Measurements of Vapor Pressures of Aqueous Amino Acid Solutions and Determination of Activity Coefficients of Amino Acids

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Vapor pressures of aqueous solutions of L-amino acids (glycine, L-alanine, L-valine, and L-serine) were measured at various concentrations at 298 K by a differential pressure static method. Activities and activity coefficients of water were determined. The activity coefficients of the amino acids in water were obtained from the activity coefficients of water using the UNIQUAC equation. The obtained activity coefficients of the amino acids were evaluated by comparison of the values obtained by a conventional virial expansion method and the literature data.

Introduction

Thermodynamic properties such as activity coefficients for the water + biochemical systems are indispensable to design efficient separation and purification processes, and drying processes in food engineering as well. These properties are also useful for studying solution chemistry and conformations of both native and denatured proteins. The number of measurements, however, is very few; especially those on the L-form of amino acids are not available, with the exception of serine.

In this work, vapor pressures of aqueous L-amino acid solutions were measured by a differential pressure method to obtain the actvity coefficient of water. The amino acids were glycine, L-alanine, L-valine, and L-serine. A new approach was used to calculate the activity coefficients of the amino acids based on the activity coefficient data of water. The activity coefficients of water are first correlated by the UNIQUAC equation (Abrams and Prausnitz, 1975), and then the activity coefficients of the amino acids are calculated indirectly via the correlated results. To evaluate this method's usefulness, it was tested in comparison with not only the literature data but also the values obtained from a conventional virial expansion method.

Experimental Section

Materials. All amino acids were supplied from Nakaraitesk Co., Ltd., and Tokyo Kasei Kogyo Co., Ltd., with a purity of 99 wt % for glycine, L-alanine, and L-valine, and 98.5 wt % for L-serine. Those values were determined by suppliers using the JIS (Japanese Industrial Standard) analytical method. Major impurities (less than 1.5 wt %) reported were sulfates and chlorides. We used all the amino acids without further purification. Water was passed through two ionic exchange columns for demineralization and then distilled.

Apparatus and Procedure. In this work, a differential pressure static method similar to that described by Patil et al. (1990) was used. The static method requires a thorough degassing of the mixture. However, there are several advantages to other methods. Smaller amounts of materials are consumed compared with the dynamic method. The apparatus and procedure are simple, and the attainment of equilibrium is much faster than the isopiestic method which sometimes requires days (Robinson and Stokes, 1965).

The method is based on the measurement of the difference of vapor pressures (ΔP) between a reference (pure water) and an aqueous solution containing a known composition of solute. The vapor pressure of the aqueous solution (P_1) was determined from ΔP and the vapor pressure (P_1°) of pure water. Since the vapor pressure of water at 298.15 K was reported with very high accuracy as 3.1690 kPa (Haar et al., 1984), the vapor pressure P_1 is obtained as follows.

$$P_1 = P_1^\circ - \Delta P = 3.1690 - \Delta P \qquad (1)$$

The reason a differential pressure method was employed instead of a total pressure one is that the latter is not suitable for detecting small changes in pressure when adding an amino acid to water. Accurate values of ΔP can be obtained with a high-precision differential pressure gauge.

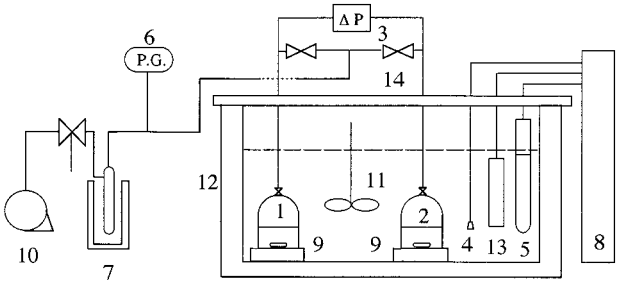

Figure 1. Schematic static apparatus for measurement of differential pressure: 1, reference cell; 2, measurement cell; 3, precise differential pressure transducer; 4, thermosensor; 5, heater; 6, pressure gauge; 7, cold trap; 8, temperature controller; 9, stirrer; 10, vaccum pump; 11, mixer; 12, water bath; 13, cooler; 14, insulating material.

The schematic apparatus is shown in Figure 1. The apparatus consisted of a precise differential pressure transducer (MKS Instruments Inc., Baratoron 220CD equipped with a five-digit meter) with a sensitivity of about 0.3 Pa. The reference and the measurement glass cells (50 mL capacity each) had magnetic stirrers. The thermostated water bath was controlled to 298.15 K with an accuracy of ±0.02 K.

The experimental procedure was as follows. The solid amino acids were dried under vacuum conditions at 333 K for 6 h. An aqueous solution containing a given mass of the amino acid was prepared, and 20 mL of this solution was transferred into the measurement cell while 20 mL of pure water was charged into the reference cell. Before the cells were immersed in the bath, degassing of water as well

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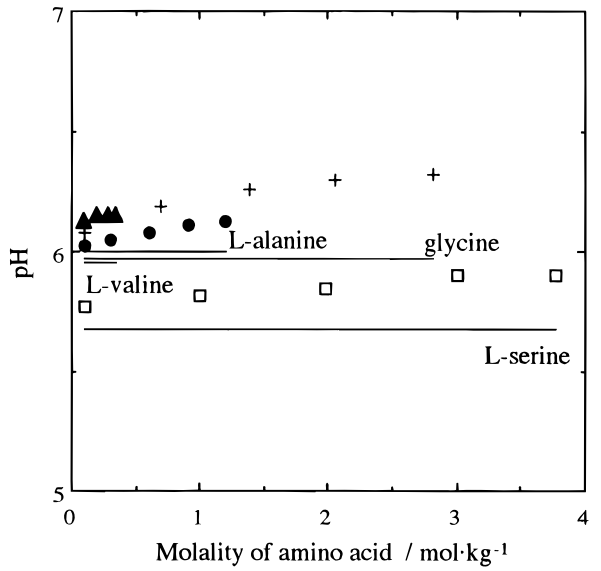

Figure 2. Dependence of pH on amino acid concentration: (—) pI; (+) glycine; (●) L-alanine; (□); L-serine; (▲) L-valine.

as the aqueous solution was carried out three times by freezing the cell contents with liquid nitrogen and then evacuating it with a vacuum pump. The total change in the amount of water during the repeated degassing processes was around −0.2 wt %. Therefore, the concentration of the solution was corrected to account for the loss of water. The impurity content (<1.5 wt %) may have an influence of 2 or 3% of error on the activity coefficients and osmotic coefficients obtained in this study. To examine the ionic form of the amino acids in water, the pHs of the aqueous solutions were measured. As shown in Figure 2, the values of the pHs of the solutions were around the isoelectric point (pI) or close to it. In consideration of the dissociation constants (pK_a and pK_b) of the amino acids and the experimental pHs, it is clear that almost 100% of the amino acids in the aqueous solution take the zwitterionic form in this work.

The degassed cells were placed in the bath and 2−3 h was allowed for achievement of equilibrium. After equilibration, the differential pressure was measured and the vapor pressure of the solution P_1 was determined by eq 1.

The differential pressure transducer was calibrated with a CEC air dead-weight tester. The temperature measurements were made with a quartz thermometer (Tokyo Denpa Co. Ltd., DMT-610) with a sensitivity of 1×10^{-3} K.

Determination of Activity and Activity Coefficients of Water and Amino Acid. The activity coefficient (γ_1) of water in the aqueous amino acid solutions was determined from the vapor pressure measurements using the following relationship.

$$a_1 = \gamma_1 x_1 = P_1/P_1^\circ \tag{2}$$

The activity of water can be related to the activity (a_2) or activity coefficient ($\gamma^*_{2,m}$) of the amino acid in the solution by the Gibbs−Duhem equation (Lewis and Randall, 1961):

$$\frac{1000}{M_1}\,\mathrm{d}\ln a_1 = -m\,\mathrm{d}\ln a_2 = -m\,\mathrm{d}\ln \gamma^*_{2,m} m \tag{3}$$

where M_1 and m refer to the molecular weight of water and the molality of amino acid, respectively, and $\gamma^*_{2,m}$ is the activity coefficient of the amino acid on the unsymmetric convention with reference to infinite dilution.

In this work a new approach based on the UNIQUAC equation (Abrams and Prausnitz, 1975), which is one of the equations satisfying the Gibbs−Duhem equation, was applied in order to determine $\gamma^*_{2,m}$ from the experimental data (a_1 or γ_1). The UNIQUAC equation is a function containing q_i (relative molecular surface area), r_i (relative molecular volume), Δu_{ji} (UNIQUAC binary interaction parameter), x_i, and T, as given in eq 4. q_i and r_i are the pure-species parameters which can be calculated by Bondi's method (Bondi, 1965), whereas Δu_{ji} are unknown parameters.

$$\ln \gamma_i = \ln \frac{\Psi_i}{x_i} + \frac{z}{2} q_i \ln \frac{\theta_i}{\Psi_i} - l_i \frac{\Psi_i}{x_i}\sum_j x_j l_j + q_i\left[1 - \ln\left(\sum_j \theta_j \tau_{ji} - \sum_j \frac{\theta_j \tau_{ij}}{\sum_k \theta_k \tau_{kj}}\right)\right] \tag{4a}$$

where

$$l_i = \frac{z}{2}(r_i - q_i) - (r_i - 1) \qquad z = 10 \tag{4b}$$

$$\theta_i = \frac{q_i x_i}{\sum_j q_j x_j} \qquad \Psi_i = \frac{r_i x_i}{\sum_j r_j x_j} \qquad \tau_{ji} = \exp\left(-\frac{\Delta u_{ji}}{RT}\right) \tag{4c}$$

If the unknown UNIQUAC binary interaction parameters (Δu_{ji}) between water and the amino acid could be determined by fitting γ_1 to the UNIQUAC equation, the activity coefficient of the other component, amino acid, may be calculated by the same equation. The activity coefficient (γ_2) calculated by the UNIQUAC equation is on the symmetric convention with reference to a pure component on a mole fraction basis. However, $\gamma^*_{2,m}$ is the activity coefficient on the unsymmetric convention with reference to an infinite dilution on a molality basis. The symmetric activity coefficient (γ_2) was converted to the unsymmetric one by the following equation:

$$\gamma^*_{2,m} = \frac{\gamma_2}{\gamma_2^\infty\left(1 + \frac{M_1 m}{1000}\right)} \tag{5}$$

where γ_2^∞ stands for the symmetric activity coefficient of amino acid at infinite dilution and is calculated by the UNIQUAC equation. Until now, the application of such a method has never been reported for the determination of activity coefficients of nonvolatile solutes such as amino acid in water.

Alternatively, the activity coefficients of amino acids were usually obtained by a conventional virial expansion method. The following conversion from experimental water activity to osmotic coefficient is necessary for the method.

$$\phi = -\frac{1000}{M_1 m}\ln a_1 \tag{6}$$

$$\phi = 1 - \frac{1}{RT}\sum_i i g_i m^i \tag{7}$$

Fitting the converted osmotic coefficient data (ϕ) to eq 7,

Table 1. Vapor Pressure of Aqueous NaCl Solutions at 298 K

m/mol·kg^{-1}	P_1/kPa (this work)	P_1/kPa (Colin et al.)	RD[a]/%
0.1974	3.1526	3.1482	0.14
0.5116	3.1185	3.1154	0.09
1.0034	3.0674	3.0629	0.14
1.4854	3.0108	3.0100	0.03
1.9837	2.9624	2.9530	0.32
2.5825	2.8808	2.8809	0.00
			0.12 (av)

[a] Relative deviation $= |P_1^{\text{this work}} - P_1^{\text{Colin et al.}}|/P_1^{\text{Colin et al.}}$ Vapor pressure of pure water at 298 K $= P_1^\circ = 3.1690$ [kPa].

Table 2. Vapor Pressure and Activity Coefficient of Water along with the Activity Coefficient of Glycine for Water (1) + Glycine (2)[a]

m/mol·kg^{-1}	P_1/kPa	γ_1^{exp}	γ_1^{calc}	$\gamma_{2,m}^{*,\text{calc}}$
0.1006	3.1625	0.9997	1.0000	0.9852
0.1485	3.1603	1.0000	1.0001	0.9783
0.2507	3.1553	1.0002	1.0001	0.9637
0.3011	3.1541	1.0007	1.0001	0.9567
0.3509	3.1514	1.0007	1.0002	0.9498
0.4024	3.1478	1.0005	1.0002	0.9428
0.4493	3.1450	1.0004	1.0002	0.9365
0.5123	3.1390	0.9996	1.0003	0.9281
0.9976	3.1174	1.0014	1.0010	0.8675
1.508	3.0945	1.0030	1.0024	0.8104
1.959	3.0677	1.0022	1.0040	0.7649
2.407	3.0545	1.0057	1.0058	0.7237
3.299	3.0225	1.0105	1.0104	0.6520

[a] Vapor pressure of pure water at 298 K $= P_1^\circ = 3.1690$ [kPa].

one can calculate the activity coefficients of amino acid by eq 8, which is derived from the Gibbs−Duhem relationship.

$$\gamma_{2,m}^{*} = \exp\left[\frac{1}{RT}\sum_i (i+1) g_i m^i\right] \tag{8}$$

where g_i is an adjustable parameter and i refers to the number of parameters needed to represent the experimental data. In this work, the activity coefficients of the amino acids were determined by the virial expansion methods. The results will be compared with those calculated by the UNIQUAC equation.

Results and Discussion

Vapor pressures of aqueous solutions of sodium chloride with a purity of 99.98 wt % were measured and compared with literature data in Table 1. The measured vapor pressures (P_1^{exp}) of the solutions are found to be in good accordance with those of literature data ($P_1^{\text{lit.}}$ of NaCl solution (Colin et al., 1985)) within the average relative deviations (ARD $= 100/N\sum_n |P_{1,n}^{\text{exp}} - P_{1,n}^{\text{lit.}}|/P_{1,n}^{\text{exp}}$) of about 0.1%. From these results, reliability as well as consistency of the proposed method including measurements and calculations were confirmed.

The experimental data of vapor pressures for aqueous solutions of glycine, L-alanine, L-serine, and L-valine at 298 K are listed in Tables 2−5. In these tables γ_1^{exp} was determined by eq 2, and γ_1^{calc} and $\gamma_2^{*,\text{calc}}$ were calculated by the UNIQUAC equation. The UNIQUAC size parameters and regressed interaction parameters are summarized in Tables 6 and 7, respectively. The correlated results are given in Table 7 along with the RMSD for γ_1. The RMSD values were between 0.03 and 0.07(%); that is, the correlated results of γ_1 were in good agreement with the experimental data for all solutions.

Table 3. Vapor Pressure and Activity Coefficient of Water along with the Activity Coefficient of L-Alanine for Water (1) + L-Alanine (2)[a]

m/mol·kg^{-1}	P_1/kPa	γ_1^{exp}	γ_1^{calc}	$\gamma_{2,m}^{*,\text{calc}}$
0.1013	3.1619	0.9996	1.0000	0.9901
0.1505	3.1591	0.9996	1.0000	0.9853
0.2007	3.1561	0.9995	1.0000	0.9805
0.2509	3.1537	0.9997	1.0000	0.9758
0.3502	3.1499	1.0003	1.0001	0.9666
0.4019	3.1462	1.0000	1.0001	0.9619
0.4505	3.1439	1.0002	1.0002	0.9576
0.5015	3.1389	0.9994	1.0002	0.9530
0.8996	3.1192	1.0003	1.0006	0.9184
1.144	3.1076	1.0008	1.0008	0.9002
1.266	3.1014	1.0010	1.0010	0.8909
1.387	3.0958	1.0013	1.0012	0.8820

[a] Vapor pressure of pure water at 298 K $= P_1^\circ = 3.1690$ [kPa].

Table 4. Vapor Pressure and Activity Coefficient of Water along with the Activity Coefficient of L-Serine for Water (1) + L-Serine (2)[a]

m/mol·kg^{-1}	P_1/kPa	γ_1^{exp}	γ_1^{calc}	$\gamma_{2,m}^{*,\text{calc}}$
0.0543	3.1651	0.9998	1.0000	0.9675
0.1515	3.1621	1.0005	1.0001	0.9143
0.2504	3.1577	1.0009	1.0003	0.8672
0.3657	3.1530	1.0016	1.0006	0.8194
0.4015	3.1489	1.0008	1.0007	0.8059
0.4665	3.1475	1.0015	1.0009	0.7828
0.5011	3.1417	1.0011	1.0010	0.7713
0.9047	3.1319	1.0044	1.0027	0.6653
1.504	3.1043	1.0061	1.0058	0.5711
2.004	3.0830	1.0080	1.0082	0.5241
2.506	3.0605	1.0094	1.0103	0.4932
3.992	2.9981	1.0141	1.0140	0.4498

[a] Vapor pressure of pure water at 298 K $= P_1^\circ = 3.1690$ [kPa].

Table 5. Vapor Pressure and Activity Coefficient of Water along with the Activity Coefficient of L-Valine for Water (1) + L-Valine (2)[a]

m/mol·kg^{-1}	P_1/kPa	γ_1^{exp}	γ_1^{calcd}	$\gamma_{2,m}^{*,\text{calc}}$
0.1008	3.1617	0.9995	0.9999	1.043
0.2000	3.1551	0.9992	0.9999	1.086
0.2471	3.1537	0.9996	0.9997	1.107
0.2930	3.1515	0.9997	0.9996	1.128
0.3428	3.1442	0.9995	0.9995	1.152
0.4011	3.1431	0.9994	0.9994	1.179
0.4543	3.1402	0.9990	0.9992	1.204

[a] Vapor pressure of pure water at 298 K $= P_1^\circ = 3.1690$ [kPa].

Table 6. UNIQUAC Size Parameters (r_i, q_i)[a]

component	r_i	q_i
H_2O	0.9200	1.400
glycine	2.6705	2.914
L-alanine	3.3441	3.450
L-serine	4.1174	4.342
L-valine	4.6921	4.526

[a] r_i and q_i were calculated by Bondi's method (Bondi, 1968).

From the determined UNIQUAC interaction parameters, the calculations of activity coefficients ($\gamma_{2,m}^{*}$) of amino acids were carried out. The calculated results for four amino acids are shown in Figure 3 and are compared with the literature data of glycine (Ellerton et al., 1964) and L-serine (Hutchen et al., 1963). The result of L-serine in this work (calculated curve in Figure 3) is underestimated from the experimental data (Hutchen et al., 1963), but it may represent the behavior qualitatively. While the activity coefficients of glycine have been investigated by many

Table 7. UNIQUAC Binary Interaction Parameters and Correlated Results γ_1 by the UNIQUAC Equation

system	Δu_{12}/J·mol^{-1}	Δu_{21}/J·mol^{-1}	RMSD[a]/%
water (1) + glycine (2)	118.2	3114	0.062
water (1) + L-alanine (2)	−903.2	2196	0.035
water (1) + L-serine (2)	3848	−2224	0.071
water (1) + L-valine (2)	−2291	−840.2	0.031

[a] $RMSD\ (\%) = 100\{\sum_k^N[(\gamma_{1,k}^{exp} - \gamma_{1,k}^{calc})/\gamma_{1,k}^{exp}]^2/N\}^{1/2}$

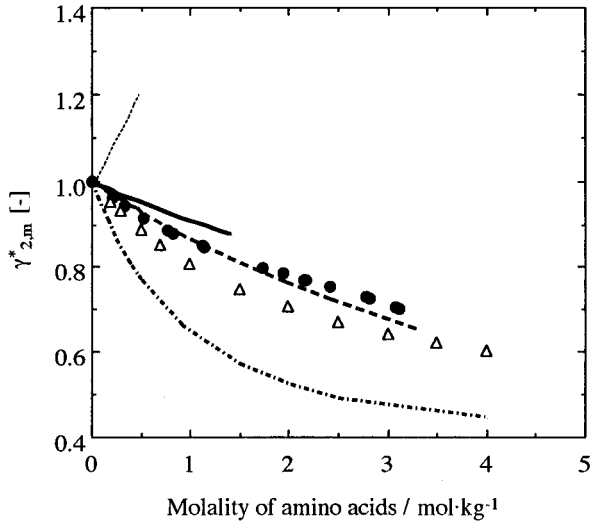

Figure 3. Activity coefficients of amino acids in water at 298 K by the UNIQUAC method. Calc: (− −) glycine; (—) L-alanine; (− · −) L−serine; (···) L-valine. Literature data: (●) glycine (Ellerton et al. 1964); (△) L-serine (Hutchen et al., 1963).

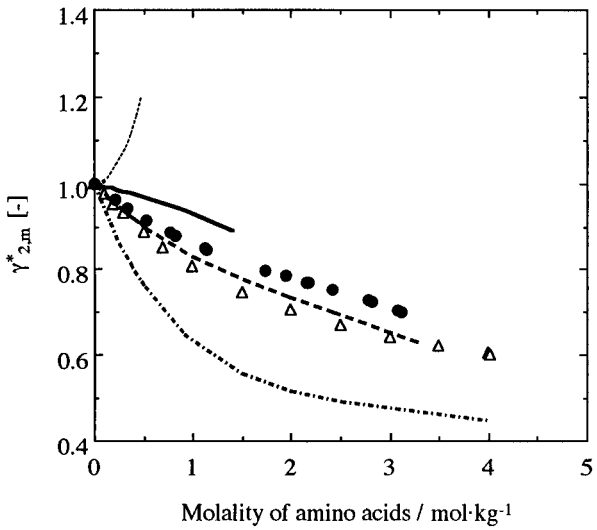

Figure 4. Activity coefficients of amino acids in water at 298 K by the virial expansion method. Calc: (− −) glycine; (—) L-alanine; (− · −) L-serine; (···) L-valine. Literature data: (●) glycine (Ellerton et al. 1964); (△) L-serine (Hutchen et al., 1963).

authors and the recommended data are available, our results of glycine in Figure 4 provide satisfactory agreement with the literature data (Ellerton et al., 1964). The $\gamma^*_{2,m}$ of L-alanine and L-valine, whose data have not been reported, are also shown in Figure 3. All the literature data of $\gamma^*_{2,m}$ except our data were measured many years ago using the isopiestic method.

The results calculated by the virial expansion method are shown in Figure 4, and the values of parameters in eq 7 or 8 are given in Table 8. The results are coincident within 1 to 3 ARD % with those by the UNIQUAC method. In addition, a comparison of the results of the UNIQUAC

Table 8. Adjustable Parameters in Eq 7 or 8 for Water (1) + Amino Acid (2)

system	g_1	g_2	g_3
water (1) + glycine (2)	289.8	−46.50	5.219
water (1) + L-alanine (2)	−165.4	440.3	0.000
water (1) + L-serine (2)	814.3	−172.9	14.83
water (1) + L-valine (2)	3704	−3970	0.000

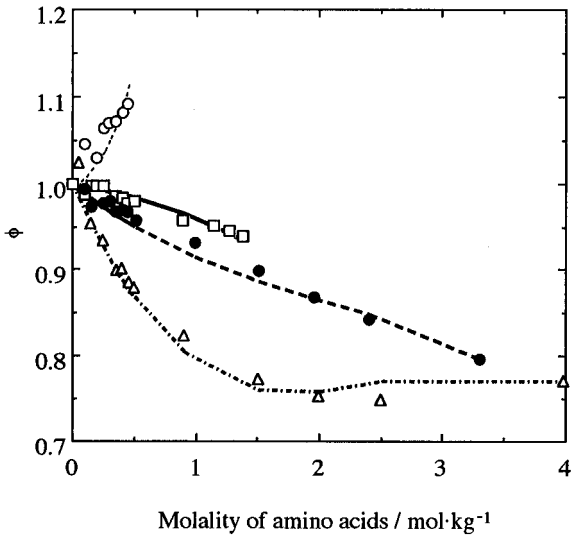

Figure 5. Comparison of the osmotic coefficients (ϕ) by the UNIQUAC method and the virial expansion method. Results of the UNIQUAC method; (●) glycine; (□) L-alanine; (△) L-serine; (○) L-valine. Results of the virial method; (− − −) glycine; (—) L-alanine; (− · −) L-serine; (···) L-valine.

model with the virial expansion model for the calculated osmotic coefficient was given in Figure 5. For the osmotic coefficient, also, the results by the UNIQUAC model were in accordance with those by the virial expansion model.

The activity coefficients from the UNIQUAC method agreed with those from the virial expansion method, and these values were rational with reference to the literature data. On the basis of the discussion mentioned above, we conclude that the proposed UNIQUAC method may provide a useful alternative procedure to the classical virial method for determination of activity coefficients of amino acids in water.

Conclusion

Vapor pressures of aqueous solutions of amino acids, glycine, L-alanine, L-valine, and L-serine, were measured by the differential pressure method. The activity and activity coefficient of water were determined from the vapor pressure data. In particular, the data for aqueous solutions of L-alanine and L-valine were first presented and then correlated very well by the UNIQUAC equation.

Moreover, the new method for determining the activity coefficients of amino acids in water was proposed. It is an indirect method, and it is based on the calculation of the activity coefficient of the amino acids via the UNIQUAC equation fitted to the experimental activity coefficient of water. The obtained activity coefficients of the amino acids were almost consistent with those calculated by the conventional virial expansion method and the literature data. The proposed method may be used to obtain activity coefficients of the nonvolatile component in water as a substitution for the classical isopiestic method.

Notation

a_i	activity of component i
g_i	adjustable parameter in eq 7 or 8
M_1	molecular weight of water
m	molality [mol·kg^{-1} of solvent]
P	vapor pressure
q_i	surface area parameter for component i
R	gas constant [J·mol^{-1}·K^{-1}]
r_i	volume parameter for component i
T	temperature [K]
Δu_{ji}	UNIQUAC binary interaction parameter [J·mol^{-1}]
x_i	liquid phase mole fraction of component i

Greek Letters

γ	activity coefficient on the mole fraction basis (symmetric convention)
γ^*	activity coefficient on the mole fraction basis (unsymmetric convention)
γ_m^*	activity coefficient on the molality basis (unsymmetric convention)
θ_i	surface area fraction of component i
τ_{ji}	Boltzmann factor
ϕ	osmotic coefficient
Ψ_i	volume fraction of component i

Superscript

calc.	calculated by the UNIQUAC equation
exp	experimental value
lit.	literature data
°	pure component
∞	infinite dilution

Subscript

1	water
2	amino acid

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