-Valine	4.660	4.633	4.778	4.642	4.833
DL-Valine	4.410	4.448	4.596	4.392	-
L-Methionine	5.139	-	-	-	5.124
DL-Methionine	5.574	-	-	-	-
L-Aspartic acid	7.300	-	-	-	7.494
DL-Aspartic acid	6.843	-	-	-	-
L-Glutamic acid	6.797	-	-	5.862	7.004
DL-Phenylalanine	6.428	-	-	–5.392	-

[a]: this work; [b]: Mortazavi-Manesh et al. [17]; [c]: Pazuki et al. [19]; [d]: Pazuki et al. [11]; [e]: experimental values obtained based on the right hand side of Eq. (34), from the Δg_F^o reported by Fasman [41]

energy change, for transferring 1 mole of amino acid from the saturated solution to a hypothetical aqueous solution at an activity of 1 m and at T_0=298.15 K, Δg_F^o, is related to Δs and Δh of Eq. (32) as the following:

$$\frac{\Delta h}{RT_0} - \frac{\Delta s}{R} = \frac{\Delta g_F^o}{RT_0} + 4.016 \tag{34}$$

Here, the left hand side of Eq. (34) denotes the value of $\Delta g/RT_o$ calculated from the Δs and Δh obtained in this work and the right hand side represents the experimental value of $\Delta g/RT_o$ which can be obtained from the value of Δg_F^o reported by Fasman [40]. The calculated Gibbs free energy changes are compared with the experimental values, as well as with those obtained by other authors, in Table 4. In this table, the results of the present work are shown to be somewhat more agreeable with experimental values than results of other authors. Further, the solubilities of several amino acids at different pH values were predicted by using Eq. (31). In Fig. 7(a), the solubilities of glycine, DL-alanine and L-valine predicted at various pH values and 298.15 K are compared with experimental data. Also, in Fig. 7(b), the prediction of the effect of pH and temperature on the solubilities of DL-methionine are shown. As shown in these figures, the solubilities of amino acids predicted in the present work are found to be in good accord with experimental data. In the above predictions of solubilities of amino acids, equilibrium constants, K_1 and K_2 of Eq. (31), are available in literature data [42] listed in Table 5.

Furthermore, we predicted the solubilities of two amino acid mixtures in water. The solubilities of an amino acid in the two amino acid mixtures are influenced by another amino acid in the solution. These changes of solubility are caused by interactions between two amino acid molecules with different chemical structures [43]. In the ternary solution composed by two amino acids and water, the solubility of an amino acids (A_1) in the aqueous solution containing other amino acids (A_2) can be described from the following solid-liquid relation based upon Eq. (32).

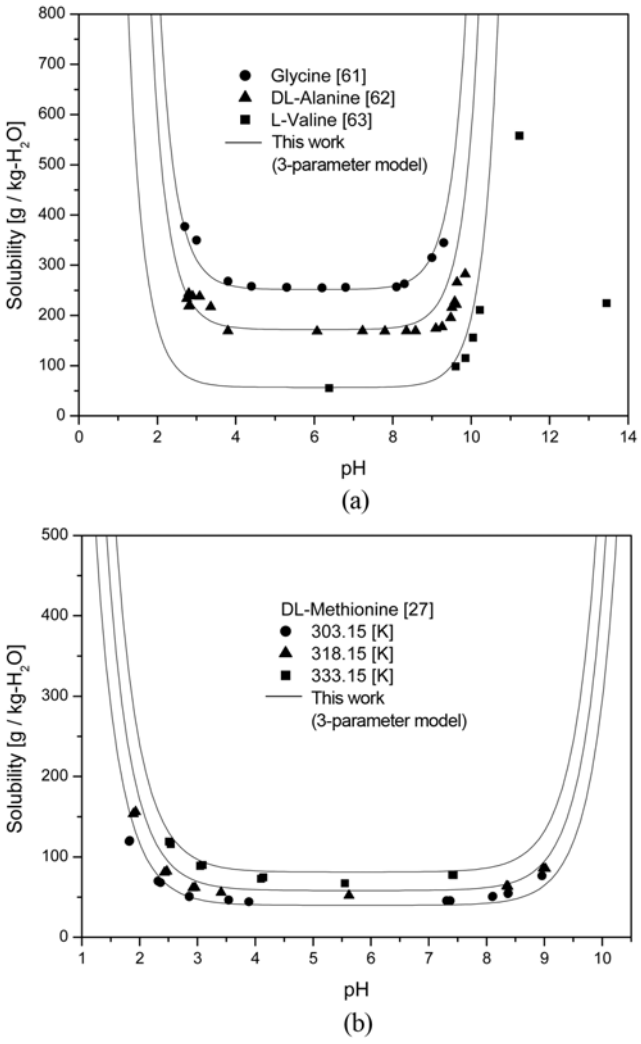

Fig. 7. (a) Predicted and experimental solubilities of amino acids at different pH values and 298.15 K: glycine, DL-alanine and L-valine. (b) Predicted and experimental solubilities of DL-methionine at different values of pH and various temperatures.

Table 5. Values of pK_1 and pK_2 for amino acids considered in this work [42]

Amino acids	pK_1	pK_2
Glycine	2.34	9.60
DL-Alanine	2.34	9.69
L-Valine	2.32	9.62
DL-Methionine	2.28	9.21

$$x_{A_1}^s \gamma_{A_1}(x_{A_1}^s, x_{A_2}, T) = \exp[\Delta s_{A_1}/R - \Delta h_{A_1}/RT] \tag{35}$$

where $\gamma_{A_1}(x_{A_1}^s, x_{A_2}, T)$ is the activity coefficient of amino acid A_1 in the saturated ternary solution. The estimated 3-parameters of amino acids presented in Table 2 can be directly applied to calculate the activity coefficients of each amino acid in the aqueous two amino acid solutions. However, for calculating activity coefficients of amino acids in the aqueous two amino acid solutions, it is needed to introduce an additional parameter, i.e., interaction parameter between

Table 6. Correlated results for solubilities of two amino acid aqueous solutions

Amino acid(A_1)/Amino acid(A_2)	Temp. [K]	$k_{A_1A_2}$	This work		Modified NRTL [44]		Data source
			RMSD (%) of $m^s_{A_1}$	RMSD (%) of $m^s_{A_2}$	RMSD (%) of $m^s_{A_1}$	RMSD (%) of $m^s_{A_2}$	
DL-Alanine/DL-Serine	298.15	0.0062	1.02	2.06	0.41	0.85	[53]
DL-Alanine/DL-Valine	298.15	–0.0028	0.95	1.42	1.09	1.16	[53]
Glycine/L-Glutamic acid	298.15	–0.1681	-	2.55	-	-	[54]
	298.15	–0.1681	-	2.64	-	6.25	[55]
	313.15	–0.1736	-	4.02	-	4.59	[55]
	333.15	–0.1837	1.44	2.23	0.64	7.42	[55]
Glycine/DL-Aspartic acid	298.15	–0.2021	0.28	6.06	0.84	9.49	[43]
Glycine/DL-Phenylalanine	298.15	–0.0526	2.04	4.22	1.97	5.64	[43]
L-Serine/L-Glutamic acid	298.15	–0.0468	2.79	5.49	2.17	16.39	[55]
	313.15	–0.0398	2.69	4.17	1.14	1.22	[55]
	333.15	–0.0370	0.64	5.09	1.91	21.87	[55]
L-Serine/L-Aspartic acid	298.15	–0.0771	2.18	8.68	1.31	19.08	[55]
	313.15	–0.0601	2.72	5.75	1.14	11.00	[55]
	333.15	–0.0573	0.84	3.43	2.01	11.50	[55]
L-Aspartic acid/L-Glutamic acid	298.15	–0.0836	1.46	3.37	1.62	1.84	[55]
	313.15	–0.0082	3.50	2.96	0.76	0.27	[55]
	333.15	–0.0389	5.98	6.23	5.15	6.18	[55]
Overall average			2.04	4.14	1.58	7.80	

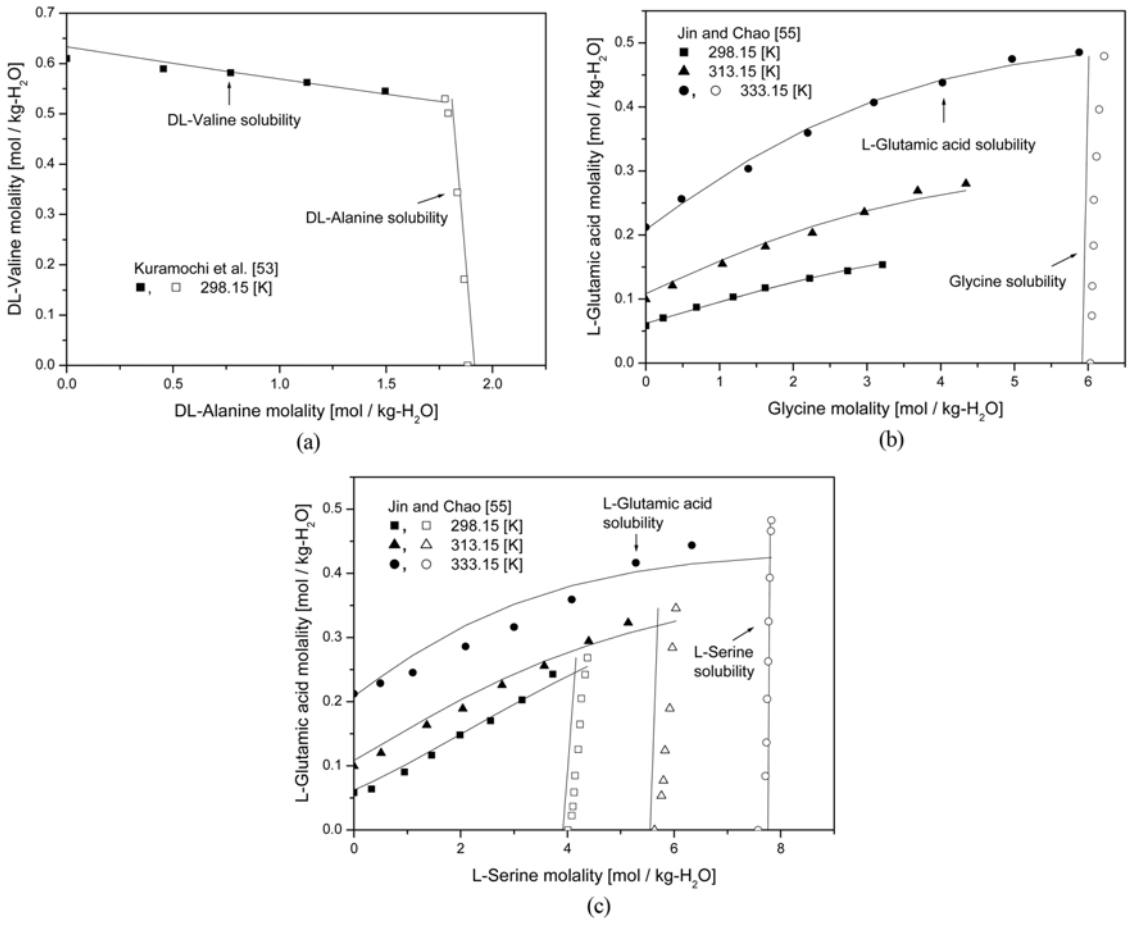

Fig. 8. (a) Correlated and experimental solubilities of amino acids in the aqueous DL-alanine/DL-valine solution. (b) Correlated and experimental solubilities of amino acids in the aqueous glycine/L-glutamic acid solution. (c) Correlated and experimental solubilities of amino acids in the aqueous L-serine/L-glutamic acid solution.

two amino acid molecules, which is required to define the dispersion Helmholtz free energy in the ternary system. In the present work, the interaction parameter between two amino acid molecules, $k_{A_1A_2}$, is treated as an adjustable parameter. Through the fitting of solubilities of amino acids calculated from Eq. (35) and experimental solubilities data, $k_{A_1A_2}$ parameter was estimated. The fitting results for 8 ternary solutions are shown in Table 6. Also, in this table, the results of present work are compared with those of modified NRTL model carried out by Sedeghi [44]. As can be seen in Table 6, the results obtained with our present model seem to be somewhat satisfactory. In Fig. 8, the calculated results of 3 ternary solutions selected among the 8 ternary solutions considered in this work are shown together with experimental data. In Figs. 8(a) and 8(b), the predicted solubilities of amino acids for the DL-alanine/DL-valine solution and glycine/L-glutamic acid solution show good agreement with experimental data. However, as shown in Fig. 8(c), the predicted solubilities for the L-serine/L-glutamic acid solution are more or less deviated from the experimental data at ranges of temperature higher than 298.15 K.

CONCLUSIONS

In the present work, assuming that zwitterions of amino acids are the associating species with two proton donor sites and two acceptor sites on their ammonium group and carboxylate group, respectively, the PC-SAFT EoS was employed to describe thermodynamic properties of aqueous amino acid solutions. Five parameters of PC-SAFT EoS for amino acids were determined by simultaneously fitting the activity coefficients of amino acids and densities data available in the literatures. Among the determined 5-parameters, the number of segment parameter, m_i, is shown to be linear with molecular weight for all amino acids treated in this work. Also the value of association volume parameter, $\kappa^{A_iB_i}$, seems to be insensitive to the regression results, considering that the estimated values of $\kappa^{A_iB_i}$ are in the narrow range from 0.023 to 0.038. Based upon these facts, in order to reduce the number of PC-SAFT EoS parameters for amino acids, it is assumed that the number of segment parameter, m_i can be defined from the linear relation with respect to molecular weight of amino acids: $m_i=0.07552M_{w,i}-1.95459$, and also association volume parameter, $\kappa^{A_iB_i}$, can be set to 0.032 for all amino acids considered in this work. Under these conditions, three parameters, the segment diameter σ_i, dispersion energy ε_i/k and association energy $\varepsilon^{A_iB_i}/k$ were determined by the method similar to the estimation of five parameters. The PC-SAFT EoS with the estimated 3-parameters of amino acids is found to satisfactorily describe the activity coefficients of amino acids and densities in the aqueous amino acid solutions. Also, the present equation was used to predict the solubility of amino acids over the temperature range from 273.15 K to 373.15 K, the solubility of amino acids at different values of pH, and the solubilities of two amino acids in the aqueous solutions. These predicted results are found to be in good agreement with the experimental data.

ACKNOWLEDGMENT

This work was supported by the research grant from Kangwon National University in 2009.

NOMENCLATURE

A : Helmholtz free energy
a_w : water activity
d_i : temperature-dependent diameter of segment of component
$\Delta g/RT_o$: Gibbs free energy change of the solute at 298.15 K
$\Delta g_F^o/RT_o$: Gibbs free energy change of the solute, for transferring 1 mole of solute from the saturated solution to a hypothetical aqueous solution at an activity of 1 molal at 298.15 K
g_{ij}^{hs} : radial distribution function in the hard sphere mixture
Δh : molar enthalpy change
K : equilibrium constant
k : Boltzmann constant
k_{ij} : binary interaction parameter between unlike segment of component i and j
M_s : molecular weight of solvent (water)
$M_{w,i}$: molecular weight of component i
m_A : molality of amino acid
m_i : number of segments per molecule of component i
m_A^s : solubility of amino acid in water (in molality scale)
N : total number of molecules in the system
P : pressure
pI : isoelectric point
pK : logarithm of equilibrium constant, $pK=-\log K$
R : gas constant
Δs : molar entropy change
T : temperature
V : volume
x_i : mole fraction of component i
x_A^s : mole fraction of amino acid in the saturated solution
X^{A_i} : mole fraction of component i not bonded at association site A

Greek Letters

$\gamma_A^{(m)}$: activity coefficient of amino acid in molality scale
γ_w : activity coefficient of water
ε_i/k : dispersion energy parameter
$\varepsilon^{A_iB_j}/k$: energy parameter of association between site A on chemical species i and site B on chemical species j
$\kappa^{A_iB_j}$: volume parameter of association between association site A on chemical species i and B site on chemical species j
μ_i : chemical potential of component i
ρ : total number density of molecules
σ_i : segment diameter of component i
ϕ : osmotic coefficient of solution

Superscripts

assoc : association term
disp : dispersion term
hc : hard-sphere chain term
hs : hard-sphere term
l : liquid state
res : residual property
s : saturated solution
^{o} : reference state

Subscripts

A : amino acid

cal : calculated value
exp : experimental value
i, j : chemical component i, j
w : water

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