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Activity coefficients of the peptide and the electrolyte in ternary systems water + glycylglycine + NaCl, + NaBr, + KCl and + KBr at 298.2 K

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Abstract

The activity coefficients of glycylglycine in four aqueous electrolyte solutions (+NaCl, +NaBr, +KCl and +KBr) were obtained at 298.2 K. The mean ionic activity coefficient of the electrolyte in aqueous solutions containing the peptide was determined from measurements of the potential differences of a cation and an anion ion-selective-electrode, each vs. a double junction reference electrode. The results show that the nature of the anion has a major effect on the activity coefficients of glycylglycine. Comparison of activity coefficient data for glycylglycine with literature data for glycine, both in aqueous NaCl solutions, indicates that the effect of the electrolyte is larger for the peptide than for the amino acid. For the peptide, in all cases, the effect of the electrolyte is more important at low molalities of the electrolyte. The Wilson equation was used to correlate the activity coefficient data obtained. The correlation results were satisfactory for the region of concentrated electrolyte.

Keywords: Peptide; Glycylglycine; Electrolytes; Activity coefficients; Ion selective electrodes

1. Introduction

Equilibrium separation and purification processes, that are key steps for the production of biochemicals, occupy a major portion of their total manufacturing costs [1]. A typical example of equilibrium based separations is the salting out from aqueous solutions of amino acids and proteins. Quantitative studies of the interactions between biomolecules and electrolytes are the first elemental steps towards compiling background information that could be of use for the design of these separation processes. In addition, a knowledge of thermodynamic properties of biomolecules in electrolyte solutions may also provide clues to understand the behavior of physiological systems.

Our group has recently reported data for the activity coefficients of amino acids various electrolyte solutions [2–7], data for the effect of indi-

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vidual ions on the solubility of amino acids [8–11] and also for the effect of one amino acid on the solubility of another amino acid [12]. The present study is a natural continuation of the above work, and explores the effect of different cations and anions on the activity coefficient of glycylglycine in aqueous electrolyte solutions. No previous study has reported experimental data on the effect of individual ions on the activity coefficient of a peptide in aqueous solutions containing a single electrolyte. A comparison of the data for the systems containing either NaCl or KCl, and of the systems NaBr or KBr would show whether there is a distinct effect of the cations sodium and potassium in the presence of a particular counterion. Similarly, the comparison of data between systems containing either NaCl or NaBr and of systems containing either KCl or KBr, would show the effect of the anion, if any. As determined in previous studies [2–11], in the case of amino acids, the effect of individual ions can be quite distinctive.

2. Theory of experiments

In this study we use the electrochemical method developed by Koshkbarchi and Vera [2]. This method measures the individual response of a cation and an anion ion-selective electrode (ISE), measured against a double junction reference electrode (DJRE). The advantages of this method over the traditional isopiestic measurements of the activity of water [13–15], is that it is much faster, and it produces more accurate data in the dilute range of concentration of the electrolyte and the biomolecule. In comparison with the electrochemical method that uses a single ion selective electrode [16–18], the method used here eliminates the uncertainty introduced by the assumption that variation of the mean ionic activity of the electrolyte can be followed by the response obtained from a single ion. As discussed in detail elsewhere [2–7], the difference ΔE between the potential E_+ of a cation ISE measured against a double junction reference electrode (DJRE) and the potential E_- of an anion ISE measured against the same DJRE, is related to the mean ionic activity coefficient of the electrolyte, at a molality m_s, by Nernst equation, namely:

$$E_+ - E_- = \Delta E = E^0 + S\ln(m_s\gamma_\pm) \tag{1}$$

where, for generality, S is introduced to replace the Nernstian slope. The term E^0 groups the values of the standard potential of the reference and the ion selective electrodes. The junction potential of the reference electrode cancels out when taking the difference $(E_+ - E_-)$. The values of E^0 and S were obtained from linear fitting system of ΔE vs. ln $(m_s\gamma_\pm)$ for a binary system of the electrolyte in water. The values of $\gamma_\pm$ each molality were obtained from the literature [19], and the values of ΔE were obtained from measurements in a cell of the type 1 below:

2.1. Electrochemical cell 1:

Cation ISE | electrolyte (m_s) | DJRE.
Anion ISE | electrolyte (m_s) | DJRE.

The potential differences in this cell and the corresponding mean ionic activity coefficients of the electrolyte in pure water are designated by a superscript one. Thus, we write:

$$\Delta E^{(1)} = E^0 + S\ln(m_s\gamma_\pm^{(1)}). \tag{2}$$

Similar measurements are carried out in a cell of type 2, containing a fixed molality m_s of electrolyte at different molalities m_p of the peptide:

2.2. Electrochemical cell 2:

Cation ISE | electrolyte (m_s) + peptide (m_p) | DJRE
Anion ISE | electrolyte (m_s) + peptide (m_p) | DJRE

For this cell type 2, containing a ternary system (water + electrolyte + peptide), we write:

$$\Delta E^{(2)} = E^0 + S\ln(m_s\gamma_\pm^{(2)}) \tag{3}$$

Combining Eq. (2) and Eq. (3), gives:

$$\ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right) = \frac{\Delta E^{(2)} - \Delta E^{(1)}}{S} \tag{4}$$

Thus, as the value of $\gamma_{\pm}^{(1)}$ is known for the particular molality m_s of the electrolyte, the value of the mean ionic activity coefficient of the electrolyte in the presence of the peptide, $\gamma_{\pm}^{(2)}$, at molality m_p, can be calculated. In fact, the important term is the ratio of the two values of the mean ionic activity coefficient. Eq. (4) clearly shows that $\gamma_{\pm}^{(2)}$ is normalized to unity with respect to the state of the electrolyte at infinite dilution in water in the absence of peptide. At any fixed value of m_s, as m_p goes to zero, $\Delta E^{(2)}$ tends to the value of $\Delta E^{(1)}$ and $\gamma_{\pm}^{(2)}$ tends to the value $\gamma_{\pm}^{(1)}$. On the other hand, at any fixed value of m_p, as m_s goes to zero, $\Delta E^{(2)}$ tends to a value different from the value of $\Delta E^{(1)}$ and while $\gamma_{\pm}^{(1)}$ tends to unity, $\gamma_{\pm}^{(2)}$ tends to a value different from unity called the trace value of the mean ionic activity coefficient. The activity coefficient of the peptide is related to the mean ionic activity coefficients of the electrolyte through the cross-differential relation [20]:

$$\left(\frac{\partial \ln \gamma_p}{\partial m_s}\right)_{m_p,T,P} = \nu\left(\frac{\partial \ln \gamma_{\pm}}{\partial m_p}\right)_{m_s,T,P} \tag{5}$$

where, for all the electrolytes used in this study, the stoichiometric number of ions produced per mol of electrolyte, $\nu = 2$. Hence, an adequate function is fitted to the numerical values obtained from Eq. (4) for experimental measurements a constant value of m_s with different values of m_p. Then, after proper differentiation and integration of this function, the values of the activity coefficients of the peptide in the aqueous electrolyte solution can be obtained using Eq. (5), together with a knowledge of the activity coefficient $\gamma_p^{(1)}$ of the peptide at molality m_p, in pure water.

3. Materials and methods

Sodium chloride, sodium bromide, potassium chloride and potassium bromide of 99% grade were purchased from A&C American Chemicals (Montreal, Quebec, Canada) and glycylglycine of purity higher than 99%, was obtained from Sigma-Aldrich (Canada). The salts were oven-dried for 72 h prior to use. The ion-selective electrodes used for sodium (model 84-11), chloride (model 94-17B), potassium (model 93-19) and the double junction reference electrode (model 92-02), were obtained from Orion (Boston, MA). An Accumet ion-selective electrode for bromide (13-620-521) was purchased from Fischer Scientific. An Orion pH/ISE meter (Boston, MA) model EA 920 with a resolution of ± 0.1 mV, was used to monitor the potential difference measurements.

All the solutions were prepared by weight. The compositions of the initial solutions were accurate within ± 0.01 wt.%. Before preparing samples, the distilled water was passed through ion exchange columns type Easy pure RF, Compact Ultrapure Water System, Barnstead Thermoline (Bubugue, IA). Deionized water with a conductivity of less than 0.8 μS/cm was used in all experiments. The electrodes were conditioned according to the manufacturer's instructions.

The experiments were performed by measuring simultaneously the potential difference of both the cation and the anion ISE, against the same double junction reference electrode, in a jacketed glass beaker containing 150 ml of solution. In this way, the effect of the junction potential of the reference electrode was minimized. All the instruments were grounded prior to and during the experiments. The presence of concentration gradients in the beaker was minimized by constant stirring of the solution during the experiments. The temperature was kept constant at 298.2 ± 0.1 K using a thermostatic bath, as shown in Fig. 1.

Each set of experiments was performed at a fixed electrolyte concentration, increasing the concentration of the peptide by additions of solid peptide accurately weighed. The readings of the potentiometer were made only when the drift of the response was less than 0.1 mV in approximately 10 min. For each set of experiments the electrodes were calibrated by measuring the re-

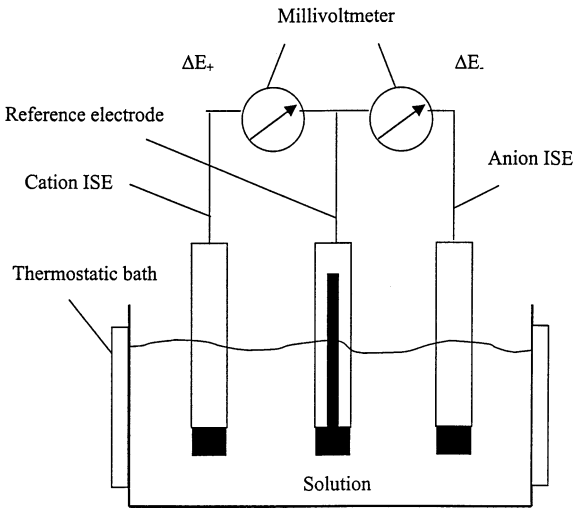

Fig. 1. Schematic view of the experimental set-up for the measurement of the mean ionic activity coefficient of an electrolyte in aqueous solution.

sponse of the electrode in the electrolyte solution without the presence of the peptide. The slope S of the response of the electrodes was independently obtained for each system measured. While the theoretical value of S at 298.2 K is 51.38 mV according to the Nernst equation, slightly different values of S were obtained for the cell containing NaCl (50.71 mV), and for the cell containing KCl (50.66 mV). For the cases of NaBr and KBr, the slopes were 45.44 and 34.50 mV, respectively.

Most of the experiments were replicated three times and the data reported are the average of the replicas. Sample variances were obtained from the replicas for each point and a pooled standard deviation was calculated using these values. The calculated pooled standard deviations, for a 95% confidence interval for the values of the ratio of the mean ionic activity coefficients of the electrolyte in the presence of the peptide and in the absence of the peptide, at the same electrolyte molality, were less than ± 0.01 for all cases.

4. Experimental results

The potentials of the corresponding cation and anion ISE, each against the same double junction reference electrode, were measured for the following four systems: H_2O + NaCl + glycylglycine; H_2O + NaBr + glycylglycine; H_2O + KCl + glycylglycine; and H_2O + KBr + glycylglycine. The highest electrolyte concentration used was 1.0 m and the highest glycylglycine concentration was 1.5 m. The experimental results for each system are presented in Tables 1–4. As discussed above, the electrochemical potentials obtained were used to calculate ratios of the mean ionic activity coefficients of the electrolyte in the presence and in the absence of the peptide. A different fitting equation was used for each system, as a single equation did not provide a satisfactory fit for all systems. In all previous studies [2–7] virial expansions with up to six parameters were used, as suggested by Scatchard and Pentiss [21]. Fol-

Table 1
Data for the ratio of the mean ionic activity coefficients of NaCl in the presence of glycylglycine to those in the absence of glycylglycine, at different molalities of NaCl and glycylglycine

Glycylglycine	$\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$ for NaCl (m)				
(m)	0.1	0.3	0.5	0.7	1.0
0.1	0.9708	0.9853	0.9925	0.9935	0.9980
0.2	0.9472	0.9734	0.9796	0.9853	0.9895
0.3	0.9266	0.9601	0.9706	0.9760	0.9837
0.5	0.8937	0.9367	0.9520	0.9595	0.9699
0.7	0.8675	0.9190	0.9365	0.9467	0.9598
1	0.8377	0.8934	0.9173	0.9282	0.9445
1.3	0.8145	0.8754	0.9024	0.9146	0.9337
1.5	0.8014	0.8663	0.8942	0.9071	0.9267

Table 2
Data for the ratio of the mean ionic activity coefficients of NaBr in the presence of glycylglycine to those in the absence of glycylglycine, at different molalities of NaBr and glycylglycine

Glycylglycine	$\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$ for NaBr (m)				
(m)	0.1	0.3	0.5	0.7	1.0
0.1	0.9395	0.9898	0.9912	0.9927	0.9931
0.2	0.9051	0.9743	0.9739	0.9747	0.9865
0.3	0.8786	0.9548	0.9615	0.9714	0.9772
0.5	0.8389	0.9265	0.9385	0.9492	0.9597
0.7	0.8031	0.8974	0.9167	0.9306	0.9447
1	0.7576	0.8676	0.8919	0.9070	0.9242
1.3	0.7266	0.8416	0.8683	0.8879	0.9064
1.5	0.7085	0.8276	0.8566	0.8753	0.8978

lowing the thesis research work done at McGill University by Hamelink [22], we use three-parameter expressions to fit the mean ionic activity coefficient data. These expressions may generate complex algebraic functions after differentiation and integration but the reduction in the number

Table 3
Data for the ratio of the mean ionic activity coefficients of KCl in the presence of glycylglycine to those in the absence of glycylglycine at different molalities of KCl and glycylglycine

Glycylglycine	$\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$ for KCl (m)				
(m)	0.1	0.3	0.5	0.7	1.0
0.1	0.9798	0.9872	0.9911	0.9915	0.9977
0.2	0.9632	0.9753	0.9817	0.9860	0.9934
0.3	0.9484	0.9657	0.9721	0.9782	0.9885
0.5	0.9247	0.9453	0.9568	0.9651	0.9791
0.7	0.9075	0.9271	0.9412	0.9521	0.9702
1	0.8868	0.9060	0.9241	0.9382	0.9584
1.3	0.8718	0.8924	0.9111	0.9265	0.9512
1.5	0.8621	0.8822	0.9048	0.9228	0.9475

Table 4
Data for the ratio of the mean ionic activity coefficients of KBr in the presence of glycylglycine to those in the absence of glycylglycine, at different molalities of KBr and glycylglycine

Glycylglycine	$\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$ for KBr (m)				
(m)	0.1	0.3	0.5	0.7	1.0
0.1	0.9401	0.9589	0.9798	0.9748	0.9892
0.2	0.8890	0.9311	0.9658	0.9697	0.9799
0.3	0.8367	0.8970	0.9466	0.9608	0.9725
0.5	0.7686	0.8473	0.9158	0.9322	0.9555
0.7	0.7079	0.8031	0.8859	0.9066	0.9337
1	0.6383	0.7457	0.8490	0.8758	0.9091
1.3	0.5893	0.7043	0.8175	0.8492	0.8892
1.5	0.5596	0.6714	0.7962	0.8334	0.8729

of parameters required justified the minor complexity added in the calculations.

4.1. Systems $H_2O + NaCl + glycylglycine$ and $H_2O + NaBr + glycylglycine$

The following semi-empirical equation proposed by Hamelink [22], was used to fit the values of mean ionic activity coefficient ratio of NaCl and NaBr, in the presence and in the absence of the peptide.

$$\nu \ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right) = \frac{a m_p}{\left(1 + b\sqrt{m_s}\right)(1 + c m_p)} \tag{6}$$

with $\nu = 2$. This function gives the expected limits, as at fixed value of m_s, as m_p goes to zero, $\gamma_{\pm}^{(2)}$ tends to the value of $\gamma_{\pm}^{(1)}$. Similarly, at any fixed value of m_p, m_s goes to zero, while $\gamma_{\pm}^{(1)}$ tends to unity, $\gamma_{\pm}^{(2)}$ tends to the trace value of the mean ionic activity coefficient. The experimental data for the mean ionic activity coefficients of NaCl and NaBr in ternary system are presented in Table 1 and Table 2, respectively. The expression for the ratio of the activity coefficients of the peptide in the presence and in the absence of the electrolyte, obtained combining Eq. (5) with Eq. (6), after rearrangement, gives [22]:

$$\ln\left(\frac{\gamma_p^{(2)}}{\gamma_p^{(1)}}\right) = \frac{2a}{b^2(1 + c m_p)^2} \times \left\{ b\sqrt{m_s} - \ln\left(1 + b\sqrt{m_s}\right)\right\}. \tag{7}$$

Table 5
Values of the parameters for the fitting equations: Eq. (6) for NaCl + glycylglycine and NaBr + glycylglycine; Eq. (8) for KCl + glycylglycine; and Eq. (10) for the KBr + glycylglycine system

	NaCl	NaBr	KCl	KBr
a	−4.9544	−293.82	−0.0783	−1.4114
b	26.128	2293.3	−2.2529	5.1640
c	0.4988	0.5250	0.5261	0.3510
R.M.S.D.[a]	0.0039	0.0100	0.0028	0.0100

[a] R.M.S.D. = root mean square deviation of the fit.

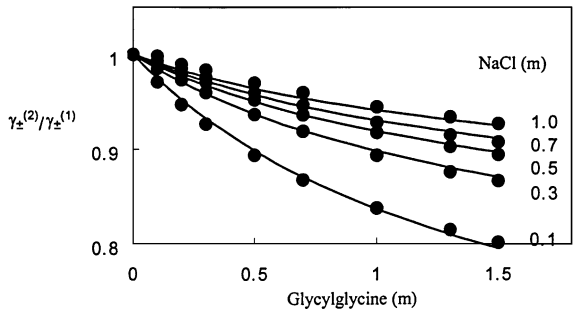

Fig. 2. Effect of glycylglycine concentration on the ratio of the mean ionic activity coefficients of NaCl, in the presence of and absence of glycylglycine, at fixed NaCl molality.

The values of the parameters a, b and c obtained from the fitting of Eq. (6) to the experimental data, have no physical meaning. Their values, together with the root mean square deviation obtained, are reported in Table 5. The empirical parameters for the system containing NaBr are 2 orders of magnitude higher than those for the system containing NaCl. These high values were required to fit all data with the same empirical equation, and should not be assigned any physical meaning. The experimental results for the system containing NaCl are shown in Fig. 2, together with the lines given by the fitting using Eq. (6). The calculated results obtained from Eq. (7) are depicted in Fig. 3. For the case of NaBr, Fig. 4 shows the experimental results and the fitting while Fig. 5 depicts the calculated ratios of activity coefficients of the peptide.

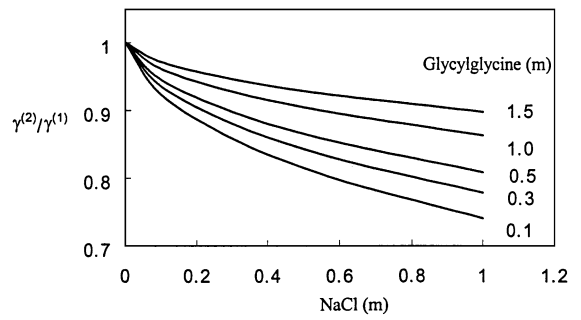

Fig. 3. Effect of NaCl concentration on the ratio of the activity coefficients of glycylglycine, in the presence of and absence of NaCl, at fixed glycylglycine molality.

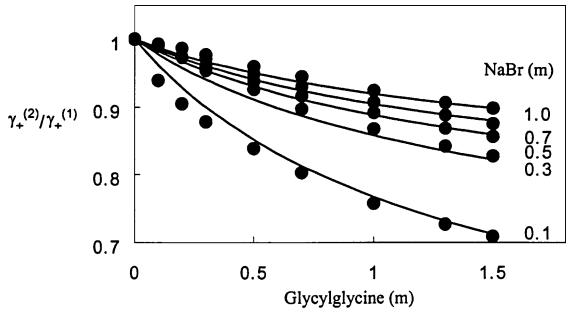

Fig. 4. Effect of glycylglycine concentration on the ratio of the mean ionic activity coefficients of NaBr, in the presence of and absence of glycylglycine, at fixed NaBr molality.

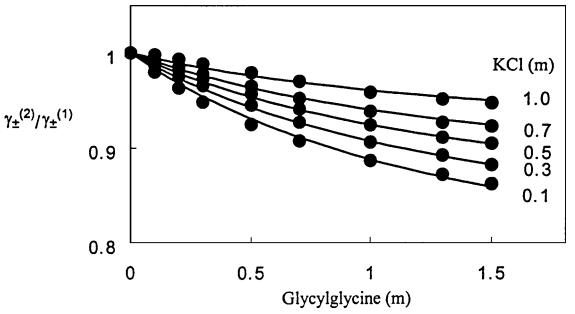

Fig. 6. Effect of glycylglycine concentration on the ratio of the mean ionic activity coefficients of KCl, in the presence of and absence of glycylglycine, at fixed KCl molality.

4.2. System of H_2O + KCl + glycylglycine

For the systems containing potassium as the cation of the electrolyte, Eq. (6) did not give a satisfactory fitting of the data. Thus, other forms of three-parameter equations were sought. The following modified form of Eq. (6) was found to give a good fit of the data for the system of H_2O + KCl + glycylglycine.

$$\nu \ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right) = \frac{a m_p (m_s + b)^2}{(1 + c m_p)}. \quad (8)$$

Eq. (8), as does Eq. (6), gives the correct limits. At zero molality of peptide, the mean ionic activity coefficient of the electrolyte has its value in pure water. As the molality of the electrolyte goes to zero, for a fixed value of the molatity of the peptide, the ratio of the activity coefficients of the electrolyte goes to a finite value that depends on the molality of the peptide. This is the so-called 'trace value' of the electrolyte mean ionic activity coefficient. Combining Eq. (8) with Eq. (5), we obtain:

$$\ln\left(\frac{\gamma_p^{(2)}}{\gamma_p^{(1)}}\right) = \frac{a}{(1 + c m_p)^2}\left\{\frac{m_s^3}{3} + b m_s^2 + b^2 m_s\right\}. \quad (9)$$

The values of the parameters a, b and c for Eq. (9), obtained from the fitting of the experimental data, together with the root mean square deviation obtained, are reported in Table 5. The experimental results for this system, together with the lines given by the fitting using Eq. (8), are presented in Fig. 6. The values of the ratios of the activity coefficients of the peptide, obtained from Eq. (9), are depicted in Fig. 7.

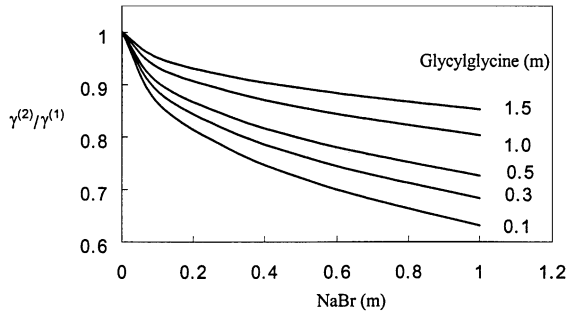

Fig. 5. Effect of NaBr concentration on the ratio of the activity coefficients of glycylglycine, in the presence of and absence of NaBr, at fixed glycylglycine molality.

4.3. System of H_2O + KBr + glycylglycine

For this system, a more complex equation was required to fit the data. After several trials, the following form was adopted:

$$\nu \ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right) = \frac{a m_p}{(1 + b m_s^{3/2})(1 + c m_p)}. \quad (10)$$

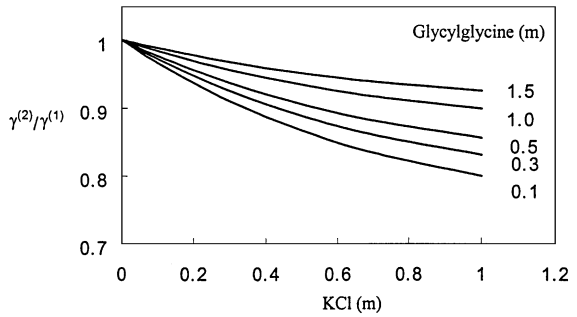

Fig. 7. Effect of KCl concentration on the ratio of the activity coefficients of glycylglycine, in the presence of and absence of KCl, at fixed glycylglycine molality.

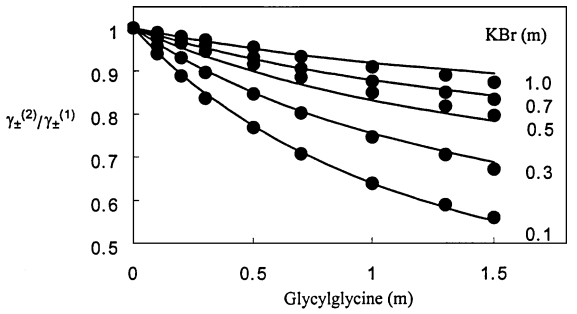

Fig. 8. Effect of glycylglycine concentration on the ratio of the mean ionic activity coefficients of KBr, in the presence of and absence of glycylglycine, at fixed KBr molality.

The expression obtained for the ratio of the activity coefficients of the peptide, in this case, takes the surprisingly complex form:

$$\ln\left(\frac{\gamma_p^{(2)}}{\gamma_p^{(1)}}\right) = \frac{a}{\sqrt{3}\,b^{2/3}(1+cm_p)^2} \times\left[2\tan^{-1}\left(\frac{-1+2b^{1/3}\sqrt{m_s}}{\sqrt{3}}\right) - 2\tan^{-1}\left(\frac{-1}{\sqrt{3}}\right) - \frac{2}{\sqrt{3}}\ln\left(1+b^{1/3}\sqrt{m_s}\right) + \frac{1}{\sqrt{3}}\ln\left(1-b^{1/3}\sqrt{m_s}+b^{2/3}m_s\right)\right] \quad (11)$$

The values of a, b and c for this system, and the root mean square deviation of the fit, are presented in Table 5. Fig. 8 depicts the experimental points and their fit with Eq. (10), while Fig. 9 presents the values calculated with Eq. (11) for the ratio of the activity coefficients of the peptide.

5. Discussion of the experimental results and calculated values

From Figs. 2, 4, 6 and 8, it is clear that in all cases the peptide has a large effect on the mean ionic activity coefficient of the electrolyte at low electrolyte concentration and that this effect decreases as the concentration of the electrolyte increases. This result suggests that higher electrolyte concentrations screen more the electrostatic ion–dipole interaction between glycylglycine and the ions. Thus, the effect of individual cations and anions is better observed from the data at molality 0.1 of the electrolyte. A comparison of Fig. 2 with Fig. 4, and also of Fig. 6 with Fig. 8, shows that the peptide has a larger effect on the mean ionic activity coefficient of the electrolyte in the presence of bromide as anion than in the presence of chloride. On the other hand, comparison of Fig. 2 and Fig. 6, and also of Fig. 4 and Fig. 8, indicate that the nature of the cation has a smaller effect on the interactions between the peptide and the electrolyte. Comparison of Fig. 2 and Fig.

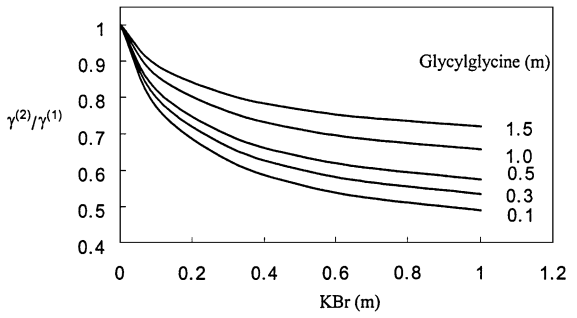

Fig. 9. Effect of KBr concentration on the ratio of the activity coefficients of glycylglycine, in the presence of and absence of KBr, at fixed glycylglycine molality.

6 at low salt concentration, indicates a slightly greater interaction with sodium as a cation when the anion is chloride, while comparison of Fig. 4 and Fig. 8 shows a stronger opposite effect when bromide is the anion. In fact, for the case of KCl and KBr as electrolytes, we have been forced to use a different *y*-scale to show the complete curves at low electrolyte concentration and still differentiate the curves at high electrolyte concentration. Clearly, the effect of the peptide on mean ionic activity coefficients is stronger for the case of KBr as electrolyte.

The fact that the interactions of glycylglycine are stronger with bromide ions than with chloride ions, can also be seen by comparing Fig. 3 with Fig. 5 or Fig. 7 with Fig. 9. On the other hand, comparison of Fig. 3 with Fig. 7 or Fig. 5 with Fig. 9, shows that the effect of the cation is somewhat smaller but still present, even at high concentrations of the peptide.

It is interesting to compare the results of this work with those reported previously [4] for glycine in aqueous solutions of NaCl at 298.2 K. Glycine is the amino acid unit of glycylglycine, and clearly the peptide has a larger effect on the mean ionic activity coefficient of NaCl than the amino acid. This indicates that the higher dipole moment of glycylglycine, compared with that of glycine, has a controlling effect on the thermodynamic behavior of the system. Comparison of the ratio of the activity coefficient of the amino acid with that of the peptide in the presence and absence of NaCl, confirms this view. At molality 1 of the biomolecule (amino acid or peptide), the effect is less noticeable. However, at one molal NaCl and low molality of the biomolecule, say 0.1, the ratio of the activity coefficient of the biomolecule, with electrolyte and without electrolyte, is close to 0.86 for glycine and close to 0.73 for glycylglycine.

Another interesting comparison is that of the trace activity coefficient of the peptide in different electrolyte solutions. As mentioned previously, the trace activity coefficient is the value of activity coefficient of the peptide at infinite dilution in an aqueous solution with fixed concentration of electrolyte. Fig. 10 depicts the trace activity coefficients, γ^{tr} of glycylglycine as a function of molality of the electrolyte for the systems

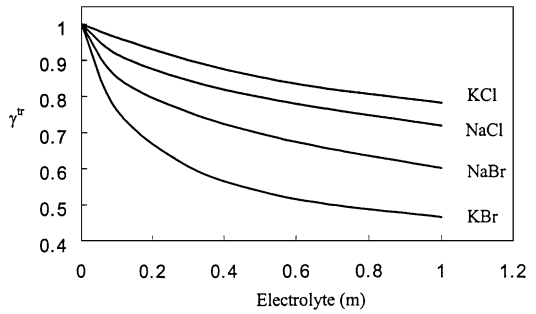

Fig. 10. Trace activity coefficients of glycylglycine in the presence of NaCl, NaBr, KCl and KBr.

studied in this work. The trace activity coefficients of the peptide were obtained by setting m_p equal to zero in Eq. (7), Eq. (9) and Eq. (11). The trace activity coefficients of glycylglycine are notably lower in the presence of bromide as anion compared with the cases having chloride. The effect of the cation, on the trace activity coefficient of the peptide, is not as strong as that of the anion. Notably, the relative positions of the curves in Fig. 10 suggests the same relative strengths of interactions as inferred from the comparison of the effect of the peptide on the mean ionic activity coefficient of the electrolyte.

6. Modeling

In this work we use, directly, a simple model proposed previously [2] for aqueous electrolyte solutions containing amino acids. In this model, the ratio of the mean ionic activity coefficient of the electrolytes takes the form:

$$\ln\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}} = \frac{1}{\nu}\left\{\ln\gamma_S^{(x)} - \lim_{x_P\to 0}(\ln\gamma_S^{(x)}) + \ln\left(\frac{1+0.001\ M_W m_S}{1+0.001\ M_W(m_S+m_P)}\right)\right\} \quad (12)$$

where the subscripts 'S', 'P' and 'W' denote electrolyte, peptide and water, M_w is the molecular weight of water, and the superscript (x) refers to the mole-fraction based activity coefficient. The ratio of the activity coefficients of the peptide in

Table 6
Parameters for the Wilson equation

Glycylglycine +	Binary parameters				
	$\Lambda_{S,P}$[a]	$\Lambda_{P,S}$[a]	$\Lambda_{S,W}$[b]	$\Lambda_{W,S}$[b]	R.M.S.D.
NaCl	−31.515	62.444	2.7276	0.3670	0.0033
NaBr	−27.958	68.974	2.6194	2.8892	0.0032
KCl	−37.064	62.743	2.9496	0.3391	0.0023
KBr	−50.808	152.58	2.8892	0.3462	0.0147

$\Lambda_{P,W} = 2.143\ 8$, $\Lambda_{W,P} = 0.4665$ evaluated from literature binary data [23].
[a] Evaluated from ternary data obtained in this work.
[b] Evaluated from literature binary data [19].

the presence and in the absence of electrolyte is given by:

$$\ln\frac{\gamma_P^{(2)}}{\gamma_P^{(1)}} = \ln\gamma_P^{(x)} - \lim_{x_S\to 0}(\ln\gamma_P^{(x)}) - \lim_{\substack{x_S\to 0\\ x_P\to 0}}(\ln\gamma_P^{(x)}) + \ln\left(\frac{1+0.001\ M_W m_P}{1+0.001\ M_W(m_S+m_P)}\right). \quad (13)$$

For the mole-fraction based activity coefficient we use here the Wilson equation [23] namely:

$$\ln\gamma_i = 1 - \ln\left(\sum_{j=1} x_j\Lambda_{ij}\right) - \sum_{j=1}\frac{x_j\Lambda_{ij}}{\sum_{k=1} x_k\Lambda_{jk}} \quad (14)$$

where Λ_{ij} is a binary parameter, with $\Lambda_{ii} = 1$. In the sums, the subscripts denote the components. We use here: 1 = S (electrolyte); 2 = P (peptide); and 3 = W (water). The binary parameters for the peptide + water system ($\Lambda_{P,W}$, $\Lambda_{W,P}$) were regressed using binary activity coefficient data for the system glycylglycine + water from the literature [24]. The binary parameters for the electrolyte + water system ($\Lambda_{S,W}$, $\Lambda_{W,S}$) were also evaluated using binary data from literature [19]. The values of binary parameters for the interaction of the peptide and electrolyte ($\Lambda_{S,P}$, $\Lambda_{P,S}$), were evaluated from the data for the ternary systems collected in this study. The data for the runs with electrolyte molality of 0.1 were not used since this model failed to reproduce the behavior of the ternary systems in the dilute region of electrolyte. Table 6 represents the binary parameters. The fact that the model failed to reproduce the data in the dilute region, further indicates that, should these data become important, a separate study for the dilute and the concentrated regions will be needed. The binary parameters for the interaction of the peptide and electrolyte are large compared with unity. This suggests a strong interaction between the peptide and electrolyte, which can be due to the ion-dipole interaction.

7. Conclusions

This study contributes information towards the more general field of amide–ion interactions recently discussed in detail by Baldwin [25]. Activity coefficient data for the systems H_2O + NaCl + glycylglycine, H_2O + KCl + glycylglycine, H_2O + NaBr + glycylglycine and H_2O + KBr + glycylglycine, measured in this work showed that the effect of the anion is more pronounced than that of the cation. The results obtained in this work are in qualitative agreement with related results available in the literature. In their studies of the effects of salts on the solubility of glycyl peptides, Nandi and Robinson [26,27] found that NaBr has a larger salting-in effect on peptides than NaCl. This fact implies that the interaction between the peptide and the electrolyte is stronger for NaBr than for NaCl and thus, the perturbation on the mean ionic activity coefficient of NaBr should be larger than for NaCl. This is observed by comparing Fig. 2 with Fig. 4. In particular, Fig. 2 and Fig. 4 show that the effects are more pronounced at low molalities of the electrolyte. At high concentration of the electro-

lyte, the effect of the individual ions is less distinctive. This is in agreement with the studies of von Hippel et al. [28], which suggest that at high concentration of the electrolyte there are stronger non-specific ion–dipole interactions.

Comparison of the data of glycylglycine in aqueous solutions of NaCl with data for glycine in the same aqueous electrolyte solution showed that the interaction of the peptide with the electrolyte.

For the purposes of this work, a model based on the Wilson equation was used to correlate the experimental data at molalities of the electrolyte equal or higher than 0.3. The model fails to reproduce the experimental data at low concentrations of the electrolyte. As more experimental data becomes available, especially for the effect of salts with larger variation in charge density, the use of more sophisticated modeling is expected. The main problem in gathering the missing data is the unavailability of proper ion-selective electrodes for bivalent ions. In addition, studies in the dilute concentration region of the electrolyte using methods other than the electrochemical present major experimental difficulties.

8. Nomenclature

DJRE	Double junction reference electrode
E^0	Standard potential of the cell
E_+	Potential of the cation ion selective electrode
E_-	Potential of the anion ion selective electrode
$\Delta E^{(1)}$	Potential difference in electrochemical cell with electrolyte but without the presence of other solutes
$\Delta E^{(2)}$	Potential difference in electrochemical cell with both electrolyte and other solutes
ISE	Ion selective electrode
m	Concentration in molality
m_p	Molality of the peptide
m_s	Molality of electrolyte
S	Slope of electrode potential
T	Absolute temperature
P	Pressure
x	Mole fraction
$\gamma^{(1)}$	Activity coefficient of the peptide in aqueous solution without another solute
$\gamma^{(2)}$	Activity coefficient of the peptide in aqueous solution with another solute
$\gamma_\pm^{(1)}$	Mean ionic activity coefficient of the electrolyte in aqueous solution without another solute
$\gamma_\pm^{(2)}$	Mean ionic activity coefficient of the electrolyte in aqueous solution with another solute
ν	Stoichiometric number of electrolyte
Λ_{ij}	Binary energy parameter between species i and j in the Wilson equation

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