Water Activity in Aqueous Amino Acid Solutions, with and without KCl, at 298.15 K

Simão P. Pinho*

Laboratory of Separation and Reaction Engineering, Escola Superior de Tecnologia e de Gestão, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5301-857 Bragança, Portugal

Using a water activity instrument meter, water activity in aqueous solutions of DL-alanine, glycine, or L-serine, with potassium chloride, molality ranging from 0.0 to 3.0, has been measured at 298.15 K. The reliability of the method was checked comparing the experimental data with literature values. The method proved to be accurate, and the water activities measured for water + amino acid systems are reproducible when compared to the data reported using the isopiestic method. Additionally, a simple theoretical approach applied to those binary systems enabled the calculation of unsymmetric molal amino acid activity coefficients in high agreement with the values found using the isopiestic measurements. Finally, the usefulness of the ternary data to extend the capabilities of thermodynamic models to higher salt and amino acid concentrations was briefly discussed.

Introduction

The thermodynamic study of aqueous systems containing amino acids is of much importance. Particularly, the knowledge of activity coefficients or water activity has been a subject of great interest. In fact, besides the relevant information concerning solute–solvent interactions that can be used, for instance, for studies on conformational stability of proteins,[1] those properties can also be very useful as a support for the efficient design and simulation of separation processes such as extraction, precipitation, or drying.[2,3]

Smith and Smith[4,5] were pioneers in the application of the isopiestic method in the experimental determination of osmotic coefficients in aqueous amino acid systems. This method is often used to obtain, indirectly, the unsymmetric molal activity coefficients of amino acids, and since then, at 298.15 K, a considerable body of experimental work has been published. However, it is time-consuming because the equilibrium time can in some cases be longer than 15 days.[1] To overcome this problem, more recently, researchers proposed vapor pressure measurements[3] or a water activity instrument meter[6] to obtain the unsymmetric molal activity coefficients of amino acids from water activity in aqueous amino acid solutions. On the other hand, for aqueous amino acid systems containing a salt, only very recently a large systematic series of measurements have been carried out on electrolyte activity coefficients in the presence of an amino acid or a peptide, which have recently been reviewed (see Ferreira et al.[7] and references therein), but no information on water activity in this type of system has yet been published.

Therefore, in this work, a program to measure water activity in aqueous amino acid systems with or without a salt was implemented. There are two main objectives: one is to study an alternative to the classical isopiestic method to find, more efficiently, reliable values for the unsymmetric molal activity coefficients of amino acids from water activity measurements in binary solutions, and the other is the presentation of new, and so far unavailable, experimental data for the water activity at 298.15 K in aqueous solutions of DL-alanine, glycine, or L-serine, with potassium chloride at three different molalities.

* Corresponding author. Fax: + 351 273313051. E-mail: spinho@ipb.pt.

Experimental Section

Chemicals. Glycine and DL-alanine were supplied by Merck with 99.7 % and 99 % purity, respectively, and L-serine was supplied by Sigma with purity higher than 99 %. The amino acids were used as received and kept in a dehydrator with silica gel to avoid water contamination. Potassium chloride, 99.5 % purity, was supplied by Merck. To avoid water salt contamination, KCl was dried at 393.15 K in a drying stove for more than 2 days and cooled after in a dehydrator with silica gel before use. In all experiments, distilled–deionized water was used.

Procedure. Approximately 120 g of a KCl aqueous solution was first prepared weighing in a balloon-flask the desired masses (± 0.1 mg) of salt and distilled–deionized water. The resulting solution was vigorously shaken to promote salt dissolution. Then, the appropriate masses of amino acid and of the already prepared aqueous KCl solution were weighed into a glass vessel (25 cm^3) to prepare about 15 cm^3 of amino acid aqueous electrolyte solution with the desired amino acid and KCl molalities. The glass vessels were then conveniently closed and the solutions homogenized using a magnetic stirrer. It should be mentioned that for solutions with an amino acid molality near the solubility limit a bland heating was applied to promote the complete dissolution of the amino acid.

After, the *Lab*MASTER-a_w water activity instrument meter[8] (Novasina, Switzerland) was used for measuring water activity in those solutions. The instrument enables measurements under controlled chamber temperature conditions (± 0.2 K) and was previously calibrated with six saturated pure salt standard solutions (water activity ranging from 0.113 to 0.973), which were included with the instrument. To measure water activity, sample dishes were charged with approximately 7 cm^3 of the solution and placed in the chamber. Immediately, the instrument was closed to start the measurement: usually 45 min was enough

10.1021/je700466s CCC: $40.75
Published on Web 11/30/2007

Table 1. Water Activity in Aqueous Amino Acid Solutions at 298.15 K

DL-alanine		glycine		L-serine	
molality	a_w	molality	a_w	molality	a_w
0.199	0.996	0.250	0.996	0.250	0.995
0.299	0.995	0.250	0.996	0.250	0.996
0.400	0.992	0.499	0.991	0.499	0.992
0.400	0.992	0.500	0.990	0.500	0.991
0.498	0.991	0.750	0.987	0.751	0.987
0.599	0.989	0.751	0.986	0.752	0.988
0.600	0.989	1.000	0.983	0.999	0.983
0.697	0.988	1.001	0.983	1.247	0.980
0.798	0.986	1.245	0.980	1.247	0.980
0.800	0.986	1.250	0.979	1.499	0.976
0.896	0.984	1.500	0.976	1.745	0.974
0.998	0.983	1.501	0.975	1.747	0.974
1.002	0.983	1.747	0.972	2.002	0.970
1.100	0.980	1.754	0.972	2.243	0.966
1.102	0.981	1.999	0.968	2.249	0.966
1.198	0.979	2.000	0.967	2.500	0.963
1.203	0.978	2.247	0.964	2.745	0.959
1.298	0.976	2.248	0.963	2.751	0.960
1.304	0.977	2.500	0.961	2.997	0.957
1.394	0.975	2.503	0.960	3.250	0.953
1.499	0.973	2.745	0.957	3.254	0.953
1.500	0.973	2.792	0.955	3.495	0.950
1.599	0.971	3.000	0.952	3.739	0.947
1.695	0.969	3.001	0.953	3.749	0.947
1.793	0.968	3.114	0.951	3.995	0.943

to reach equilibrium in the chamber, and the water activity value was registered. It is important to notice that for solutions with high amino acid molality or for those presenting water activity close to 1 the equilibrium time can be a little longer.

To improve accuracy, each day that measurements were carried out, a calibration curve was also built. At least six KCl aqueous solutions were prepared at distinct molalities. The KCl molalities were chosen based on the expected values for the water activity to be measured in the aqueous solutions containing amino acids. After, the measured values were plotted against the water activity of aqueous KCl solutions at 298.15 K calculated applying the fundamental equations given in the extensive review by Archer.[9] Using the originally observed values, the calibration curve allows the recalculation of water activity in the aqueous solutions containing amino acids. Each experimental point was an average of two different measurements presenting a maximum standard deviation of 0.001.

Results and Discussion

Binary Systems. In Table 1, the measured water activities for DL-alanine, glycine, or L-serine aqueous solutions at 298.15 K and different amino acid molalities are reported. All three water + amino acid systems were studied to near the saturation molality. From Figure 1, it is possible to observe that for amino concentrations higher than 1 *m* water activity in amino acid solutions at the same concentration follows the sequence L-serine > glycine > DL-alanine, which is exactly the same sequence that is observed for amino acid solubility in water at the same temperature.[7,10] This behavior reflects the balance between hydrophobicity and self-association. Several hydrophobicity scales have been proposed,[11] and even if they are not always consistent, it is generally accepted that DL-alanine is more hydrophobic than L-serine. For this reason, it would be expected that at the same amino acid concentration more "free" water molecules would be available for the DL-alanine aqueous solution; that is, a higher water activity should be observed. However, as shown by their very low activity coefficients,

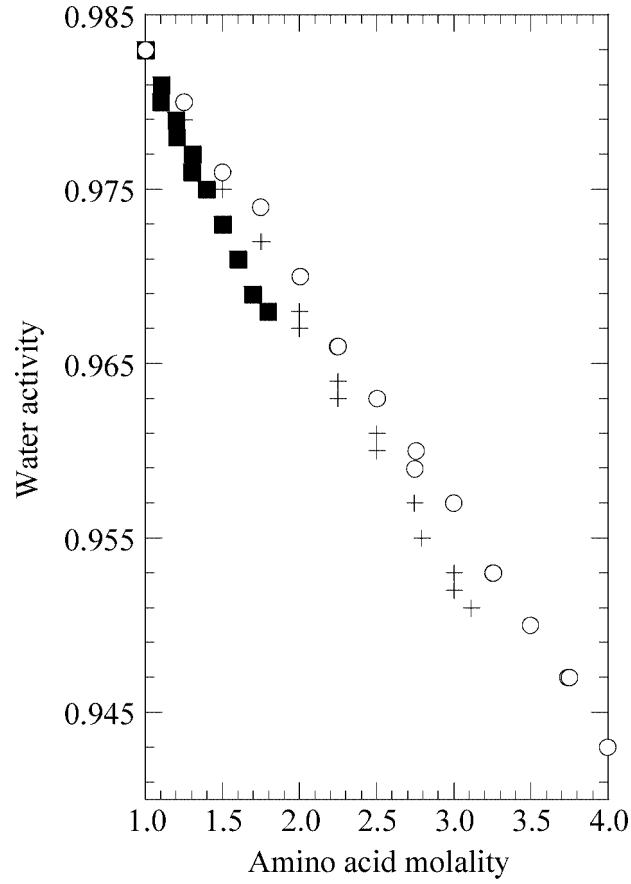

Figure 1. Comparison of water activity in aqueous amino acid solutions at 298.15 K: ■, DL-alanine; +, glycine; ○, L-serine.

glycine and, more evidently, L-serine molecules present a tendency for self-association[5,12] which results in a high water activity.

The quality of the measured data may be investigated by comparing it with values reported in the open literature. Due to the large amount of consistent data available measured by the isopiestic method[4,13] for the water + glycine system, it is possible to make an average curve fit for a better graphical comparison with the more recent published data. In Figure 2, it is possible to observe the very good agreement of the water activity data measured in this work at 298.15 K with the average curve. On the contrary, the data measured by Ninni and Meirelles,[6] using equipment identical to the one used in this work, show a systematically positive deviation to that curve. Indeed, their procedure seems very similar to the one implemented in this work, but a major change has been introduced here by finding a calibration curve for each new set of measurements, using KCl solutions as standards. Ninni and Meireles[6] preferred to calibrate the instrument once, using the saturated pure salt standard solutions included with the instrument, but from the experience acquired during the development of this work, that is not enough and can never give the desired accuracy. So, this can be the main reason for their deviation from the average curve. Concerning the water activity data obtained by Kuramochi et al.,[3] using vapor pressure measurements, only at high glycine molality, the deviations to the average curve are significant.

Kuramochi et al.[3] used their water activity data to estimate the UNIQUAC[14] parameters from which they could calculate the unsymmetric molal activity coefficients of the amino acids. They also applied a virial expansion in the amino acid molality

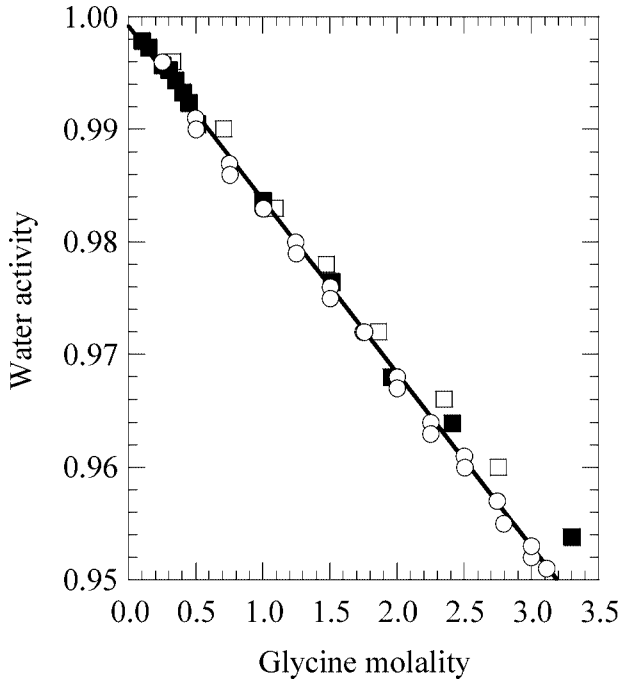

Figure 2. Comparison of water activity in aqueous glycine solutions at 298.15 K: ■, Kuramochi et al.;[3] □, Ninni and Meirelles;[6] ○, this work. The line represents the smoothed curve.

Table 2. Model Parameters and rmsd's for Water Activity in Amino Acid + Water Systems at 298.15 K

	$w_{1,n}$	$u_{1,n}$	rmsd
DL-alanine	4.0035	−1.6586	0.0005
glycine	−11.267	4.6221	0.0006
L-serine	−23.818	9.6327	0.0005

and verified the independence of the calculated values from the thermodynamic model chosen. Similarly, in this work, the UNIQUAC and the Pitzer−Simonson−Clegg equations[15,16] were applied to correlate water activity data, and the calculated molal activity coefficients were also independent of the model. As the Pitzer−Simonson−Clegg model is much simpler than the UNIQUAC, containing two unknown interaction parameters, only its results will be presented. In this model, the excess Gibbs energy (g^E) for a water + amino acid mixture is simply a Redlich–Kister[17] expansion given as

$$g^E/RT = x_1 x_n [w_{1,n} + u_{1,n}(x_1 - x_n)] \quad (1)$$

where R is the ideal gas constant; T is the absolute temperature; x_1 and x_n are the mole fraction of water and amino acid, respectively; and $w_{1,n}$ and $u_{1,n}$ are the coefficients for the description of water−amino acid interactions, which are the parameters to be estimated.

Water activity data (a_w) measured in this work were used to estimate the interaction parameters minimizing the following objective function (Fob)

$$\text{Fob} = \sum_k \left[\left(\frac{\ln a_w}{m_n} \right)_k^{\text{calcd}} - \left(\frac{\ln a_w}{m_n} \right)_k^{\text{exptl}} \right]^2 \quad (2)$$

where m_n is the amino acid molality and calcd and exptl mean calculated values according to the model and experimental values, respectively. Table 2 lists the parameters estimated together with the root-mean-square deviation (rmsd) for each system.

Using those parameters, the unsymmetric molal activity coefficients of the amino acids are readily calculated. The results

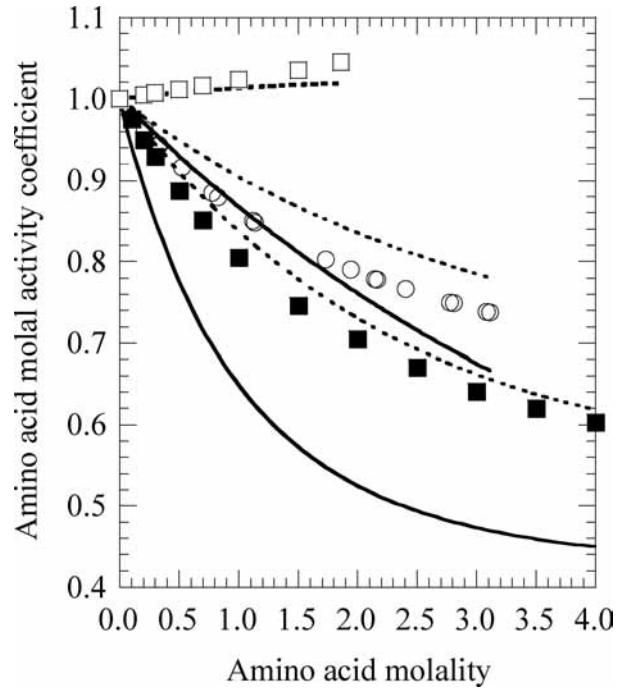

Figure 3. Molal activity coefficients of amino acids in water at 298.15 K. Comparison of the calculated values by Kuramochi et al.[3] (—) and this work (-----) with the experimental data; □, DL-alanine (Fasman[18]); ○, glycine (Ellerton et al.[13]); ■, L-serine (Hutchens et al.[19]).

for the three amino acids are compared in Figure 3 with literature data[13,18,19] obtained by the classical isopiestic method and with the results calculated by Kuramochi et al.[3] Generally, the amino acid activity coefficients calculated in this work are in good agreement with the literature data. The rmsd's between those two sets are 0.013, 0.038, and 0.023 for DL-alanine, glycine, and L-serine aqueous systems, respectively. On the other hand, while for the molal activity coefficient of glycine the values calculated by Kuramochi et al.[3] present quality (rmsd 0.036) similar to those calculated in this work, Figure 3 shows that for L-serine the activity coefficients are significantly underestimated with respect to the experimental data (rmsd 0.148). It must be stressed that the experimental method proposed, which is much faster than the isopiestic, allows the calculation of amino acid activity coefficients in good accordance to the values obtained by the isopiestic measurements a long time ago.

Ternary Systems. Tables 3, 4, and 5 present the measured water activity at 298.15 K for aqueous KCl solutions at 1.0, 2.0, and 3.0 salt molalities containing DL-alanine, glycine, or L-serine, respectively.

As mentioned before for ternary systems, no data for comparison were found in the literature. However, it is possible to use thermodynamic models to calculate this property and compare it to the observed values. Recently, Ferreira et al.[10] showed the good capabilities of the Pitzer−Simonson−Clegg equations[15,16] in the thermodynamic description of the ternary systems water−KCl with glycine, DL-alanine, or L-serine at different temperatures. The only thermodynamic properties used to estimate the parameters representing the ternary interactions were amino acid solubility and electrolyte activity coefficients. Figure 4 shows a comparison between the measured and predicted water activities in a 1 *m* aqueous KCl solution with amino acids. Excluding the L-serine system that presents more evident deviations for higher amino acid molalities, globally the agreement is very good. The rmsd's found were 0.0004 for DL-alanine, 0.0008 for glycine, and 0.0018 for L-serine system.

Table 3. Water Activity in Aqueous DL-Alanine Solutions with KCl at 298.15 K

m_{KCl} = 1.000		m_{KCl} = 2.000		m_{KCl} = 3.000	
DL-alanine molality	a_w	DL-alanine molality	a_w	DL-alanine molality	a_w
0.200	0.964	0.199	0.931	0.200	0.899
0.200	0.965	0.214	0.931	0.200	0.900
0.398	0.961	0.399	0.928	0.349	0.897
0.399	0.961	0.409	0.928	0.400	0.895
0.596	0.957	0.599	0.925	0.499	0.893
0.600	0.957	0.749	0.922	0.599	0.891
0.799	0.954	0.798	0.921	0.789	0.888
0.999	0.950	0.999	0.917	0.800	0.888
1.001	0.950	1.002	0.918	1.000	0.884
1.186	0.946	1.200	0.914	1.002	0.884
1.201	0.946	1.201	0.914	1.194	0.880
1.399	0.942	1.323	0.911	1.200	0.880
1.602	0.939	1.398	0.910	1.293	0.878
1.637	0.938	1.597	0.906	1.397	0.876
1.747	0.936	1.600	0.906	1.502	0.874
1.772	0.935	1.742	0.903	1.598	0.871
		1.799	0.903	1.737	0.869

Table 4. Water Activity in Aqueous Glycine Solutions with KCl at 298.15 K

m_{KCl} = 1.000		m_{KCl} = 2.000		m_{KCl} = 3.000	
glycine molality	a_w	glycine molality	a_w	glycine molality	a_w
0.250	0.965	0.249	0.934	0.250	0.900
0.500	0.959	0.499	0.929	0.499	0.897
0.749	0.956	0.748	0.926	0.749	0.893
1.000	0.952	1.002	0.923	0.999	0.890
1.247	0.949	1.247	0.918	1.251	0.886
1.500	0.945	1.252	0.918	1.494	0.884
1.746	0.941	1.498	0.915	1.747	0.879
1.750	0.942	1.504	0.914	1.999	0.877
2.001	0.939	1.749	0.911	2.253	0.873
2.247	0.935	1.995	0.907	2.499	0.870
2.498	0.931	2.242	0.904	2.753	0.865
2.743	0.927	2.503	0.899	3.001	0.863
3.002	0.924	2.750	0.896	3.002	0.862
		2.994	0.893		

The higher deviation for the L-serine system is not surprising taking into consideration how the ternary interaction parameters were estimated. Ferreira et al.[10] based their parameter estimation mainly on electrolyte activity coefficient data in the presence of an amino acid, for which the maximum KCl concentration was always 1 *m*, whereas for amino acids the corresponding maximum molalities were 1.6, 2.4, and 0.4 for DL-alanine, glycine, or L-serine, respectively. Therefore, extrapolating the calculation of water activity in aqueous 1 *m* KCl solutions at 298.15 K up to 4 *m* is, perhaps, too demanding.

Naturally, a similar analysis done for the aqueous 3 *m* KCl solutions at 298.15 K reveals much higher deviations between the calculated and the experimental water activity values: the rmsd's are now an order of magnitude larger, which for this particular thermodynamic property is very considerable. Therefore, it is important to state that the measured ternary data can be very useful to extend consistently the thermodynamic description of these highly complex systems to broader salt and amino acid molalities.

Conclusions

A new simple, fast, and reliable experimental procedure was proposed to measure water activity in aqueous amino acid solutions with or without a salt. A high number of new experimental data have been measured for aqueous KCl solutions at 0.0, 1.0, 2.0, and 3.0 salt molalities containing DL-alanine, glycine, or L-serine at 298.15 K. Generally, for binary water + amino acid systems, water activity data are reproducible when compared to the values reported in the literature obtained by the classic isopiestic method.

Additionally, the simple theoretical approach implemented allows the calculation of unsymmetric molal amino acid activity coefficients in water at 298.15 K, highly consistent with those calculated applying a virial expansion to fit osmotic coefficients measured by the isopiestic method. These promising results suggest that the proposed methodology may be used to obtain the activity coefficients of a nonvolatile component in water in a much quicker way than the isopiestic method.

Table 5. Water Activity in Aqueous L-Serine Solutions with KCl at 298.15 K

m_{KCl} = 1.000		m_{KCl} = 2.000		m_{KCl} = 3.000	
L-serine molality	a_w	L-serine molality	a_w	L-serine molality	a_w
0.300	0.964	0.250	0.933	0.251	0.899
0.486	0.961	0.500	0.930	0.500	0.896
0.802	0.957	0.501	0.930	0.751	0.893
0.972	0.954	0.750	0.928	0.997	0.890
1.298	0.951	0.998	0.923	1.247	0.888
1.501	0.948	0.999	0.924	1.495	0.885
1.803	0.944	1.250	0.919	1.748	0.882
1.940	0.941	1.498	0.916	1.753	0.882
1.944	0.942	1.499	0.916	2.000	0.877
2.251	0.937	1.749	0.913	2.005	0.878
2.422	0.936	2.000	0.910	2.247	0.875
2.505	0.934	2.000	0.909	2.249	0.876
2.752	0.931	2.246	0.907	2.496	0.871
2.999	0.928	2.493	0.902	2.503	0.870
2.999	0.927	2.502	0.903	2.746	0.868
3.244	0.924	2.756	0.900	3.002	0.864
3.247	0.923	2.998	0.896	3.004	0.864
3.494	0.921	3.003	0.896	3.242	0.861
3.507	0.921	3.240	0.893	3.499	0.859
3.747	0.917	3.498	0.890	3.499	0.858
3.883	0.915	3.741	0.886	3.747	0.855
3.999	0.914	3.993	0.883	3.987	0.853
		4.000	0.883	3.998	0.853

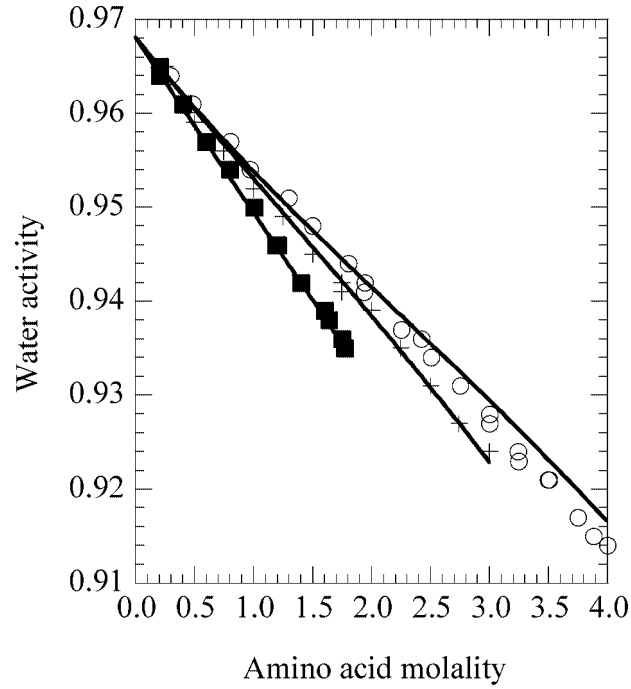

Figure 4. Water activity in aqueous 1 *m* KCl solutions containing amino acids at 298.15 K: —, calculated with the model[10] and experimental data; ■, DL-alanine; +, glycine; ○, L-serine.

Finally, it is also important to mention the usefulness of the water activity measured in the ternary systems to extend, consistently, the thermodynamic description of water + amino acid + electrolyte systems to broader salt and amino acid molalities.

Acknowledgment

I thank Eugénia Macedo for helpful discussions.

Literature Cited

(1) Romero, C. M.; González, M. E. Osmotic and Activity Coefficients of Glycine, DL-α-alanine, and DL-α-aminobutyric Acid in Aqueous Solutions at Temperatures between 288.15 and 303.15 K. *Fluid Phase Equilib.* **2006**, *250*, 99–104.

(2) Kamali-Ardakani, M.; Modarress, H.; Taghikhani, V.; Khoshkbarchi, M. K. Activity Coefficients of Glycine in Aqueous Electrolyte Solutions: Experimental Data for (H_2O + KCl + Glycine) at T = 298.15 K and (H_2O + NaCl + Glycine) at T = 308.15 K. *J. Chem. Thermodyn.* **2001**, *33*, 821–836.

(3) Kuramochi, H.; Noritomi, H.; Hoshino, D.; Nagahama, K. Measurements of Vapor Pressures of Aqueous Amino Acid Solutions and Determination of Activity Coefficients of Amino Acids. *J. Chem. Eng. Data* **1997**, *42*, 470–474.

(4) Smith, E. R. B.; Smith, P. K. The Activity of Glycine in Aqueous Solution at Twenty-Five Degrees. *J. Biol. Chem.* **1936**, *117*, 209–216.

(5) Smith, P. K.; Smith, E. R. B. Thermodynamics Properties of Solutions of Amino Acids and Related Substances. II. The Activity of Aliphatic Acids in Aqueous Solution at Twenty-Five Degrees. *J. Biol. Chem.* **1937**, *121*, 607–613.

(6) Ninni, L.; Meirelles, J. A. Water Activity, pH and Density of Aqueous Amino Acid Solutions. *Biotechnol. Prog.* **2001**, *17*, 703–711.

(7) Ferreira, L. A.; Macedo, E. A.; Pinho, S. P. The Effect of KCl and Na_2SO_4 on the Solubility of Glycine and DL-Alanine in Water at 298.15 K. *Ind. Eng. Chem. Res.* **2005**, *44*, 8892–8898.

(8) http://www.novasina.ch/wEnglisch/Produkte/Wasseraktivitaet/Items/a_LabMaster_aw.php.

(9) Archer, D. G. Thermodynamics Properties of the KCl + H_2O System. *J. Phys. Ref. Data* **1999**, *28*, 1–17.

(10) Ferreira, L. A.; Macedo, E. A.; Pinho, S. P. KCl Effect on the Solubility of Five Different Amino Acids in Water. *Fluid Phase Equilib.* **2007**, *255*, 131–137.

(11) Karplus, P. A. Hydrophobicity Regained. *Protein Sci.* **1997**, *6*, 1302–1307.

(12) Lin, R.; Hu, X.; Ren, X. Homogeneous Enthalpic Interaction of Amino Acids in DMF-H_2O Mixed Solvents. *Thermochim. Acta* **2000**, *352–353*, 31–37.

(13) Ellerton, H. D.; Reinfelds, G.; Mulcahy, D. E.; Dunlop, P. J. Activity, Density, and Relative Viscosity Data for Several Amino Acids, Lactamide, and Raffinose in Aqueous Solution at 25 °C. *J. Phys. Chem.* **1964**, *68*, 398–402.

(14) Abrams, D. S.; Prausnitz, J. M. Statistical Thermodynamics of Liquid Mixtures: A New Expression for the Excess Gibbs Energy of Partly or Completely Miscible Systems. *AIChE J.* **1975**, *21*, 116–128.

(15) Clegg, S. L.; Pitzer, K. S. Thermodynamics of Multicomponent, Miscible, Ionic Solutions: Generalized Equations for Symmetrical Electrolytes. *J. Chem. Phys.* **1992**, *96*, 3513–3520.

(16) Hu, Y.-F.; Guo, T.-M. Thermodynamics of Electrolytes in Aqueous Systems Containing Both Ionic and Nonionic Solutes. Application of the Pitzer-Simonson-Clegg Equations to Activity Coefficients and Solubilities of 1:1 Electrolytes in Four Electrolyte-Non-Electrolyte-H_2O Ternary Systems at 298.15 K. *Phys. Chem. Chem. Phys.* **1999**, *1*, 3303–3308.

(17) Prausnitz, J. M.; Lichenthaler, R. N.; de Azevedo, E. G. *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed.; Prentice-Hall: Englewood Cliffs, 1998.

(18) Fasman, G. D. *Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data*, 3rd ed.; CRC Press: Cleveland, 1976.

(19) Hutchens, J. O.; Figlio, K. M.; Granito, S. M. An Isopiestic Comparison Method for Activities. *J. Biol. Chem.* **1963**, *238*, 1419–1422.

Received for review August 15, 2007. Accepted November 03, 2007. Financial support for this work was in part provided by national research grant FCT/POCTI/40179/EQU/2001 and by LSRE financing by FEDER/POCI/2010, for which the author is thankful.

JE700466S