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Effect of the cation and the anion of an electrolyte on the solubility of DL-aminobutyric acid in aqueous solutions: measurement and modelling

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Abstract

The solubilities at 298.2 K of DL-aminobutyric acid in aqueous solutions of NaCl, KCl, $NaNO_3$ and KNO_3 were measured. The solubility of DL-aminobutyric acid was found to be influenced by the concentration and by the nature of both the cation and the anion of the electrolyte. Comparison of the results obtained in this study and those for other amino acids reported in the literature, indicates that the structure of the hydrocarbon backbone of an amino acid plays an important role in the interactions of an amino acid with an electrolyte. A thermodynamic model has been used to correlate the solubilities of DL-aminobutyric acid in aqueous electrolyte solutions. The activity coefficients of the amino acid in the electrolyte solutions, were represented by a model proposed by Khoshkbarchi and Vera [M.K. Khoshkbarchi, J.H. Vera, AIChE J. 42 (1996) 2354; M.K. Khoshkbarchi, J.H. Vera, Ind. Eng. Chem. Res. 35 (1996) 4755]. This model, which considers a combination of both long- and short-range interactions, contains only two adjustable parameters. All other parameters are available in the literature. The model can accurately correlate the solubility of DL-aminobutyric acid in aqueous solutions of electrolytes.

Keywords: DL-Aminobutyric acid; Aqueous solutions; Thermodynamic model

1. Introduction

The effect of electrolytes on the solubilities of biomolecules is well described in the literature [1]. Electrolyte-induced separation of biochemicals has recently attracted attention due to its simplicity and economy [2]. Separation processes based on precipitation and crystallization have been widely used for the concentration and separation of biomolecules [3]. These simple and efficient techniques, however, depend on various complex interaction phenomena. A better understanding of the nature of these interactions is

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essential for the efficient use of electrolyte-induced separation techniques, and for widening their range of applications.

Experimental data of the solubilities of amino acids in aqueous electrolyte solutions [1,4,5] indicate that the increase (salting-in) or the decrease (salting-out) in solubility with an increase in the electrolyte concentration depends, to a large extend, on the nature of both the amino acid and the electrolyte present. In order to model the solubility of an amino acid in aqueous electrolyte solutions, it is necessary to know the values of the activity coefficient of the amino acid. Kirkwood [6,7] developed two models which can qualitatively represent the behaviour of the water–electrolyte–amino acid systems at low electrolyte and amino acid concentrations. Chen et al. [8] used their own version of the electrolyte NRTL model [9] to model the solubilities of four amino acids in electrolyte solutions. Their model contains a long-range interaction term, represented by a Pitzer–Debye–Hückel form [10], and a short-range interaction term given by a modified form of the NRTL equation [11]. Fernández-Mérida et al. [12] and Rodriguez-Raposo et al. [13] applied the modified form of the Pitzer model [14] for aqueous solutions of an electrolyte and a non-electrolyte. Khoshkbarchi and Vera [15,16] have recently proposed two models for the activity coefficients of amino acids in aqueous solutions containing an electrolyte. The first model [15] is a combination of a short-range interaction term represented by the NRTL model [11] or the Wilson model [17] and a long-range interaction term represented by the Bromley [18] or the K-V model [19]. The second model is based on the perturbation theory with a hard sphere reference system. Both models accurately correlate the activity coefficient of an amino acid, over a wide range of amino acid and electrolyte concentrations.

The purpose of this study is to investigate the effects of the anion and the cation of an electrolyte on the solubility of DL-aminobutyric acid. Solubility measurements of DL-aminobutyric acid were performed at 298.2 K in aqueous solutions of NaCl, KCl, $NaNO_3$ and KNO_3. The solubilities of the amino acid in aqueous electrolyte solutions were correlated using the activity coefficients obtained from a model proposed by Khoshkbarchi and Vera [15].

2. Materials and experimental techniques

DL-Alpha-amino-*n*-butyric acid (DL-aminobutyric acid) with a purity of +99% was obtained from ICN (Barcelona, Spain). Sodium chloride of 99% purity, potassium chloride of 99% purity, sodium nitrate of 99% purity, and potassium nitrate of 99% purity, were obtained from SIGMA (Madrid, Spain). The amino acid was used as received. The salts were oven-dried for 72 h prior to use. All solutions were prepared based on molality and the water was also weighed. The compositions of the initial solutions were accurate within ± 0.01 wt.%. In all experiments deionized water with a conductivity of less than 0.8 μS cm^{-1} prepared by a Milli-Q Plus system was used. The temperature of the samples was kept constant at 298.2 ± 0.02 K using a thermostatic bath, coupled with a bath cooler.

Solutions were prepared at different molalities of electrolyte, with the amino acid added in excess to the amount required for saturation. Jacketed glass containers, containing 40 ml of solution, were continuously stirred with teflon coated magnets. The temperature of the solutions were first maintained for 3 h at 303.2 K, and then lowered to 298.2 K. The mixing was continued for 48 h to reach equilibrium, and stopped for 7 h to settle the undissolved amino acid particles. The results of experiments, with different mixing times, showed that there were no detectable difference in solubility after 24 h. A period of 48 h for mixing was employed to insure that the equilibrium was attained. After settling the undissolved amino acid particles, a sample of the supernatant phase was withdrawn with a plastic syringe, and filtered through a 0.2-μm, Minisart Sartorious disposable filter, into a previously weighed aluminum dish. These filters were previously tested with solutions of known concentration of the amino acid, and were found not to adsorb the amino acid. The aluminum dishes were then capped and weighed. The caps were also weighed. After removing the caps, the dishes were placed

in an oven for 24 h at 323.2 K and for 72 h at 353.2 K to evaporate the water. After the evaporation, the aluminum dishes with the dry samples were weighed. The molality of amino acid dissolved was calculated from the knowledge of the initial concentration of the electrolyte present in the solution and the weights of the caps and of the aluminum dishes empty, with solution and with the dry samples. Various tests were performed to determine the effect of possible interference factors such as adsorption of the amino acid to the filter and syringe, sublimation of DL-aminobutyric acid during drying, and purity of the solid phase. The details of these tests have been discussed elsewhere [4]. The results of these tests showed that these factors have no effect on the experimental data.

All experiments were replicated at least three times, and in some cases four times. The data reported are the average of the replicates. Sample variances were obtained from the replicates for each point and a pooled standard deviation was calculated using these values. The calculated pooled standard deviations for the values of the solubilities of DL-aminobutyric acid in aqueous solutions of NaCl, KCl, $NaNO_3$ and KNO_3 were calculated to be ± 0.003, ± 0.004, ± 0.011 and ± 0.009 molality, respectively.

3. Experimental results

Fig. 1 and Table 1 present the solubilities of DL-aminobutyric acid in aqueous solutions of NaCl, KCl, $NaNO_3$ and KNO_3 at various electrolyte concentrations. As can be seen from Fig. 1, the effect of the presence of an electrolyte on the solubility of DL-aminobutyric acid depends on the nature of both the cation and the anion of the electrolyte. This figure shows that the solubility of DL-aminobutyric acid is more sensitive to the nature of the cation of the electrolytes with nitrate ion than chloride ion as the anion. Notably, over the whole range of electrolyte concentration studied, the electrolytes containing nitrate ion have a salting-in effect whereas the electrolytes containing chloride ions show a salting-out effect. It can also be seen that the effect of the cation of the electrolyte is smaller than the effect of the anion of the electrolyte. The effect of the electrolytes on the solubility of DL-aminobutyric acid increases as the concentration of the electrolytes increase.

It is interesting to compare the solubilities of DL-alanine and DL-valine in the presence of KCl reported previously [4], with the data for DL-aminobutyric acid and KCl measured in this work. The DL-aminobutyric acid has one $-CH_3$ group more than DL-alanine, and DL-valine has one more branched $-CH_3$ group than DL-aminobutyric acid in its hydrocarbon backbone. All three of these amino acids are alpha amino acids. Fig. 2 compares the ratios of the solubilities of these amino acids in the presence of KCl to that in pure water, S/S_0. As shown in this figure, the effect of an electrolyte on the solubility of an amino acid depends not only on the nature of the cation and the anion of the electrolyte, but also on the structure of the hydrocarbon backbone of the amino acids.

The chemical structure of the hydrocarbon backbone of the amino acid plays an important role in its interactions with water molecules and with different ions. A comparison of the solubilities of glycine, DL-alanine and DL-valine in water reported by Fasman [20] shows that, as expected, a larger hydrocarbon backbone in the amino acid leads to a lower solubility in water. In aqueous solutions the carboxyl group of the amino acid loses a proton and becomes negatively charged, while the amino group gains a proton and becomes positively charged. This results in the formation of a strong electrostatic field around the amino acid molecules, which gives rise to impor-

Table 1
Solubilities of DL-aminobutyric acid in aqueous solutions of NaCl, KCl, $NaNO_3$ and KNO_3 (molality)

Electrolyte molality	NaCl	KCl	$NaNO_3$	KNO_3
0.0	2.044	2.044	2.044	2.044
0.5	2.026	2.028	2.106	2.104
1.0	1.977	1.988	2.138	2.111
1.5	1.929	1.922	2.165	2.102
2.0	1.874	1.865	2.174	2.102
2.5	1.819	1.802	2.178	2.091
3.0	1.745	1.723	2.160	2.049

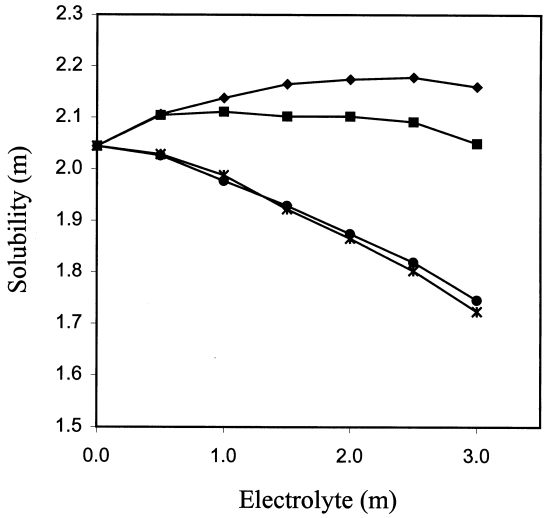

Fig. 1. Effect of electrolyte nature and concentration on the solubility of DL-aminobutyric acid in aqueous solutions. ♦, $NaNO_3$; ■, KNO_3; ●, NaCl; ∗, KCl.

tant ion–amino acid interactions. The ion–amino acid interactions can be classified in long-range interactions, which are purely electrostatic in nature, and the short-range interactions which depend on the nature of the of ions. The long-range

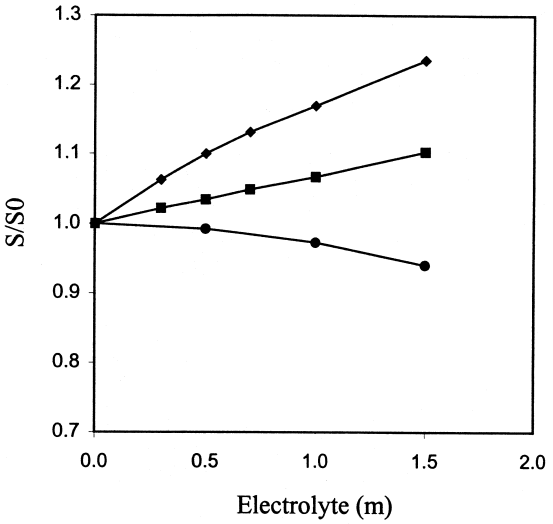

Fig. 2. Effect of amino acid hydrocarbon backbone structure and KCl concentration on the solubility of an amino acid in aqueous KCl solutions. ♦, DL-valine; ■, DL-alanine; ●, DL-aminobutyric acid.

interactions are responsible for the condensation of the ions on the amino or carboxyl groups of the amino acid. Whereas, the short-range effects are due to the effect of the ion pairs formed as a result of the ion condensation phenomenon. Higher electrolyte concentrations screen more the electrostatic ion–dipole interactions between amino acid molecules and the ions, favoring the formation of more ion–pair complexes, as a result of the mass action law. Thus, the ion–pair complexes formed with different ions affect more the solubilities of the amino acid at higher concentrations. The formation of these ion–pair complexes, which may also occur because of adsorption of ions on the non-polar side chains of amino acid molecules, also changes the physicochemical characteristics of amino acids leading to change in their solubilities. In addition, high ion concentrations increase the interactions of the ions with the charge resonance of the carboxyl group making more pronounced the effect of the ions on the solubility of amino acids.

4. Modelling of the solubility

The modelling of the solubility of amino acids in electrolyte solutions by different authors, has been discussed by Khoshkbarchi and Vera [4]. As shown previously [4], the solubility of an amino acid in an aqueous solution containing an electrolyte can written as:

$$m_{AA} = \frac{m^{\circ}_{AA}\gamma^{\circ}_{AA}}{\gamma_{AA}} \times \frac{f^{S}_{AA}}{f^{\circ S}_{AA}} \quad (1)$$

where m°_{AA} is the saturation molality in an aqueous solution, γ°_{AA} and γ_{AA} are the molality scale unsymmetrically normalized activity coefficient of the amino acid in the absence and in the presence of electrolyte, respectively. Previous studies have shown that the fugacity of the amino acid in the solid phase in equilibrium with an aqueous electrolyte solution saturated in amino acid, f^{S}_{AA}, is different from the fugacity of the amino acid in a solid phase in equilibrium with an aqueous solution without an electrolyte, $f^{\circ S}_{AA}$. Since there is no independent information of the values of γ_{AA},

with the purpose of correlating the solubilities of the amino acid, in this work we assume that the ratio $f^{S}_{AA}/f^{\circ S}_{AA}$ is equal to unity, or equivalently, that both solid phases are pure amino acid in the same crystalline form. Hence, in this case, Eq. (1) simplifies to:

$$m_{AA} = \frac{m^{\circ}_{AA}\,\gamma^{\circ}_{AA}}{\gamma_{AA}} \tag{2}$$

Eq. (2) implies that once the solubility of the amino acid in pure water, and its activity coefficient at saturation in pure water and in the water–electrolyte systems are known, the solubility of the amino acid in an aqueous solution containing an electrolyte can be calculated. The activity coefficients in Eq. (2) are represented here by a model proposed by Khoshkbarchi and Vera [15]. The model, which considers the contributions of long- and short-range interactions to the excess Gibbs free energy, G^E, of the system, has the form:

$$G^E = G^E_{LR}(x_W, x_S, x_{AA}=0) + G^E_{SR}(x_W, x_S, x_{AA}) - G^E_{SR}(x_W, x_S, x_{AA}=0) \tag{3}$$

where subscripts LR and SR stand for the long- and short-range interaction terms, respectively, and x_W, x_S, x_{AA} are the mole fractions of water, electrolyte and amino acid, respectively. The symbols in brackets indicate the composition variables considered in each term. The long-range interaction term in Eq. (3) takes into account all interactions in a binary water–electrolyte system, whereas the short-range interaction terms account for all interactions between water–amino acid, amino acid–electrolyte and also for the change in the interaction between the electrolyte and water, due to the presence of the amino acid. The structure of the model is such that the excess Gibbs free energy of the system reduces to the excess Gibbs free energy of the binary water–electrolyte system in the absence of amino acid and to the excess Gibbs free energy of the binary amino acid–water system in the absence of electrolyte. The activity coefficients of the amino acids can then be obtained by proper differentiation of Eq. (3) [15]. Since at neutral pH the amino acid molecules are predominantly in their zwitterionic form, the net charge on the amino acid is zero. Thus, the amino acid is not considered to contribute to the ionic strength of the system. As the long-range interaction term of the model is considered to be only a function of ionic strength, there is no contribution from this term to the activity coefficient of the amino acid. In this study, the unsymmetrically normalized, molal based, NRTL model is employed to represent the short-range interaction term. The mole fraction symmetrically normalized NRTL model is written as:

$$\ln \gamma_i^{NRTL} = \frac{\sum_{j=1} \tau_{ji} G_{ji} x_j}{\sum_{j=1} G_{ji} x_j} + \sum_{j=1} \frac{x_j G_{ij}}{\sum_{k=1} x_k G_{kj}} \left(\tau_{ij} - \frac{\sum_{k=1} x_k \tau_{kj} G_{kj}}{\sum_{k=1} x_k G_{kj}} \right) \tag{4}$$

with the parameter G_{ij} defined as:

$$G_{ij} = \exp(-\alpha \tau_{ij}) \tag{5}$$

where α is the non-randomness factor, τ_{ij} is the binary energy parameter with $\tau_{ii} = 0$. The derivation of this model has been discussed in detail by Khoshkbarchi [15]. The activity coefficient of the amino acid in this framework is obtained from the following relation:

$$\ln \gamma_{AA} = \ln \gamma^{NRTL}_{AA} - \lim_{x_{AA}\to 0, x_S \to 0} \ln \gamma^{NRTL}_{AA} - \ln[1 + 0.001\, M_W (m_{AA} + m_S)] \tag{6}$$

The activity coefficients given by Eq. (6), are normalized in the unsymmetric convention, i.e. with $\gamma_i \to 1$ as $x_i \to 0$. The reference state for water is its pure state while that of the electrolyte and amino acid are their states at infinite dilution in water. The NRTL model for a ternary amino acid–electrolyte–water system contains three sets of binary interaction parameters, i.e. water–amino acid, amino acid–amino acid and electrolyte–

water parameters. The long-range interaction of the water–electrolyte system is considered in the first term in Eq. (3) and it does not contribute to Eq. (6). The two binary interaction parameters for the water–electrolyte pair included in the NRTL model, account for the change in the interactions between the electrolyte and water due to the presence of amino acid molecules. This change in interactions of the water–electrolyte pair is mainly caused by the formation of weak physical bonds between charged groups of amino acid molecules and the free ions of the electrolyte. The model used here assumes that the weak physical bonds between charged groups of amino acids and the ions do not form new molecules, and that the activity coefficients of all amino acid molecules are the same.

5. Results of the calculations

The model proposed in this study was employed to correlate the solubilities of DL-aminobutyric acid in the solutions of the four electrolytes studied here. The value of the non-randomness parameter, α, in the NRTL model and the water–DL-aminobutyric acid binary parameters are those reported by Khoshkbarchi and Vera [15]. The binary electrolyte–DL-aminobutyric acid and electrolyte–water parameters were considered to be symmetric, $\tau_{ij} = \tau_{ji}$, and were treated as two adjustable parameters. These parameters were evaluated by the fitting the parameters of the model to the experimental data. The list of the parameters, along with the root mean square deviation error, are presented in Table 2. The results of the modelling are shown in Fig. 3. As can be seen from this figure, the model can accurately represent the experimental data for solubility of DL-aminobutyric for all four electrolytes studied here, over the whole range of electrolyte concentration. It is important to mention that the values of the adjustable parameters in the model may be influenced by the fact that at high electrolyte concentrations the weak physical bonds between the ions and charged amino and carboxyl groups of the amino acid become more strong and the ion-pair complexes act as independent molecules. This renders the activity coefficient model less accurate and therefore reduces the accuracy of the solubility model. High electrolyte concentrations also increase the adsorption of ions on the solid DL-aminobutyric acid phase and therefore changes the crystallographic shape of the crystalline in the solid phase. This, in turn, changes the fugacity of the solid phase compared to a solid phase in equilibrium with a water–DL-aminobutyric acid solution. As a result of this phenomenon, the accuracy of the parameters of the model decreases for systems with high electrolyte concentrations. This phenomenon has been discussed in detail by Khoshkbarchi [4].

Table 2
Regressed binary interaction parameters for the solubility of DL-aminobutyric acid (AA) in water (W)–electrolyte (S) solutions

Interaction parameters	NaCl	KCl	$NaNO_3$	KNO_3
$\tau_{AA-S} = \tau_{S-AA}$	−2.392	−4.369	−1.229	−1.796
$\tau_{W-S} = \tau_{S-W}$	−3.742	−3.000	−2.006	−2.786
r.m.s. d × 100	0.49	0.50	0.39	0.82

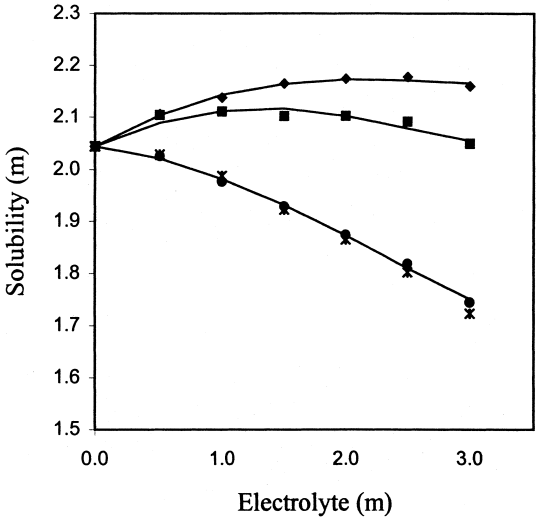

Fig. 3. Modelling of the effect of electrolyte nature and concentration on the solubility of DL-aminobutyric acid in aqueous solutions. ♦, $NaNO_3$; ■, KNO_3; ●, NaCl; ∗, KCl; ———, result of the modelling.

6. Conclusions

The solubilities at 298.2 K of DL-aminobutyric acid in aqueous solutions at various NaCl, KCl, $NaNO_3$ or KNO_3 concentrations, up to 3.0 molality, were measured. The results showed that both the concentration of electrolyte and the nature of its cation and anion affect the solubilities of DL-aminobutyric acid. Notably, nitrate ions produce a salting-in effect and the chloride ions a salting-out effect. With chloride anion, the effect of the cation is weak. With nitrate anion, however, sodium has a larger salting-in effect than potassium. Comparison of the data measured in this study and those obtained from a previous work [4] revealed the nature of the hydrocarbon backbone of the amino acid has also a major effect on the solubilities of amino acids in aqueous electrolyte solutions.

A two-parameter model was developed to correlate the solubility of amino acids in aqueous electrolyte solutions. The activity coefficient of amino acids required by the solubility model was represented with an unsymmetric, molal based NRTL model as proposed by Khoshkbarchi and Vera [15]. It was shown that the model developed in this study can accurately correlate the solubilities of amino acids in aqueous electrolyte solutions over a wide range of electrolyte concentration.

7. Notation

f, fugacity;
G^E, excess Gibbs free energy;
G_{ij}, NRTL parameter;
m, saturation molality;
S, solubility;
x, mole fraction;
α, non-randomness factor;
γ, activity coefficient; and
τ, binary interaction parameter;

Superscripts and subscripts

AA, amino acid;
LR, long-range interactions;
NRTL, NRTL model;
S, solid phase;
SR, short-range interactions;
W, water;
o, Water–amino acid system; and
θ, standard state

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