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Experimental data and modelling of apparent molar volumes, isentropic compressibilities and refractive indices in aqueous solutions of glycine + NaCl

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Abstract

Experiments have been performed at 298.15 K to measure the density, sound velocity and refractive index of glycine in aqueous solutions of NaCl over a wide range of both glycine and NaCl concentrations. The values of apparent molar volume and isentropic compressibility of glycine were calculated from the measured data. The results show a positive transfer volume of glycine from an NaCl solution to a more concentrated NaCl solution. This indicates that the size of a glycine molecule is larger in a solution with higher NaCl concentration. The negative values of apparent isentropic compressibility imply that the water molecules around the glycine molecules are less compressible than the water molecules in the bulk solution. These effects are attributed to the doubly charged behaviour of glycine and to the formation of physically bonded ion-pairs between the charged groups of glycine and sodium and chloride ions. The formation of ion-pairs, whose extents of binding reactions depend on the concentrations of both NaCl and glycine, alter the hydration number of glycine. This also explains the reason for the increase in the size of glycine with an increase in the NaCl concentration. A model based on the Pitzer formalism has been developed to correlate the activity coefficient, apparent molar volume and isentropic compressibility of glycine in aqueous solutions of NaCl. The results show that the model can accurately correlate the interactions in aqueous solutions of glycine and NaCl.

Keywords: Molar volume; Isentropic compressibility; Refractive index; Glycine; NaCl

1. Introduction

Interactions of proteins with their surrounding environment play an important role in their conformational characteristics. These interactions are mainly between the protein molecules and the solvent, ions, and side chains of other protein molecules. Most of these interactions, such as hydrogen bonding and electrostatic interactions, have non-covalent natures. The study of these interactions provides an important insight into

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the conformational stability and unfolding behaviour of globular proteins. However, proteins are complex molecules and their behaviour in solutions is governed by a combination of many more specific interactions. One approach that reduces the degree of complexity in the study of these interactions and requires less complex measurement techniques is to study the interactions in systems containing smaller biomolecules, such as amino acids. As amino acids are the building blocks of the proteins, their study provides important information, which can be related to the behaviour of larger biomolecules such as proteins.

Measurement of the density and sound velocity in aqueous solutions of amino acids and peptides without the presence of an electrolyte has been the subject of some investigations [1–5]. In all these studies efforts have been made to generalise the measured values for simple amino acids to more complex biomolecules and attribute the observed phenomena to the interactions in the systems under investigation. For example Iqbal and Verrall [2] showed that the partial specific volumes of some proteins can be calculated, with a reasonable accuracy, from the partial specific volume of its constituent amino acids. Although the effect of electrolytes on the behaviour of biomolecules has been known for a long time and exploited for their salt-induced separation, few studies have been conducted to measure the interactions and physicochemical properties in these systems [6,7]. The results of these studies have revealed that the presence of an electrolyte drastically affects the behaviour of amino acids in solutions, which can be used for their separation and purification.

In this study we have measured the density, sound velocity and refractive index in water + glycine + NaCl systems at 298.15 K. Experiments have been performed over a wide range of glycine and NaCl concentrations. The values of apparent molar volume, V_ϕ, and apparent molar isentropic compressibility, K_ϕ, were then calculated from the measured data. A model based on the Pitzer formalism [8] has been developed to correlate the activity coefficient, V_ϕ and K_ϕ of glycine in aqueous solutions of NaCl. It is shown that the model can accurately correlate the interactions in water + glycine + NaCl systems

2. Materials and methods

Sodium chloride with nominal purity of > 99.8 mass% was purchased from SIGMA (Madrid, Spain) and was oven-dried for 72 h prior to use. Glycine with a purity of > 99.0 mass% was purchased from ICN (Barcelona, Spain) and was used without further purification. In all experiments deionized water produced by a Milli-Q water deionizer was used. Prior to use, the deionized water was ultrasonically degassed.

All the solutions were prepared by weight on a Mettler AE 240 balance precise to within ±0.0001 g. In order to prepare the solutions under investigation, aqueous solutions of each salt at different concentrations were first prepared. A known amount of glycine was then added to a salt solution prepared in advance and stirred until a homogeneous solution was obtained.

The densities and sound transmission speeds of the mixtures were measured on an Anton Paar DSA-48 densimeter and sound analyser. The instrument precision was within ± 0.0001 g/cm^3 for the density and ±1.0 m/s for the velocity of sound. The refractive indices were measured on an ATAGO RX-1000 refractometer precise to within ±0.0001. In order to maintain the sample temperature constant during the experiments, all the instruments were equipped with a thermostatic bath and the temperature was kept constant at 298.15 ± 0.02 K throughout the experiments. All measurements were repeated three times for each sample, and were repeatable to within the precision quoted for each apparatus.

3. Results and discussions

Experiments were performed at 298.15 K to measure the density, sound velocity and refractive index in water + glycine + NaCl systems. The measured experimental data for density, sound velocity and refractive index are presented in Table 1. The apparent molar volumes, V_ϕ, and apparent molar isentropic compressibility, K_ϕ, of glycine at different NaCl molalities were calculated from the density and sound velocity experimental data according to the following relations:

Table 1
Measured values of density, sound velocity and refractive index and calculated values of apparent molar volume and isentropic compressibility in water + glycine + NaCl system

Glycine molality	Density (kg/m^3)	Sound velocity (m/s)	Refractive index	$V_\phi \times 10^6$ (m^3/mol)	$K_\phi \times 10^{15}$ (m^3/kPa per mol)
NaCl concentration = 0.0 m					
0.000	997.0	1497	1.3325	–	–
0.4972	1012.4	1523	1.3389	43.46	−25865.1
1.0226	1027.4	1548	1.3454	44.05	−23312.9
1.4867	1039.9	1569	1.3508	44.36	−21830.6
1.9724	1052.2	1591	1.3562	44.67	−19818.5
2.4091	1062.6	1609	1.3606	44.94	−19074.2
2.9291	1074.3	1630	1.3657	45.24	−17724.4
NaCl concentration = 0.2 m					
0.000	1005.2	1510	1.3343	–	–
0.4975	1020.1	1535	1.3408	44.38	−21529.7
1.0038	1034.3	1559	1.3469	44.69	−19870.9
1.4771	1046.7	1580	1.3523	45.02	−18453.8
1.9545	1058.4	1601	1.3575	45.34	−17370.8
2.4064	1069.0	1619	1.3620	45.55	−16397.4
2.908	1080.0	1639	1.3668	45.82	−15324.7
NaCl concentration = 0.4 m					
0.000	1013.2	1523	1.3363	–	–
0.4968	1027.6	1547	1.3425	45.21	−19344.3
1.0077	1041.5	1570	1.3485	45.46	−17528.0
1.4603	1053.1	1590	1.3536	45.68	−16618.8
1.9653	1065.3	1611	1.3590	45.91	−15522.2
2.4890	1077.2	1632	1.3642	46.13	−14433.5
2.9521	1087.1	1650	1.3687	46.33	−13762.1
NaCl concentration = 0.6 m					
0.000	1021.0	1535	1.3383	–	–
0.5013	1035.2	1558	1.3445	45.72	−16972.7
0.9827	1048.0	1580	1.3499	45.95	−16332.5
1.4533	1059.8	1600	1.3550	46.16	−14641.7
1.9959	1072.5	1622	1.3608	46.43	−13988.0
2.4717	1083.0	1641	1.3654	46.63	−12932.2
2.9309	1092.6	1658	1.3697	46.81	−12149.2
NaCl concentration = 0.8 m					
0.0000	1028.6	1546	1.3401	–	–
0.4939	1042.3	1569	1.3461	46.15	−15464.1
1.0087	1055.6	1591	1.3520	46.46	−14428.3
1.4861	1067.2	1611	1.3570	46.68	−13240.9
1.9150	1077.1	1628	1.3615	46.84	−12544.6
2.5167	1090.1	1651	1.3673	47.07	−11611.1
3.0780	1101.5	1672	1.3724	47.25	−10544.1

Table 1

Glycine molality	Density (kg/m^3)	Sound velocity (m/s)	Refractive index	$V_\phi \times 10^6$ (m^3/mol)	$K_\phi \times 10^{15}$ (m^3/kPa per mol)
NaCl concentration = 1.0 m					
0.0000	1036.2	1558	1.3419	–	–
0.4941	1049.6	1580	1.3479	46.59	−13454.7
0.8911	1059.7	1597	1.3524	46.82	−12999.2
1.4558	1073.1	1620	1.3583	47.16	−11762.9
1.8365	1081.7	1635	1.3623	47.30	−10961.1
2.455	1094.9	1658	1.3681	47.49	−10178.6
3.1226	1108.2	1682	1.3741	47.66	−9211.7

$$V_\phi = \frac{M}{\rho} - \frac{\rho - \rho_0}{m \rho \rho_0} \tag{1}$$

$$K_\phi = \frac{M \kappa_S}{\rho} - \frac{\kappa_{S0}\, \rho - \kappa_S\, \rho_0}{m \rho \rho_0} \tag{2}$$

where κ_{S0} and κ_S are isentropic compressibilities of solvent and water + NaCl solution, respectively. The isentropic compressibility is defined as:

$$\kappa_S = \frac{1}{u^2 \rho} \tag{3}$$

In Eqs. (1)–(3), M denotes the molecular weight of glycine, m is the molality of glycine, u is the sound velocity and ρ and ρ_0 are the densities of the solution and solvent, respectively. For systems containing both NaCl and glycine, the water + NaCl is considered as the solvent. The values of V_ϕ and K_ϕ of glycine at different NaCl molalities, calculated from Eqs. (1)–(3), are presented in Table 1. The values of the infinite dilution apparent molar volume, V_ϕ^0, and infinite dilution apparent molar isentropic compressibility, K_ϕ^0, of glycine are also two important properties. They provide valuable information about the interactions in the water + glycine + NaCl system and can be calculated from the values of V_ϕ and K_ϕ. The results show that at a constant NaCl molality, the values of both V_ϕ and K_ϕ of glycine vary almost linearly with the molality of glycine. To calculate the values of V_ϕ^0 and K_ϕ^0 of glycine, the values of V_ϕ and K_ϕ at each NaCl molality were fitted by a least square method to the following equations:

$$V_\phi = V_\phi^0 + \chi_v m_A \tag{4}$$

$$K_\phi = K_\phi^0 + \chi_S m_A \tag{5}$$

where χ_v and χ_S are two empirical constants calculated from the fitting of the experimental data to Eqs. (4),(5) and m_A is the molality of glycine. The values of V_ϕ^0 and K_ϕ^0 of glycine along with the values of χ_v and χ_S at different NaCl concentrations are presented in Table 2. The value of the infinite dilution apparent molar

Table 2
Calculated values of V_ϕ^0, K_ϕ^0 and the slopes of Eqs. (4),(5), χ_v and χ_S, obtained from the fitting of the experimental data

NaCl molality	$V_\phi^0 \times 106$ (m^3/mol)	$\chi_v \times 10^6$ (kg/m^3 per mol^2)	$K_\phi^0 \times 10^5$ (m^3/kPa per mol)	$\chi_S \times 10^5$ (kg m^3/kPa mol^{-2})
0.0	43.235	0.70817	−26954.0	3305.03
0.2	43.737	0.722481	−22662.9	2596.02
0.4	45.005	0.452466	−20045.4	2223.81
0.6	45.504	0.452625	−18011.0	2036.61
0.8	46.013	0.416585	−16268.1	1883.69
1.0	46.475	0.406124	−14273.0	1664.53

volume of glycine in pure water measured in this study was compared with the values reported in the literature [2] and our measurement was found to be different by 0.02% from the average of the reported values in the literature.

Fig. 1 shows the effect of glycine concentration on its apparent molar volume at different levels of NaCl concentration. As shown in this figure the value of V_ϕ for glycine increases as the concentration of either glycine or NaCl increases. This indicates that glycine molecules are larger in size in solutions with higher NaCl concentration. This effect can be attributed to the fact that the amino and carboxyl groups of glycine dissociate in aqueous solutions and become respectively, negatively and positively charged. The dissociation of glycine molecules in the presence of NaCl leads to the formation of physically bonded ion-pairs between the charged groups of glycine and sodium and chloride ions produced from the dissociation of NaCl. The formation of the ion-pairs, on the one hand, increases the apparent molar volume of glycine and, on the other hand, reduces the electrostatic interactions between glycine molecules and water molecules and ions. This in turn reduces the interactions of water molecules and ions with the hydrocarbon backbone of glycine resulting in an increase in the number of water molecules attached to the amino and carboxyl groups of glycine in the form of hydration water. This effect can also be seen from the increasing values of V_ϕ^0 with an increase in the NaCl concentration presented in Table 2. An increase in the ionic strength also gives rise to the clustering effect in which the glycine molecules form aggregates. This is a result of a repulsive force between the hydrophobic hydrocarbon backbone of glycine and its densely charged environment. It should be noted that the clustering effect occurs at the microscopic level and not in a phase-split form. It is important to mention that although values of V_ϕ^0 for glycine in pure water measured in this work and the value reported by Bhat and Ahluwalia [6] are different by only 0.04%, their difference increases to 4.2% in a solution of 1.0 molal NaCl.

Fig. 2 shows the effect of glycine concentration on its apparent molar isentropic compressibility at different levels of NaCl concentration. As shown in Fig. 2 the value of K_ϕ of glycine is negative throughout the entire range of NaCl and glycine concentration and it increases as the concentration of glycine increases. It can also be seen from Fig. 2 that for a constant glycine concentration, the value of K_ϕ is larger at higher electrolyte concentrations. It is important to note that although V_ϕ to some extent reflects the interactions between molecules in the solution, they can be highly influenced by the size of the molecules. The intrinsic size of a molecule in a solution may be influenced by different factors, such as a change in the bond angles, which in turn can be a result of a change in the physicochemical properties of the solvent at different salt concentrations. On the other hand, K_ϕ can better reflect the effect of the interactions between the solute and the solvent and the hydration of the molecules. Hydration number particularly plays an important role in interactions in water + amino acid + electrolyte systems. The doubly charged behaviour of glycine in aqueous solutions and the presence of charged simple ions in the solution are the main reasons for the importance of the hydration on the interactions in water + glycine + NaCl systems. In fact, the values of K_ϕ can be related to the values of V_ϕ through the following relation:

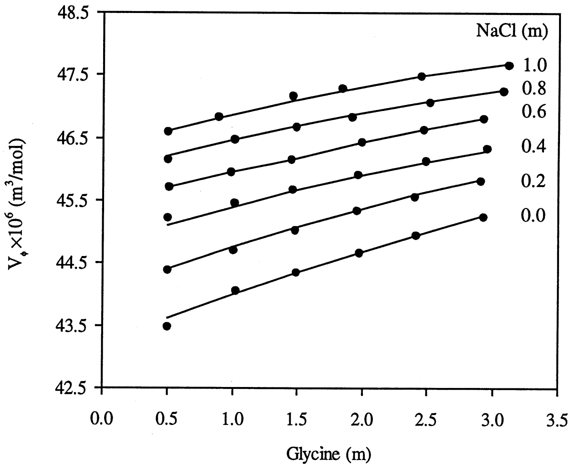

Fig. 1. Effect of glycine concentration on the values of V_ϕ at different levels of NaCl concentration. ●, Experimental data; ——, results obtained from Eq. (11).

$$K_{\phi} = -\left(\frac{\partial V_{\phi}}{\partial P}\right)_S \tag{6}$$

The negative values of K_{ϕ} and K_{ϕ}^0, presented in Tables 1 and 2, indicate that the water molecules around the glycine molecules are less compressible than the water molecules in the bulk solution. This can be due to the fact that the water molecules around a glycine molecule are under the influence of the electrostatic field arisen from the charged groups of the glycine. As mentioned before, at higher NaCl concentrations, the charges on more glycine molecules are neutralized as a result of the formation of ion-pairs between the sodium and chloride ions and charged groups of glycine. This suppresses the electrostatic interactions between glycine and water molecules and renders the water molecules more compressible.

Fig. 3 shows the effect of the glycine concentration on the refractive index in water + glycine + NaCl solutions at different levels of NaCl concentration. The change of the refractive index by a change in either glycine or NaCl concentration is an indication of the interactions between water, glycine and NaCl in the solutions. As the refractive index reflects the interactions in a system it can be related to other properties which also reflect the interactions in the system. For example, activity coefficient is an important thermodynamic property, which represents the interactions in a mixture. Fig. 4 shows the relation between the refractive index in water + glycine + NaCl system and the activity coefficient of glycine at the same concentration of glycine and NaCl. As can be seen from this figure, refractive index can be directly related to the activity coefficient. Unfortunately, theories relating refractive index to these interactions, particularly for systems containing electrolytes, are rather complex and in some cases are not well developed.

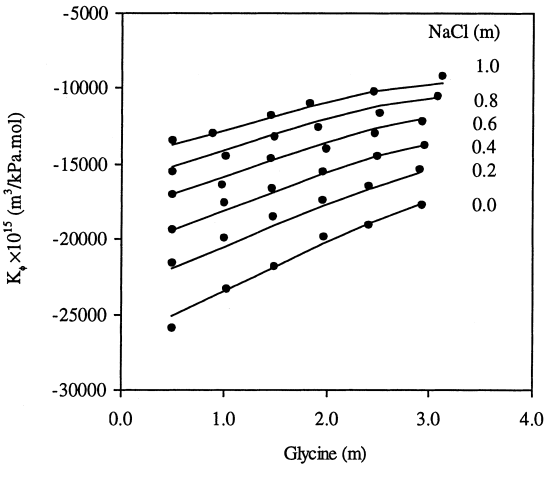

Fig. 2. Effect of glycine concentration on the values of K_{ϕ} at different levels of NaCl concentration. ●, Experimental data; ——, results obtained from Eq. (12).

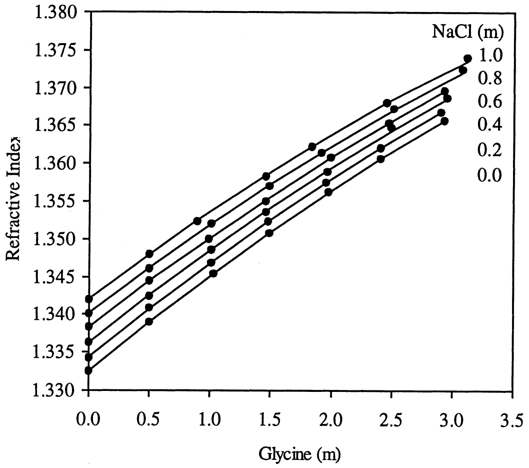

Fig. 3. Effect of glycine concentration on the refractive index in water + glycine + NaCl system at different levels of NaCl concentration. ●, Experimental data; ——, results obtained from Eq. (7).

Here, we relate the refractive index, n, to molalities of glycine and NaCl by a virial expansion type equation as:

$$n - n_0 = 0.01331m_A - 0.000676m_A^2 + 0.0096m_S - 0.00112m_S m_A \tag{7}$$

where n_0 is the refractive index in pure water equal to 1.3325 and m_S and m_A are the molalities of NaCl and glycine, respectively. The solid lines in Fig. 3 are the results of the calculations using Eq. (7).

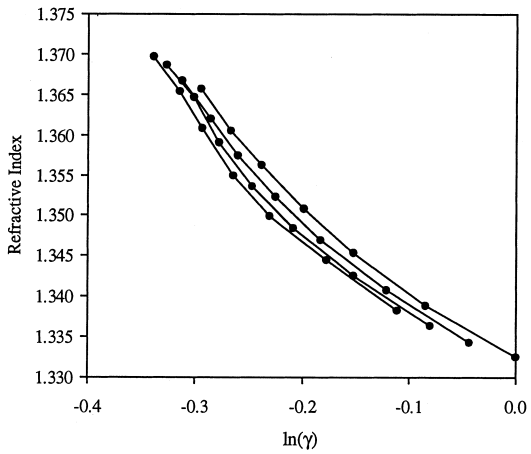

Fig. 4. Relation between the activity coefficient of glycine and the refractive index in water + glycine + NaCl system at different levels of NaCl concentration. ●, Experimental data; ——, solid lines to lead the eyes.

4. Modelling framework and results

The Pitzer formalism has been employed to model the experimental data of glycine in aqueous NaCL solutions obtained in this study and the activity coefficient data reported in the literature. According to the Pitzer formalism, the excess Gibbs free energy of a system containing a mixture of an electrolyte and a non-electrolyte in a solvent is written in the form of a virial expansion in terms of the molalities of the solutes [8]. The relation for other properties such as activity coefficient, K_ϕ and V_ϕ can be derived from the excess Gibbs free energy relation. Here, for simplicity, the derivations are given only for the ternary water + glycine + NaCl system. According to the Pitzer formalism the excess Gibbs free energy, G^E, for a system containing water, glycine and NaCl can be written as [8]:

$$\frac{G^E}{RT} = f(m_S) + \lambda_{SS} m_S^2 + \mu_{SSS} m_S^3 + \lambda_{AS} m_S m_A + \lambda_{AA} m_A^2 + \mu_{AAA} m_A^3 + \mu_{AAS} m_A^2 m_S + \zeta_{ASS} m_S^2 m_A \tag{8}$$

where the function f represents the Pitzer–Debye–Huckel model, which accounts for the long-range interactions in the system due to the presence of ions, and the parameters ζ, λ and μ are the virial coefficients. The subscripts A and S refer to the glycine and NaCl, respectively. The corresponding relations for the activity coefficient, K_ϕ and V_ϕ of glycine can respectively be derived by combining Eq. (8) and the following thermodynamic differential equations:

$$\ln\gamma_A = \frac{1}{RT}\left(\frac{\partial G^E}{\partial n_A}\right)_{T,P,n_S},$$

$$V_\phi - V_\phi^0 = \left(\frac{\partial \ln\gamma_A}{\partial P}\right)_T,$$

$$K_\phi - K_\phi^0 = -\left(\frac{\partial\left(V_\phi - V_\phi^0\right)}{\partial P}\right)_S \tag{9}$$

Consequently, the following equations for activity coefficient, K_ϕ and V_ϕ can be derived as:

$$\ln\gamma_A = 2\lambda_{AA} m_A + 3\mu_{AAA} m_A^2 + \frac{\partial \mu_{AAA}}{\partial m_A} m_A^3 + \lambda_{AS} m_S + \zeta_{ASS} m_S^2 + 2\mu_{AAS} m_S m_A \tag{10}$$

$$V_\phi = V_\phi^0 + 2\lambda_{AA}^V m_A + 3\mu_{AAA}^V m_A^2 + \frac{\partial \mu_{AAA}^V}{\partial m_A} m_A^3 + \lambda_{AS}^V m_S + \zeta_{ASS}^V m_S^2 + 2\mu_{ASS}^V m_S m_A \tag{11}$$

$$K_\phi = K_\phi^0 + 2\lambda_{AA}^K m_A + 3\mu_{AAA}^K m_A^2 + \frac{\partial \mu_{AAA}^K}{\partial m_A} m_A^3 + \lambda_{AS}^K m_S + \zeta_{ASS}^K m_S^2 + 2\mu_{AAS}^K m_S m_A \tag{12}$$

where a superscript V denotes that the parameter is the derivative of the corresponding parameter in Eq. (10) with respect to pressure at constant temperature and a superscript K denotes that the parameter is the derivative of the corresponding parameter in Eq. (11) with respect to pressure at constant entropy. In order to enhance the capability of Eqs. (10)–(12) to correlate the experimental data of glycine both in pure water and in

NaCl solution, the following modifications to the μ_{AAA} and μ_{AAS} parameters have been introduced.

$$\mu_{AAA} = \mu^0_{AAA} + \mu^1_{AAA} e^{-m_A} \tag{13}$$

$$\mu_{AAS} = \mu^0_{AAS} + \mu^1_{AAS} e^{-m_S} \tag{14}$$

The model developed here was employed to correlate the experimental data of K_ϕ and V_ϕ of glycine in aqueous solutions of NaCl obtained in this study and the activity coefficient data reported previously [4,9]. In order to evaluate the parameters of Eqs. (10)–(12), the respective independently measured experimental data for glycine in pure water [10] were first fitted. The relations for the properties of glycine in pure water were obtained by setting to zero the molality of NaCl in Eqs. (10)–(12). After setting the values of the parameters to the obtained values, Eqs. (10)–(12) were applied to correlate the experimental data in the water + glycine + NaCl ternary system. The solid lines in Figs. 1,2 and 5 show the results of the model. The values of the parameters of the model together with their relative root mean square deviation (r.m.s.d.) for water + glycine and water + glycine + NaCl systems are presented in Tables 3 and 4. As can be seen from Figs. 1,2 and 5 and the small values of r.m.s.d., presented in Tables 3 and 4, the model proposed in this study can accurately correlate the experimental data of activity coefficient, K_ϕ and V_ϕ of glycine in NaCl solutions over a wide range of concentration.

Table 3
Parameters of Eqs. (10)–(12) obtained from the fitting of the experimental data of water + glycine systems

λ_{AA}	μ^0_{AAA}	μ^1_{AAA}	r.m.s.d. × 100
−0.09204	0.00946	0.01695	0.08
λ^V_{AA}	$\mu^{0,\mathrm{V}}_{AAA}$	$\mu^{1,V}_{AAA}$	r.m.s.d. × 100
0.34911	−0.00083	0.08698	0.09
λ^K_{AA}	$\mu^{0,K}_{AAA}$	$\mu^{1,K}_{AAA}$	r.m.s.d. × 100
2263.9570	−153.5918	−750.2457	1.5

5. Conclusions

Experimental data at 298.15 K of density, sound velocity and refractive index of glycine in aqueous solutions of NaCl have been reported. The values of apparent molar volume and isentropic compressibility of glycine were calculated from the measured data. The results indicate that the interactions between glycine and NaCl highly influence the properties of their solutions. These interactions result in a positive transfer volume of glycine from solutions with lower concentration of NaCl to solutions with higher concentration of NaCl. This may indicate that an increase in the NaCl concentration increases the size of the glycine molecules in the solution. The negative values of apparent isentropic compressibility also imply that the water molecules around the glycine molecules are less compressible than the water molecules in the bulk solution. These effects are attributed to the doubly charged behaviour of glycine and the formation of physically bonded ion-pairs between the charged groups of glycine and sodium and chloride ions. The formation of these ion-pairs affects the charged behaviour of glycine and consequently the long-range interactions in the system. They also contribute to the change of the hydration number of the glycine molecules at different NaCl and glycine concentrations.

A model based on the Pitzer formalism has

Table 4
Parameters of Eqs. (10)–(12) obtained from the fitting of the experimental data of water + glycine + NaCl systems

λ_{AS}	ζ_{AAA}	μ^0_{AAS}	μ^1_{AAS}	r.m.s.d. × 100
−0.20840	0.06311	0.01002	0.01438	0.03
λ^V_{AS}	ζ^V_{AAA}	$\mu^{0,V}_{AAS}$	$\mu^{1,V}_{AAS}$	r.m.s.d. × 100
4.66152	−1.50690	0.04637	−0.59494	0.21
λ^K_{AS}	ζ^K_{AAA}	$\mu^{0,K}_{AAS}$	$\mu^{1,K}_{AAS}$	r.m.s.d. × 100
17835.05	−5809.1	−210.95	−390.82	0.05

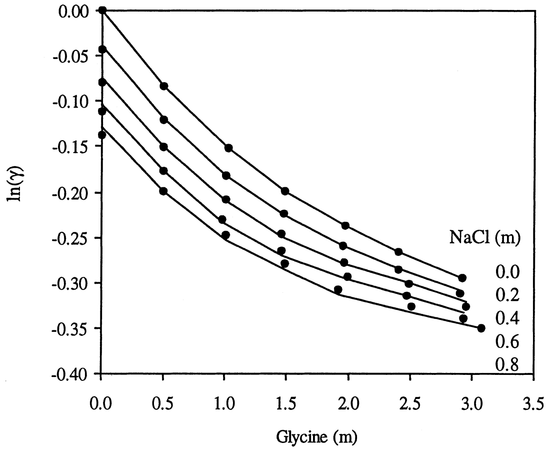

Fig. 5. Effect of glycine concentration on its activity coefficient in water + glycine + NaCl system at different levels of NaCl concentration. ●, Experimental data; ——, results obtained from Eq. (10).

been developed to correlate the activity coefficient, apparent molar volume and isentropic compressibility of glycine in aqueous solutions of NaCl. The results show that the Pitzer's model can accurately correlate the interactions in aqueous solutions of glycine and NaCl.

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