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Activity coefficients of antibiotics in aqueous NaCl solutions at 298.2 K

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Abstract

Data at 298.2 K for aqueous NaCl solutions containing either D-phenylglycin, D-(*p*-hydroxy)phenylglycine, 6-amino penicillanic acid (6-APA), amoxicillin or ampicillin, are reported. The mean ionic activity coefficients of NaCl were determined from measurements of the responses of a sodium and a chloride ion-selective electrode. The activity coefficients of the precursors or the antibiotics were calculated from the values of the mean ionic activity coefficients using the exact cross differential relation between them. The correlation of solubility data using the activity coefficients measured in this work shows the same puzzling results previously observed in systems containing amino acids.

Keywords: Antibiotics; Activity coefficients; Electrolyte solutions; Ion selective electrodes

1. Introduction

The discovery of antibiotics and the development of their semi-synthetic derivatives had a major impact on therapeutic medicine. Antibiotics are substances, which, at low concentrations, can inhibit the growth of microorganisms [1]. More than two thousand antibiotics are presently known. Even though there seems to be no limits to new discoveries, modifications and applications of antibiotics, semi-synthetic penicillins are still the working horse and are expected to remain so for the next decade. Therefore, price competition will increase and the rational development of novel, improved processes is a must for the antibiotics industry [2]. The work reported here must be seen in that framework. Semi-synthetic penicillins are made by adding side chains to the 6-amino penicillanic acid nucleus. The addition of the side chains D-phenylglycine or D-(*p*-hydroxy)phenylglycine leads to the formation of ampicillin or amoxicillin, respectively.

Ampicillin, amoxicillin and their precursors 6-amino penicillanic acid (6-APA), D-phenylglycine and D-(*p*-hydroxy)phenylglycine present the typical amino acid behavior in aqueous solutions. In the absence of a proton donor or a proton acceptor, up to 99% of the molecules are in their zwitterionic

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form [3]. In this form, the carboxyl group loses a proton and becomes negatively charged, whereas the amino group gains a proton and becomes positively charged.

The worldwide need for semi-synthetic penicillins has led to the development of large-scale production processes. Due to economical restrictions and environmental requirements, the production process based on a synthetic application of biocatalysis has gained wide acceptance. Potential economic savings can be obtained from a proper design of the downstream separation processes of the semi-synthetic penicillins, as the separation of unconverted reactants is troublesome due to the fairly similar structure of the compounds [4]. Rational design of separation processes requires knowledge of the phase behavior of semi-synthetic antibiotics in aqueous electrolyte solutions. Systematic collection of data and the development of a unifying thermodynamic framework are essential elements required to support the design of efficient separations.

In this work, we study the thermodynamics of aqueous NaCl solutions containing ampicillin, amoxicillin, 6-APA, D-phenylglycine and D-(*p*-hydroxy)phenylglycine. The activity coefficients of the electrolyte and of the antibiotics in aqueous solutions have been determined.

2. Determination of activity coefficients

There are no data reported in the literature for the activity coefficients of antibiotics and its precursors in aqueous electrolyte solutions. On the other hand, a pletora of experimental data are reported for the activity coefficients of amino acids in aqueous electrolyte solutions. These molecules are the building blocks of important bio molecules like peptides, proteins and also antibiotics. Two major techniques have been used to determine activity coefficients in aqueous electrolyte solutions containing amino acids: the isopiestic method and the electrochemical method.

The isopiestic method is a simple method to determine the solvent activity of a solution containing non-volatile solutes. The activity coefficients of the solutes can be obtained through the use of the Gibbs–Duhem equation. The major drawbacks of this method are that attainment of equilibrium takes a long time and that the accuracy is low at low solute concentrations.

A recent variation of the electrochemical method uses an electrochemical cell with two ion-selective electrodes [5], to measure the activity coefficient of the electrolyte in the solution. The activity coefficients of the other solutes, and of the solvent if needed, can be obtained through the use of the Gibbs–Duhem equation. This method is fast and has proved to give reliable results for the determination of activity coefficients, mainly in the all-important dilute region. Antibiotics have a low solubility, are non-volatile and can degrade when dissolved in water. Thus, the electrochemical method seems to be the most adequate procedure to determine the activity coefficients of antibiotics in aqueous electrolyte solutions.

3. Theory of the experimental method

The method to measure the mean ionic activity coefficients of electrolytes using ion-selective electrodes was standardized by Haghtalab and Vera [6,7]. The method was later extended to determine the activity coefficients of amino acids in aqueous electrolyte solutions [5,8–12]. Shortly stated, the difference of the potentials of a cation and an anion ion selective electrodes, both measured against a common reference electrode, is related to the mean ionic activity coefficient of the electrolyte by the Nernst equation, written as:

$$\Delta E = E^{\circ} + S \ln(m_S \gamma_{\pm}) \quad (1)$$

where, considering that studies with different ion selective electrodes have shown deviations to a Nernstian response [6,13], the slope S is used instead of the value of 51.38 mV at 25 °C. As the potential of the reference electrode cancels out when taking the difference, an electrochemical cell in which the potential of the cation and the anion selective electrode are directly read one against the other, gives the same results. The determination of the activity coefficient in aqueous electrolyte solutions of a second solute is done by measuring the mean ionic activity coefficient of the electrolyte in different electrochemical cells. A cell of type (1) contains only the electrolyte and water

and a cell of type (2) contains the electrolyte, water and the second solute. In the electrochemical cell of type (1):

Cation ISE|electrolyte (m_S)|Anion ISE

the measured potential difference, $\Delta E^{(1)}$, is related to the mean ionic activity coefficient of the electrolyte at molality m_S, $\gamma_{\pm}^{(1)}$, as follows:

$$\Delta E^{(1)} = E^{\circ} + S \ln(m_S \gamma_{\pm}^{(1)}) \quad (2)$$

where the superscript (1) indicates the electrochemical cell of type (1). The values of S and E°, in Eq. (2), are obtained from a fitting of the experimental values of $\Delta E^{(1)}$ as function of $\ln(m_S \gamma_{\pm}^{(1)})$. The values of $\gamma_{\pm}^{(1)}$ at each molality m_S are obtained from the literature [14]. The potential difference of an electrochemical cell changes in the presence of other solutes due to the change in the mean ionic activity coefficient of the electrolyte. In the electrochemical cell of type 2:

Cation ISE|electrolyte (m_S)
+solute(m_A)|Anion ISE

the difference in potential between a cation and an anion ISE is related to the mean ionic activity coefficient of the electrolyte by:

$$\Delta E^{(2)} = E^{\circ} + S \ln(m_S \gamma_{\pm}^{(2)}) \quad (3)$$

where the superscript (2) indicates the electrochemical cell of type (2). As the same ISEs are used in both cells (1) and (2), the values of S and E° are the same in Eqs. (2) and (3). Combining Eqs. (2) and (3), the logarithm of the ratio $\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$ can be determined by:

$$\ln \frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\bigg|_{m_S} = \frac{\Delta E^{(2)} - \Delta E^{(1)}}{S} \quad (4)$$

The subscript m_S on the left-hand side of Eq. (4) indicates that both $\gamma_{\pm}^{(2)}$ and $\gamma_{\pm}^{(1)}$ are evaluated at the same molality m_S of the electrolyte. As the molality of the antibiotic solute m_A goes to zero, at any fixed value of the molality of the electrolyte m_S, the right hand side of Eq. (4) tends to zero and $\gamma_{\pm}^{(2)}$ tends to the value of $\gamma_{\pm}^{(1)}$ at the particular molality of the electrolyte. On the other hand, at a fixed molality of the antibiotic, m_A, as the molality m_S of the electrolyte goes to zero, the right hand side of Eq. (4) does not vanish and while the value of $\gamma_{\pm}^{(1)}$ tends to unity, the value of $\gamma_{\pm}^{(2)}$ tends to a value different from unity called the trace value of the activity coefficient. Clearly, the mean ionic activity coefficient of the electrolyte is normalized to unity with respect to the state of the electrolyte at infinite dilution in pure water. The activity coefficient of the antibiotic is related to the mean ionic activity coefficient of the electrolyte through the exact cross-differential relation:

$$\left(\frac{\partial \ln \gamma_A}{\partial m_S}\right)_{m_A,T,p} = \nu \left(\frac{\partial \ln \gamma_{\pm}}{\partial m_A}\right)_{m_S,T,p} \quad (5)$$

for sodium chloride, the stoichiometric number of ions ν is equal to 2. Thus, fitting the experimental results to a function of the form:

$$\ln \frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\bigg|_{m_S} = f(m_S, m_A) \quad (6)$$

differentiation with respect to m_A, and subsequent integration with respect to m_S, gives a functional form to obtain the activity coefficient of the second solute γ_A. The function used to the experimental data of $\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$, should satisfy the following boundary conditions:

$$\ln \frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\bigg|_{m_S} = \begin{cases} 0 & \text{as } m_A \to 0 \text{ at fixed } m_S \\ \ln \gamma_{\pm,\text{trace}} \neq 0 & \text{as } m_S \to 0 \text{ at } m_A \neq 0 \end{cases} \quad (7)$$

In addition, after the differentiation and integration implied in Eq. (5), the resulting expression for $\gamma_A^{(2)}/\gamma_A^{(1)}$ should satisfy the following conditions:

$$\ln \frac{\gamma_A^{(2)}}{\gamma_A^{(1)}}\bigg|_{m_A} = \begin{cases} 0 & \text{as } m_A \to 0 \text{ at fixed } m_S \\ \ln \gamma_{A,\text{trace}} \neq 0 & \text{as } m_S \to 0 \text{ at } m_A \neq 0 \end{cases} \quad (8)$$

The subscript m_A in the right hand side of Eq. (8) indicates that both $\gamma_A^{(1)}$ and $\gamma_A^{(2)}$are evaluated at the same molality m_A of the antibiotic. The value of the trace activity coefficient of the electrolyte in Eq. (7), or the trace value of the acitivity coefficient of the antibiotic in Eq. (8), are not known a priori and they are determined from the

limiting value of the corresponding function arising from Eq. (6). By establishing the proper limits in the integration step, the activity coefficient of the antibiotic is normalized with respect to the state of the antibiotic at infinite dilution in pure water.

4. Materials and methods

The ternary systems studied in this work, using the electrochemical method, are the aqueous NaCl solutions containing the antibiotic precursors D-phenylglycine, D-(*p*-hydroxy)phenylglycine and 6-APA, and the aqueous NaCl solutions containing the antibiotics amoxicillin and ampicillin. The experiments were performed in the concentration ranges between 0.01 and 1.5 mol/kg for NaCl, and between 0 mol/kg to the solubility limit of the antibiotic precursors or the antibiotics.

For all experiments sodium chloride of analytical grade, 99% purity, A and C American Chemicals (Montreal, Que., Canada), was used. The NaCl was oven-dried at least 72 h prior to use. D-phenylglycine, D-(*p*-hydroxy)phenylglycine, 6-APA, ampicillin trihydrate and amoxicillin trihydrate were obtained from DSM Research (Geleen, The Netherlands), and used as received. During all experiments deionized water with a conductivity of less than 0.8 μS/cm was used. All concentrations are based on molality, therefore all additions of chemicals, including water, were weighed. The solutions were kept at a constant temperature of 298.2 ± 0.1 K using a 250-ml jacketed glass beaker connected to a thermostatic bath. During the experiments the solutions are stirred constantly with a magnetic stirrer to avoid temperature and concentration gradients in the solution.

To obtain emf-values in NaCl solutions a Na^+-ISE, a Cl^--ISE and a double junction reference electrode were used. Two different sodium and two different chloride ion-selective electrodes were used. For sodium we used an Orion Ross Glass membrane electrode, model 8411BN purchased from Fisher Scientific, Canada (Catalogue number 13-641-822), and a Sentek Glass membrane, model 315-75, purchased from Fisher Scientific, The Netherlands. For chloride we used an Accumet Epoxy Chloride Indicating Half-Cell electrode, model 13-620-518, purchased from Fisher Scientific, Canada, and a Sentek Half-Cell chloride selective membrane electrode, model 301-75, purchased from Fisher Scientific, The Netherlands (Catalogue number FSN-205-255C). In most runs, the potential difference between each ISE and a reference electrode was measured. An Accumet double-junction ISE reference electrode, model 13-620-47, purchased from Fisher Scientific was used for these measurements. In some runs the potential difference between both ion-selective electrodes was directly determined. The potential differences were monitored using an Orion mV-meter, model EA 920, with an accuracy of ± 0.1 mV. While not in use, the ion selective electrodes were stored in a NaCl-H_2O solution with a molality of $m \approx 0.1$-mol/kg water.

Prior to each measurement for the ternary system, a calibration was performed in the binary aqueous electrolyte solution, in type (1) cells, by measuring the emf at different salt molalities. Using literature data for the mean ionic activity coefficient of the electrolyte the values of E° and S of the cell were determined by fitting Eq. (2) to the data presented in Fig. 1. Experiments were performed in two different ways. Some sets of experiments were performed using constant salt molality and varying the molality of the antibiotics, and other sets of experiments were performed at constant antibiotic molality changing the molality of the electrolyte. While the first technique gives a better stability of the response of the ion selective electrodes, the latter makes better use of the antibiotics samples. For experiments at constant electrolyte molality, the response of the electrodes is calibrated using electrolyte concentrations close to the one used in the measurements. For experiments at constant antibiotic concentration, the calibration has to cover the whole range of molality to be used in the experimental run. Both procedures were additionally tested in runs with and without reference electrode. In the latter case the response of the anion and cation ISE's were measured directly, one against the other, instead of measuring separately the response of each electrode against the double junction reference electrode. The Sentek electrodes were used only for the measurements of the system containing amox-

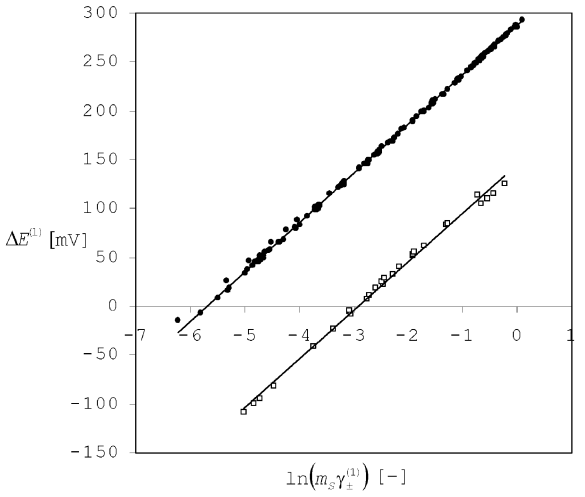

Fig. 1. Calibration of the response of the electrodes in aqueous NaCl solutions at 298.2 K. All experimental points collected during calibrations in this work have been included. Full circles correspond to the experimental points for the cell formed by a Ross sodium glass ISE and an Accumet chloride solid state ISE. Open squares correspond to the cell formed by a Sentek sodium glass ISE and a Sentek chloride solid state ISE. The lines correspond to an overall linear fitting of all points for the cell.

icillin. Their response was validated by measuring the mean ionic activity coefficient of NaCl in aqueous solutions containing glycine and comparing the results obtained with those reported in the literature. Differences were within the margin of the experimental error.

5. Results and discussion

As stated above, calibrations of the response of the ion-selective electrodes in the binary H_2O-NaCl system were performed prior to every measurement of a system containing an antibiotic. All experimental points obtained in the calibrations are presented in Fig. 1. The two lines presented correspond to the two different sets of electrodes used. The overall calibration constants for the cell with a Ross sodium glass ISE and Accumet chloride solid state ISE are $E^\circ = 287.60$ mV and $S = 50.592$ mV. For a cell with a Sentek sodium glass ISE and Sentek chloride solid state ISE the constants are equal to $E^\circ = 145.26$ mV and $S = 49.726$ mV. For a particular calibration made before a ternary run, the values of the constants could be slightly different from these overall values, as they are sensitive to experimental circumstances such as the concentration of the solution in which the ISE are stored and time of storage. Table 1 presents the values of the standard deviation σ, determined assuming a normal distribution, calculated as:

$$\sigma = \sqrt{\frac{\sum_i^n (\Delta E^{(1)}_{\text{meas},i} - \Delta E^{(1)}_{\text{fit},i})}{n-1}} \quad (9)$$

where n is the number of experimental data points. Although there is not much difference between using or not using a reference electrode, there seems to be a small reduction in the drift of the response of the cell when using the reference electrode. The larger standard deviation found for the electrochemical cell using the Sentek ISE is probably due to the fact that less experimental points were measured.

A detailed discussion of the results obtained for each ternary system is presented below.

5.1. System $NaCl + H_2O + 6$-APA

Mean ionic activity coefficients of the electrolyte in the system $NaCl + H_2O + 6$-APA were obtained from measurements in runs at fixed salt molality, and also in runs at fixed antibiotic molality. The experimental results for the ratio $\gamma_\pm^{(2)}/\gamma_\pm^{(1)}$ obtained from experiments at constant antibiotic molality, m_A are presented in Fig. 2 together with the results obtained at constant m_S. The agreement of both sets of measurements is

Table 1
Number of points (n) and standard deviation (σ) for the calibration of the electrodes

Electrochemical Cell	n	σ
Ross Na^+ and Accumet Cl^-, ISEs with an Accumet reference electrode	72	0.884
Ross Na^+ and Accumet Cl^-, without a reference electrode	252	1.083
Sentek Na^+ and Sentek Cl^-, without a reference electrode	25	3.959

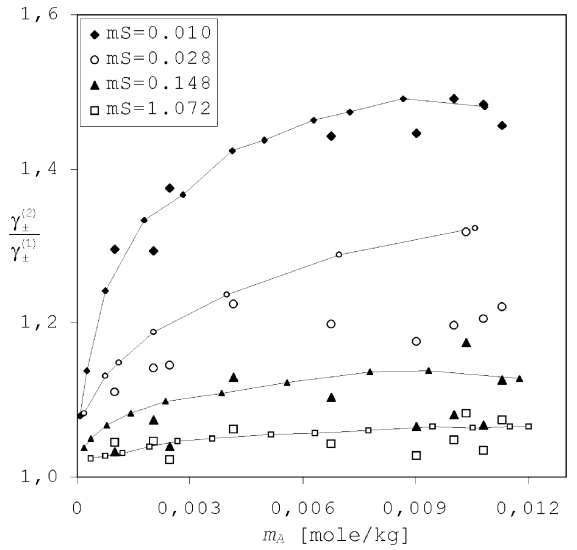

Fig. 2. Ratio of the mean ionic activity coefficients of sodium chloride in the presence and in the absence of 6-APA as a function of the molality of the antibiotic. Large symbols correspond to measurements performed changing the molality of NaCl at constant molality of the 6-APA. Small symbols correspond to measurements performed changing the molality of 6-APA at constant molality of the electrolyte. Lines are used to indicate points of the same set.

acceptable considering the fact that the set of measurements at constant antibiotic molality requires either a new calibration of the cell before each measurement or the use of an overall calibration performed before the complete set of measurements. The results obtained at constant electrolyte concentration are considered to be more reliable, as $\Delta E^{(1)}$ is a constant at constant m_S and it is measured before each run. Fig. 2 shows that the mean ionic activity coefficient ratio, $\gamma_{\pm}^{(2)}/\gamma_{\pm}^{(1)}$, increases with increasing m_A at constant m_S. The results at constant molality of the electrolyte were fitted with different empirical functions. The following function was found to fit well the experimental results using the least number of adjustable parameters:

$$\ln\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\bigg|_{m_S} = \frac{am_A}{(1+b\sqrt{m_S})(1+cm_A)} \qquad (10)$$

This function, that has no physical model behind, meets the limiting conditions required by

Table 2
Constants for Eqs. (10) and (12)

System	a	b	c	n	σ
NaCl + H_2O + 6-APA	3769	61.4	1149	77	0.0219
NaCl + H_2O + ampicillin	1733	74.2	837	68	0.0177
NaCl + H_2O + amoxicillin[a]	299	8.2	1431	57	0.0065

[a] System measured with Sentek ion-selective electrodes.

Number of points used in the fit (n), and standard deviation of the fit (σ).

Eq. (7). The parameters a, b and c are reported in Table 2 together with the number of points considered and the standard deviation of the fit, defined as:

$$\sigma = \frac{\sqrt{\sum_i^n \left(\ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right)_{\text{meas},i} - \ln\left(\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\right)_{\text{fit},i}\right)^2}}{n-1} \qquad (11)$$

The experimental points at constant molality of the electrolyte together with the curves obtained from Eq. (10) are presented in Fig. 3.

The ratio of the activity coefficients of the antibiotic in the presence and in the absence of electrolyte, obtained combining Eq. (5) and Eq. (10), has the form:

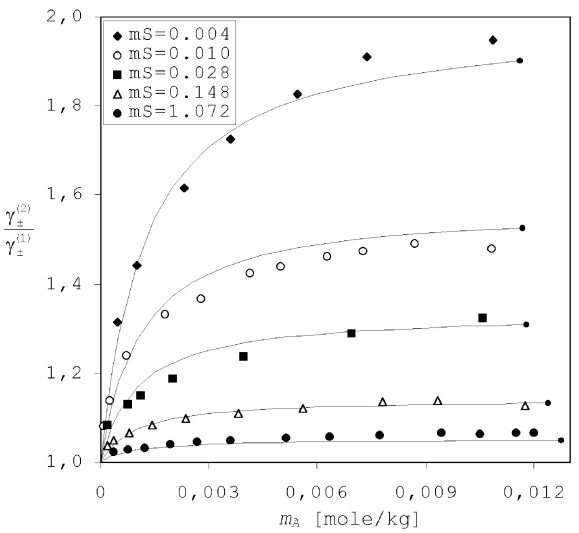

Fig. 3. Fitting of the experimental results with Eq. (10) for the system NaCl + H_2O + 6-APA. Symbols are experimental points; lines are the results of the fit.

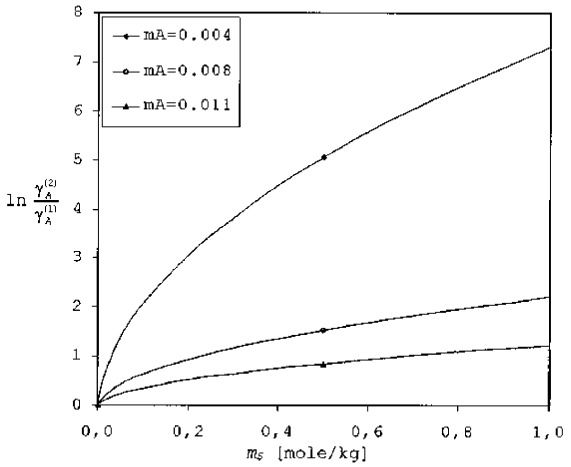

Fig. 4. Values of the ratio of the activity coefficients of 6-APA, in the presence and in the absence of sodium chloride, as obtained from Eq. (12). Points are not experimental, they are used to identify the molality of 6-APA.

$$\ln\frac{\gamma_A^{(2)}}{\gamma_A^{(1)}}\bigg|_{m_A} = \frac{2\nu a}{b^2(1+c\ m_A)^2} \times\left(b\sqrt{m_S}-\ln\left(1+b\sqrt{m_S}\right)\right) \qquad (12)$$

As in Eq. (5), we have kept the stoichiometric number of ions of the electrolyte ν, as a symbol although for sodium chloride its value is $\nu=2$. The results calculated with Eq. (12) for the ratio of the activity coefficients of 6-APA in aqueous solutions of sodium chloride and in pure water, at the same antibiotic molality, are depicted in Fig. 4. The lines representing the calculated results have been extrapolated up to the saturation molality of the antibiotic in the corresponding electrolyte solution.

5.2. Systems $NaCl+H_2O+$ampicillin and + amoxicillin

All experiments for the systems containing ampicillin and amoxicillin in aqueous NaCl solutions were performed at constant molality of NaCl, m_S. At each molality of electrolyte, before starting the weighed additions of antibiotic, the response of the ion selective electrodes was calibrated using the type 1 cell. The experimental results are presented in Figs. 5 and 6, respectively. Although

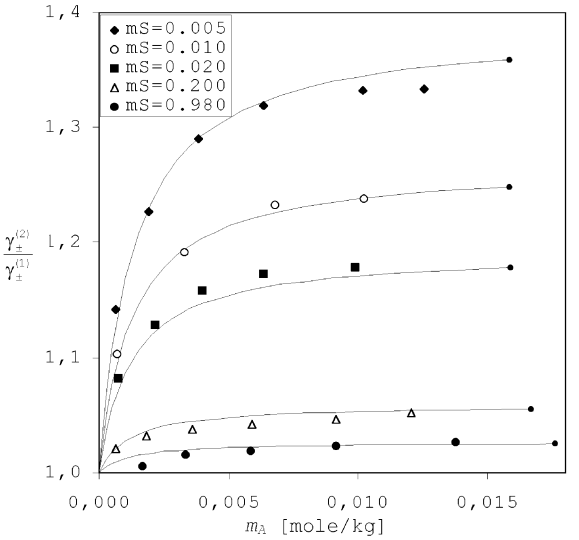

Fig. 5. Fitting of the experimental results with Eq. (10) for the system $NaCl+H_2O+$ampicillin. Symbols are experimental points, lines are the results of the fit.

these systems behave similar to the system containing 6-APA, the effect of the antibiotic on the ratio of the mean ionic activity coefficient of the electrolyte decreases in the order: 6-APA, ampicillin, amoxicillin, as shown by the change in the *y*-

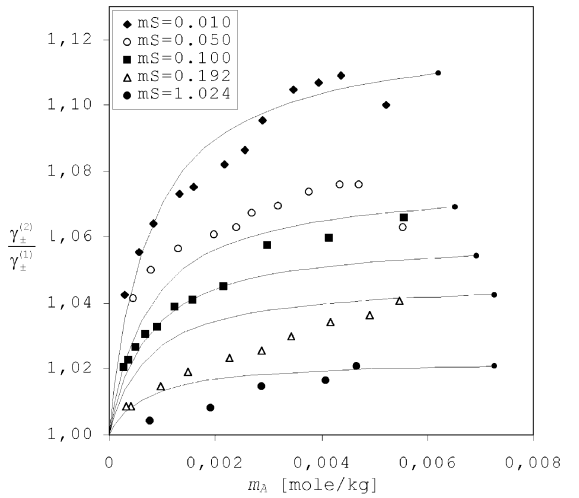

Fig. 6. Fitting of the experimental results with Eq. (10) for the system $NaCl+H_2O+$amoxicillin. Symbols are experimental points; lines are the results of the fit.

Table 3
Constants for Eqs. (13) and (14)

System	*a*	*b*	*c*	*n*	σ
NaCl + H_2O + D-phenylglycine	−165.4	248.3	2.581	29	0.0118
NaCl + H_2O + D-(p-hydroxy) phenylglycine	6.26	0.147	−0.509	354	0.0144

Number of points used in the fit (n), and standard deviation of the fit (σ).

axes of Figs. 3–6. The experimental results were fitted with Eq. (10) and the values of the adjustable parameters are reported in Table 2, together with the number of experimental points collected and the standard deviation of the fit as given by Eq. (11). The ratio of the activity coefficient of the antibiotic in a sodium chloride solution to the activity coefficient of the antibiotic in pure water is, again, given by Eq. (12).

5.3. Systems $NaCl+H_2O+$ D-phenylglycine and + D-(p-hydroxy)phenylglycine

Most experiments of systems containing D-phenylglycine and D-(*p*-hydroxy)phenylglycine were performed at constant antibiotic molality, m_A. As discussed previously, this technique produces less precise results than the measurements at constant electrolyte composition. However, its use is justified here due to the insensitivity of the ratio of activity coefficients of the precursors to the molality of the electrolyte. In addition, for the case of D-phenylglycine the ratio of the mean ionic activity coefficients is also quite insensitive to the concentration of D-phenylglycine, and, thus, close to unity. For the case of D-(*p*-hydroxy)phenylglycine, the mean ionic activity coefficient of the electrolyte decreases with the addition of the precursor and reaches a minimum value of approximately 0.99 at a 0.04 molality of D-(*p*-hydroxy)phenylglycine, and then increases up to a value of 1.03 at 0.12 molality of the precursor. After some preliminary trials, the following function was selected to fit the data:

$$\ln\frac{\gamma_{\pm}^{(2)}}{\gamma_{\pm}^{(1)}}\bigg|_{m_S} = \frac{am_A^2 + cm_A}{1 + b\sqrt{m_S}} \tag{13}$$

The values of the adjustable parameters are reported in Table 3 together with the number of points measured and the standard deviation of the fit, as defined by Eq. (10). The ratio of the activity coefficient for the antibiotic precursor, in the presence and in the absence of the electrolyte, obtained from Eqs. (13) and (5), takes the form:

$$\ln\frac{\gamma_A^{(2)}}{\gamma_A^{(1)}}\bigg|_{m_A} = \frac{2\nu(2am_A + c)}{b^2} \times\left(b\sqrt{m_S} - \ln\left(1 + b\sqrt{m_S}\right)\right) \tag{14}$$

Again here $\nu = 2$, although for generality we have kept the symbol in Eq. (14).

6. Modeling the solubility of antibiotics in electrolyte solutions

The modeling of the solubility of amino acids in electrolyte solutions has been the subject of recent studies [15–18]. We consider here the case of a binary system formed by a pure compound *A* solid in equilibrium with a saturated solution of compound *A* in a solvent. The difference between the chemical potential of a pure solid *A* and the standard state chemical potential of compound *A* in the liquid mixture, in molality scale, can be written as:

$$\Delta\mu^0 = RT\ln(m_A^{\mathrm{sat},0}\gamma_A^{\mathrm{sat},0}) \tag{15}$$

where $m_A^{\mathrm{sat},0}$ is the solubility of solute *A* in the solvent and $\gamma_A^{\mathrm{sat},0}$ is the corresponding activity coefficient at saturation. The superscript 0 is used here to denote the binary system. Addition of the second solute, the electrolyte in the case considered here, to a solution saturated in compound *A*, does not change the value of $\Delta\mu^0$ if the solute is pure *A* and the morphology of the solid phase does not change. The activity coefficients of compound *A* are evaluated in all cases with respect to the reference state of compound *A* at infinite dilution

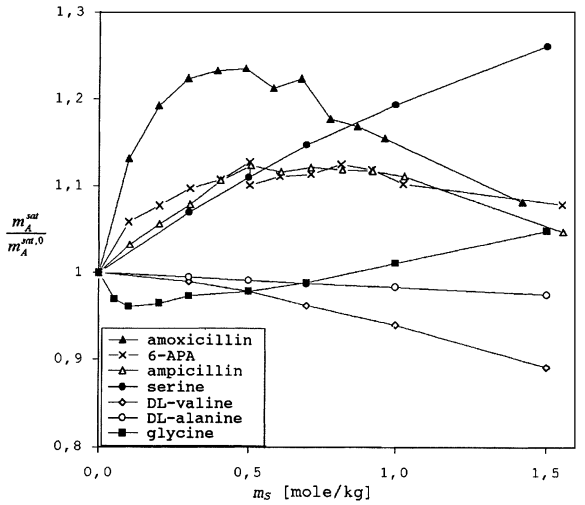

Fig. 7. Ratio of the molality of a biological molecule at saturation in an aqueous electrolyte solution to the molality at saturation in pure water.

in the pure solvent. In order to verify the invariance of the chemical potential of the solid phase, for the case of a solution saturated in A in the presence of another solute we write the equivalent to Eq. (15) as:

$$\Delta\mu = RT\ln(m_A^{\mathrm{sat}}\gamma_A^{\mathrm{sat}}) \tag{16}$$

Whether the solute changes in its solid state can be shown by subtracting Eq. (16) from Eq. (15), to obtain:

$$\frac{\Delta\mu - \Delta\mu^0}{RT} = \ln\frac{m_A^{\mathrm{sat}}\gamma_A^{\mathrm{sat}}}{m_A^{\mathrm{sat,0}}\gamma_A^{\mathrm{sat,0}}} \tag{17}$$

We emphasize that the standard state for the activity coefficients of the antibiotic is the same in Eqs. (15) and (16). For the antibiotics and precursors, the ratio of the activity coefficients was evaluated by extrapolation of Eq. (12) or Eq. (14), depending on the case. Thus, plotting the right hand side of Eq. (17) vs. the molality of the second solute detects the variability or invariability of the chemical potential of the compound A in the solid phase. In order to show the dramatic effect of the activity coefficients of the biochemical compounds, Fig. 7 shows the values of the ratio $(m_A^{\mathrm{sat}}/m_A^{\mathrm{sat,0}})$ as a function of the molality of the electrolyte. The values of the saturation molality of the compounds studied in this work are those obtained by Rudolph [19]. Data for four amino acids reported in the literature [15–18] have also been included in the figure to show the effect of molecular weight. Fig. 8 depicts the values of the right hand side of Eq. (17), as a function of the molality of the electrolyte. Notably, the difference between the chemical potentials of the solid phase is quite large for amoxicillin and for 6-amino penicillanic acid. In fact, Fig. 8 shows that the increase in chemical potential is larger for the compounds with larger solubilities.

7. Discussion and conclusions

Activity coefficient data for the NaCl-H_2O systems containing the antibiotic precursors D-phenyl glcyine, D-(*p*-hydroxy)phenylglycine and 6-APA and for the NaCl-H_2O systems containing the antibiotics ampicillin and amoxicillin were obtained using the electrochemical method. Additional details on the background of the method and the experimental results, including the testing of a different set of ion selective electrodes, are given elsewhere [20].

The ratio of the mean ionic activity coefficient in the NaCl-H_2O systems containing the anti-

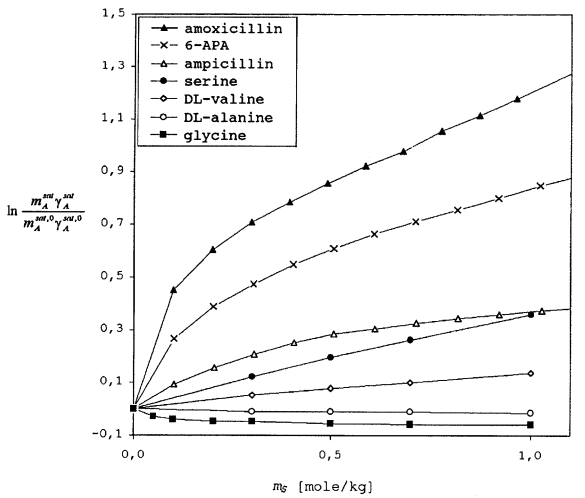

Fig. 8. Ratio of the activity of a biological molecule at saturation in an aqueous electrolyte solution to the activity at saturation in pure water.

biotic precursors D-phenylglcyine and D-(*p*-hydroxy)phenylglycine was found to be rather insensitive to changes in the NaCl concentration. The mean ionic activity coefficient in the NaCl-H_2O system containing D-phenylglycine is also only slightly sensitive to changes in the D-phenylglycine concentration and, as a result, the ratio of the mean ionic activity coefficients in both cells is close to unity. The mean ionic activity coefficient in the NaCl-H_2O system containing D-(*p*-hydroxy)phenylglycine shows a small dependence on the precursor concentration.

The ratio of the mean ionic activity coefficient in the NaCl-H_2O systems containing 6-APA, ampicillin and amoxicillin show a similar behavior. The ratio of the mean ionic activity coefficient decreases with increasing salt concentration and increases with increasing antibiotic concentration. The effect of the antibiotic on the ratio of the mean ionic activity coefficient decreases in the order 6-APA, ampicillin, amoxicillin. The antibiotic activity coefficients for these systems show also the same qualitative behavior. The ratio of the antibiotic activity coefficient increases with increasing salt concentration and decreases with increasing antibiotic concentration.

Combination of the activity coefficient data with available solubility data for the antibiotics and precursors [19] made it possible to calculate the difference in chemical potential of the solid state in the H_2O-solute and the H_2O-solute-NaCl system. The ratios of the activity coefficients at saturation were evaluated by extrapolation of Eq. (12) or Eq. (14), depending on the case. Based on available information [19], the activity coefficients of the biomolecules at saturation in pure water were set at unity. This assumption is exactly equivalent to the use of Eq. (12) or Eq. (14) with the right hand side evaluated at the saturation molality of the biomolecule in the electrolyte solution. Both of these ways of looking at the correction introduced by the ratio of activity coefficients seem reasonable. Thus, the fact that the chemical potential difference increases with an increase in the molality of the electrolyte is intriguing and it remains unexplained. This phenomenon was previously observed in the study of the activity coefficients of amino acids in aqueous electrolyte solutions [15]. Precipitation experiments of glycine in aqueous sodium chloride solutions were carried out and, while no change in the electrolyte concentration was detected by precipitation, the crystallographic form of the glycine precipitate clearly changed depending on the electrolyte concentration in the solution [15]. At low salt concentrations, glycine precipitates in the form of fine particles and, as the salt concentration increases, the crystals become needle-like and grow in size [15]. The logical conclusion, with the information available, was that the fugacity (chemical potential) of the solid phase glycine changed with the concentration of salt of the mother liquour [15]. Although this explanation is reasonable, it is also possible that the activity coefficients of the biological molecule determined by the electrochemical method have a systematic error that increases with an increase in the concentration of the electrolyte. Experiments were carried out by Gao [21] to measure the activity coefficients of DL-Serine and KNO_3 in aqueous solutions by the isopiestic method and by the two-ion-selective-electrode method. The small difference in the results obtained cannot possibly explain the effect discussed above. The minor difference in results obtained by both methods was thought to be due to inaccuracy of the isopiestic experiments that took several weeks to attain equilibrium. In additional experiments, Gao [22] precipitated DL-alanine by cooling down to 298.2 K aqueous solutions saturated in the amino acid at 348 K at different concentrations of NaCl, well below the saturation concentration of the salt. The purpose of these experiments was to observe the solid phase amino acid crystals in contact with the mother solution from which they were formed. Again, it was observed that crystals formed at low salt concentration were quite different from crystals formed at higher salt concentrations. While these simple experiments, that are easy to test in any laboratory, justify the assumption that the observed changes in the solid phase of an amino acid may explain its solubility behavior, in this work with antibiotics and their precursors, a similar explanation is not suitable. In fact, crystallographic studies on the solid phase of antibiotics formed by precipitation from electrolyte solutions at different salt concentrations have failed to show a difference

in the crystallographic structure. For the systems studied here, the effect of the approximation introduced by the extrapolation of Eqs. (12) and (14) requires further study. Work on this area is presently in progress.

8. Nomenclature

E^0 : Standard potential of the cell
$\Delta E^{(1)}$: 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 units
m_A: Molality of the antibiotic or precursor
m_S: Molality of electrolyte
S: Slope of electrode potential
T: Absolute temperature
P: Pressure

Greek letters:

$\gamma_A^{(1)}$: Activity coefficient of the antibiotic or precursor in aqueous solution without another solute
$\gamma_A^{(2)}$: Activity coefficient of the antibiotic or precursor 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
Δ: Difference
ν: Stoichiometric number of ions per mole of electrolyte
μ: Chemical potential

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