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EFFECT OF ANIONS, ACIDS AND BASES ON THE SOLUBILITY OF AMINO ACIDS IN AQUEOUS SOLUTIONS

by

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To My Parents

Abstract

The effect of acids and bases on the solubility of amino acids at 298.2 K, within the pH range (2-10) and out of the pH range was determined. The amino acids studied were glycine, DL-alanine, DL-valine and DL-serine. The acids used were HCl and HNO_3 and the bases NaOH and KOH. It was observed that in the pH range (2-10) there is no significant difference in the solubility of DL-alanine in the presence of different ions such as, Na^+ or K^+, at high pH, and Cl^- or NO_3^-, at low pH. However, in concentrated acids the solubility is higher in nitric acid than in hydrochloric acid. Also in concentrated bases the solubility is higher in KOH than in NaOH.

The solubilities of these amino acids in electrolyte solutions containing either $NaNO_3$ or KNO_3 were also measured at 298.2 K. The effect of anions was determined by comparing these results, with those reported in the literature for the effect of NaCl and KCl. For each cation, the solubility is higher with nitrate than with chloride anion. This is the first systematic study reporting the effect of anions on the solubilities of amino acids in water.

Résumé

L'action d'acides ou de bases sur la solubilité d'amino acide a été déterminée à 298.2 K avec un pH variant dans un cas entre 2 et 10 et dans un autre cas se situant en dehors des limites usuelles de définition du pH. Dans cette étude, les amino acides étudiés ont été la glycine, la DL-alanine, la DL-valine et la DL-serine. Les acides et bases utilisés ont été respectivement HCl, HNO_3, NaOH et KOH. Les résultats obtenus avec un pH variant entre 2 et 10 ont été les suivants. A des pH élevés, aucune différence importante entre les ions Na^+ et K^+ sur la solubilité de la DL-alanine n'a été notée. Il en est de même entre les ions Cl^- et NO_3^- à bas pH. Cependant l'étude à des pH trés élevés a montré que la solubilité en milieu acide concentré est plus élevée avec l'acide nitrique qu'avec l'acide chlorhydrique. De même en milieu basique concentré, la solubilité est plus grande avec la potasse qu'avec la soude.

Les solubilités pour les différents acides aminées en solution électrolytique contenant soit du nitrate de soude soit du nitrate de potassium ont également été mesurées à 298.2 K. L'action des anions pour NaCl et KCl a été déterminée en comparant les résultats obtenus à ceux de la littérature. Pour chaque cation, la solubilité s'est avérée plus grande avec l'anion NO_3^- qu ávec l'anion Cl^-. Ce travail est le première étude systématique analysant l'action d'anions sur les solubilités d'amino acides en milieu aqueux.

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I am grateful to the following people for assisting in carrying out various tasks related to my thesis:

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Nomenclature

A = amino acid

K_D, K_1 and K_2 = equilibrium constants of reactions (1.1), (1.2) and (1.3) respectively

x = mole fraction

f = fugacity

Δs and Δh = change in the molar entropy and enthalpy of the amino acid between the standard state and the solid state

R = gas constant

T = absolute temperature

m = molality

M = molarity

A^- = anion of either an acid or electrolyte

C^+ = cation of either the base or electrolyte

AA^-, AA^+ = charged form of an amino acid

a = adjustable parameter

b = adjustable parameter

G= Gibbs free energy

Greek letters

γ = activity coefficient

τ = binary interaction parameter

α = nonrandomness factor

Superscripts

L= liquid phase

S = solid phase

O = property in the water-amino-acid system

Subscripts

A= amino acid

S = electrolyte

W = water

$\pm$ = zwitterion

+ = cation

- = anion

Chapter 1: Introduction

1.1 General Aspects

The separation of biomolecules is an important aspect in the production of biochemicals in the food, pharmaceutical and chemical industries. The conventional ways of concentration and separation are fractional precipitation, crystallization, and ion exchange. The cost involved in these separation processes, is as high as 50% of the total cost of manufacturing (Eyal and Bressler, 1993). Due to the advancements in the biotechnology industry more sophisticated and efficient separation techniques are required. To design separation units that are reliable, it is important to have a thorough knowledge of the properties of the biosystems involved. The majority of the commercially available biochemicals are produced in the crystalline form, so knowing their solubility behavior is essential.

The thermodynamic properties of aqueous systems containing salts and charged molecules are important in the biotechnology industry (Prausnitz,

1989). By studying the interactions of biochemical molecules, in different electrolyte solutions, the thermodynamic behavior can be modeled. The interaction of biomolecules with electrolytes has application in separation processes, such as salt induced precipitation and reverse micellar extraction.

1.2 Amino acids

There are twenty basic amino acids found in all living organisms. In fact, amino acids are the basic building blocks of proteins and peptides, and the study of their solubility behavior in the presence of different electrolytes may be of use for the understanding of the behavior of more complex biomolecules. Although amino acids are among the simplest biochemicals, they have many similarities with more complex biomolecules such as antibiotics (Orella and Kirwan, 1991).

For the study of the structure of proteins, the protein is first hydrolyzed into its component amino acids. The process of separation and identification of the amino acids is difficult. Methods such as electrophoresis and ion exchange chromatography are used for these processes. Applying these methods requires the knowledge of the acid-base properties of amino acids at a given pH.

Experimental data have been reported by Hitchcock, 1924; Dalton and Schmidt, 1933; Greenstein and Winitz, 1961; Needham et al., 1971; Orella and Kirwan, 1989; Zumstein and Rousseau, 1989; Gatewood and Rousseau, 1989. Additional data on the pH effect were reported by Carta and Tola, 1996. Recently, solubility data of amino acids in aqueous solutions of electrolytes such as NaCl, KCl were reported by Khoshkbarchi (1996).

Aliphatic amino acids exhibit the properties of strong electrolytes when dissolved in solvents of high dielectric constant, such as water or alcohol (Kirkwood, 1934). Unlike electrolytes, amino acids do not produce single charged ions. Amino acids have an amino group and a carboxyl group, which are ionized in aqueous solutions. These bear a separate positive and a negative charge, respectively. The powerful electric fields surrounding these charged ions gives rise to important interactions. Thus in the neutral pH range, the amino acids have both a positive and a negative charge, and are thus called zwitterions. The following reactions take place when an amino acid is present in the aqueous phase:

$$NH_2RCOOH(s) \Leftrightarrow NH_3^+RCOO^- \quad (1.1)$$

$$NH_3^+RCOOH \Leftrightarrow H^+ + NH_3^+RCOO^- \quad (1.2)$$

$$NH_3^+RCOO^- \Leftrightarrow H^+ + NH_2^+RCOO^- \quad (1.3)$$

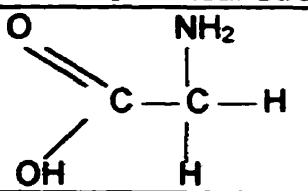
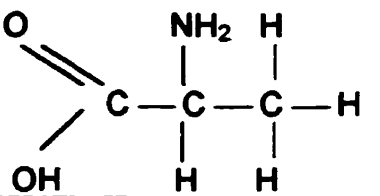
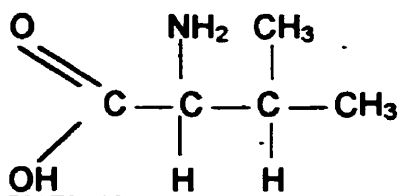
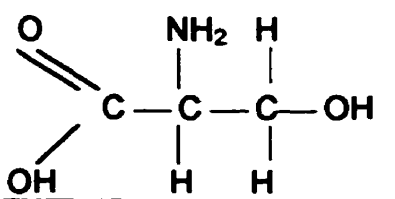
Once the carboxyl group of the amino acid loses a proton, and the amino group gains a proton, the zwitterionic molecules act as strong dipoles causing important ionic interactions. Thus the solution behavior of amino acids, especially those with relatively small side chains like glycine and alanine, is similar to that of electrolytes (Edwin et al., 1934).

In this study we focus our attention on the solubility of four aliphatic amino acids: glycine, DL-alanine, DL-valine and DL-serine. Table 1.1 depicts the difference in the chemical structure of these four amino acids. Glycine has the simplest chemical structure with only one CH_2 group, DL-alanine and DL-valine have one and three additional CH_2 groups respectively, and DL-serine is similar to DL-alanine except that it has an OH group attached to its hydrocarbon backbone.

1.3 Motivation

Previous work by Khoshkbarchi (1996) studied the effect of different ions on the activity coefficient and on the solubility of amino acids. It was found that the presence of different cations in the aqueous solutions of amino acids could lead to a salting-out or salting-in effect, depending on the amino acid. The salt dissociates in the aqueous solution and leads to a decrease or increase in the solubility of the amino acids. The effect of cations was

Table 1.1: Chemical structure of amino acids studied.

Amino acid	Chemical Structure
glycine	
DL-alanine	
DL-valine	
DL-serine	

studied using sodium chloride and potassium chloride. The results reported showed that for all the amino acids studied here, the solubility was higher in the presence of potassium than sodium. DL-alanine and DL-valine both lead to salting-out in the presence of sodium chloride and salting-in effect in potassium chloride. Glycine on the other hand showed salting-out at lower concentrations and salting-in at higher concentrations. The amino acid DL-serine had salting-in effect in both sodium and potassium chloride. It was thus expected that these cations would have a similar effect in the presence of different anions. No systematic studies of the effect of anions on the solubility of amino acids have been reported in the literature. Experimental data of the effect of pH were reported by Hitchcock, 1924, Dalton and Schmidt, 1933, Needham et al., 1971, Carta and Tola, 1996. Their work concentrated on the effect of pH adjusting it with NaOH and HCl. The effect of different cations, in the high pH range, or of different anions, in the low pH range, has not been studied. As seen in the work of Khoshkbarchi (1996) the presence of sodium and potassium did affect the solubility of the amino acids in aqueous solutions. Thus the study of the effect of cations in the high pH range using NaOH, KOH and of anions in the low pH range using HCl and HNO_3 is a natural continuation of the work. One would expect to find similar salting-in and

salting-out behavior than with other strong electrolytes.

1.4 Objectives

The literature survey showed that no data had been reported on the effect of anions and on the effect of pH (using different acids and bases) on the solubility of amino acids.

Thus the two objectives of this study were:

A) To study the effect of anions on the solubility of amino acids using aqueous solutions of $NaNO_3$ or KNO_3, and to compare the results with data reported for systems containing NaCl or KCl. Data for the solubility of amino acids in aqueous solutions of NaCl and KCl have been reported by Khoshkbarchi, (1996).

B) To study the influence of pH on the solubility of amino acids by adjusting the pH with different acids and bases. It was expected that at the same pH, the effect of different cations, such as sodium and potassium, or different anions, such as chloride or nitrate, would lead to differences in solubilities of the amino acids.

Chapter 2: Methods of Analysis for Solubility of Amino Acids

To define the structure of a protein or a peptide, it is a prerequisite to determine its amino acid composition. There is no analytical panacea applicable to the assaying of amino acids. A method could prove to be more effective than another, in specific situations. Often the analytical technique chosen by a researcher depends on the equipment available (Mackenzie L.S., 1990).

In this study, three methods were used to determine the solubility of amino acids. A comparative study was done, and then the experiments were carried out based on the method selected. The analytical techniques were:

- U-V Spectroscopy
- Carbon Analyzer
- Gravimetry

2.1 U-V Spectroscopy

Organic compounds are capable of absorbing electromagnetic radiation as they have valence electrons that can be excited to higher energy levels. As

the energy associated with the electrons in the formation of single bonds is high; the absorption is restricted to the vacuum ultraviolet region. Due to experimental difficulties associated with the vacuum ultraviolet region for most spectroscopic investigations the wavelength used is greater than 180 nm.

Unsaturated organic compounds, when subjected to ultraviolet light of the correct wavelength, absorb radiant energy. This is because π electrons move to their first excited state. Beer's law is used for the measurement of concentration. This law postulates that there exists a linear relationship between absorbency at a fixed wavelength and the concentration of a compound. The utility of a plot of absorbency versus concentration is to have a standard curve, and then use it for determining unknown concentrations.

Roth (1971) proposed a fluorescence reaction for all amino acids. Wang (1994) used this o-phthaldialdehyde (OPA-labeling) procedure for amino acid extraction experiments. This method involves the addition of a strong reducing agent for producing bright fluorescence. The principle of this OPA labeling procedure is that the amino acid released reacts with o-phthaldialdehyde and 2-mercaptoethanol to form an abduct that strongly absorbs at 340 nm (Wang, 1994).

2.1.1 Experimental Procedure:

The OPA reagent was prepared by mixing 0.8 g o-phthaldialdehyde with 20 ml of ethanol and then 10 g of sodium tetraborate, 2 ml 2-mercaptoethanol and 1000 ml of water. This reagent has to be used immediately after preparation. For determining the solubility, 20 ml of this OPA reagent was used with 0.5 ml of a sample of the supernatant aqueous phase containing the amino acid. The concentration of the amino acids measured ranged from 2.0 to 3.5 mM. The absorbency at 340 nm (the optimal wavelength) was then measured 5 minutes after mixing. A calibration curve for the analysis was prepared, and then the concentrations were determined. The absorbency at each concentration of the amino acid was measured with a CARY 1/3 UV-spectrophotometer (Varian Techtron Pty Ltd., Victoria, Australia).

2.1.2 Results

The result obtained by this method for glycine is shown in Table A.1 (Appendix A). At high concentrations of sample the degree of absorption is high and very little radiant energy falls on the detector. Then, at lower concentrations the amount of radiation falling on the detector is high, and the absorption is low. The error in the percent absorption leads to an error in the

determination of concentration from the calibration curve. Thus at high or low concentrations of the sample, significant error could be introduced (Robinson, 1995). As seen in Table A.1 the solubilities determined by this method were lower than the literature values. The errors involved in this process are associated with sample handling, dilution errors in solution preparation, and the intrinsic spectrophotometric error. The errors in the measurement of radiation intensity directly lead to errors in the measurement of the concentration when using calibration curves.

2.2 Carbon Analyzer

This is an organic elemental analysis method. It involves the combustion of the element carbon in the amino acid. The combustion is performed in the presence of a catalyst. The operating principle is as follows: acidified potassium persulphate reagent is pumped from an external reservoir to the injection port and then into the bottom of the UV reactor, which is a constant volume reactor. The liquid in the reactor is continuously sparged and this carrier gas flows out at the top of the reactor to the non-dispersive infrared detector. When a sample is thus injected, it is carried by the reagent into the reactor. Oxidation occurs rapidly and the resultant carbon dioxide is sparged from the liquid and carried to the detector. The detector produces an electrical

output that is integrated, and scaled by the number processor, and then displayed and printed.

2.2.1 Experimental procedure

The concentration of the sample was measured using a Dohrmann DC-80 Total Organic Carbon Analyzer in combination with a Dohrmann DC-85 NDIR detector module. Suitable standards were injected in the calibration mode that enabled direct concentration measurements. The range of concentrations detected by this instrument is from 1ppm to 2000 ppm.

Samples were injected using a 50 μl syringe. The calibration factor memory was cleared. The 400 ppm standard was then injected, and the digital display showed increasing number of counts. When the detector signal was back at the original base line, the analysis terminated automatically. The diluted samples of the upper phase of the aqueous amino acid solutions were then injected. A standard was injected from time to time. The carbon concentration measured was then converted to give the solubility of the amino acid.

2.2.2 Results

The results obtained from the carbon analyzer are shown in Table A.1 (Appendix A). As seen in this table, the values of the solubilities were much lower than the reported literature values. The accuracy of the analyzer is dependent on the accuracy of the standards plus the precision and linearity of the analyzer. Combustion techniques such as these, also are dependent on the attainment of complete combustion (Sibilia, 1996).

2.3 Gravimetric Analysis

The gravimetric method is one of the most accurate and precise methods of macro quantitative analysis (Christian, 1971). Factors such as temperature, volume of the solution, concentration of other species, and pH are important in preparing the solution.

2.3.1 Experimental procedure

A sample solution of glycine was prepared by adding the amino acid in a quantity well in excess to that corresponding to saturation. Then, 20 ml of water was added to the sample bottles. The sample bottles were then sealed, and agitated using magnetic stir bars in a thermostatic water bath at 298.2 K. The mixing was done for 48 hours, after which the solutions were allowed to

settle for 7 hours. Samples of the supernatant liquid were then taken using a plastic syringe. These samples were filtered using a MSI 0.22 μm, HPLC disposable filter. The filtered samples were loaded in a weighed aluminum dish, weighed and then dried in an oven for 48 hours at about 308 K. The solubility in the sample was then determined by the knowledge of the weights.

2.3.2 Results

As seen from Table A.1 (Appendix A), the results of the gravimetric method are closer to the reported literature values. In the literature survey done, it was seen that the gravimetric method had been used by Needham et al., 1971, Carta and Tola, 1996; and by Khoshkbarchi, 1996.

2.4 Comparative study of the analytical methods

In order to have a better comparison of the three analytical methods; the following study was done. Samples of known concentration (0.9 times the saturation concentration) were prepared. These were then analyzed using the above three methods, and the results compared. For all the experiments three replicates were taken, and then averaged. Table A.1 (Appendix A) shows the results, and compares them with the known value.

As seen from the confidence interval values and the proximity of the gravimetric result to the known value this method seems to be more reliable. The results from the carbon analyzer and the UV-spectroscopy were lower than the known value as was seen in the earlier sections 2.1.2 and 2.2.2 in comparison with the literature value. In addition, as compared to the UV-spectroscopy, the gravimetric method was less expensive. From this comparative study we concluded that the gravimetric method was to be used for further studies.

Chapter 3: Effect of Acids and Bases on the Solubility of Amino Acids

3.1 Introduction

One of the objectives of this thesis was to determine the effect of acids and bases on the solubility of amino acids, within the pH range (pH from 2 to10) and outside the pH range. In this chapter the solubility of DL-alanine at different pH values is reported. The pH was adjusted using acids such as HCl and HNO_3 and bases such as NaOH and KOH. The solubility of glycine, DL-alanine, DL-valine and DL-serine at higher concentrations of the same acids and bases were also studied.

Amino acids are usually recovered from the hydrolysis of keratinic material. These processes are carried out in the presence of a strong acid or base and the amino acids are obtained as precipitates by neutralization of the solution. In such cases the information of the solubility of amino acids in the presence of acids, bases and salt (formed during neutralization) is required for the rational design of the separation process (Carta and Tola, 1996).

Amino acids have an amino group and a carboxyl group, which are ionized in aqueous solutions in the neutral pH range giving rise to zwitterionic amino acid molecules, as shown in reactions (1.1-1.3) reproduced here,

$$NH_2RCOOH\ (s) = NH_3^+RCOO^- \qquad K_D$$

$$NH_3^+RCOOH = H^+ + NH_3^+RCOO^- \qquad K_1$$

$$NH_3^+RCOO^- = H^+ + NH_2RCOO^- \qquad K_2$$

The equilibrium constants K_D, K_1, and K_2 for these reactions, are defined as

$$K_D=\frac{[NH_3^+RCOO^-]}{[NH_2RCOOH]} \qquad (3.1)$$

$$K_1=\frac{[H^+][NH_3^+RCOO^-]}{[NH_3^+RCOOH]} \qquad (3.2)$$

$$K_2=\frac{[H^+][NH_2RCOO^-]}{[NH_3^+RCOO^-]} \qquad (3.3)$$

The values of pK_1 and pK_2 reported in the literature (Greenstein and Winitz, 1961) for three of the amino acids are reproduced in Table 3.1. Using pK_1 and pK_2, the values of K_1 and K_2 are obtained using the equation

$$pK = -\log_{10} K \qquad (3.4)$$

The value of K_D is $10^{5.41}$ for DL-alanine as reported (Greenstein and Winitz, 1961), this large value indicates that in aqueous solutions at neutral pH, the

Table 3.1: **Values of pK_1, pK_2 for the amino acids studied in this work (Greenstien and Winitz, 1961).**

Amino acid	pK_1	pK_2
glycine	2.350	9.778
DL-alanine	2.348	9.867
DL-valine	2.286	9.718

amino acid molecules are in their zwitterionic form. The square brackets in equations (3.1) to (3.3) of these ionic species represent the activities.

The concentration of the anionic and cationic forms of the amino acid are negligible at neutral pH. Once the carboxyl group of the amino acid loses a proton, and the amino group gains a proton the zwitterionic molecules have a large dipole moment (Cohn and Edsall, 1965) which gives rise to important ionic interactions. When an acid or a base is added, the counterion of the acid or base is introduced to the solution. This counterion interacts with the charged amino acid molecules. Thus, not only does the concentration of the zwitterion change, but the formation of ion-complexes changes the activity coefficients of the amino acids as well.

Data were obtained for the four amino acids: glycine, DL-alanine, DL-valine and DL-serine. The solubility of DL-alanine was measured at different pH values obtained by adjusting the pH with HCl or HNO_3 and with NaOH or KOH. The effect of these acids and bases, at higher concentrations, on the solubility of all four amino acids was also measured.

3.2 Experimental work

Glycine, DL-alanine, DL-valine and DL-serine, 99% purity, sodium hydroxide, 97.94% purity and potassium hydroxide, 85% purity, were obtained from A&C Chemicals Ltd. (Montreal, Quebec, Canada). Nitric acid 70%, with 16.91 ppm impurities was obtained from Anaechemia (Montreal, Quebec, Canada) and Hydrochloric acid 37% with maximum impurities up to 72.21 ppm was obtained from Fisher Scientific (Nepean, Ontario, Canada). The amino acids, acids and bases were used as received.

Deionized water, with a conductivity of less than 0.8 $\mu S\ cm^{-1}$, passed through ion exchange columns of East pure RF, (Compact Ultrapure Water System, Barstead Thermoline, Bubugue, IA) was used to make the solutions. Vials of outer diameter 24 mm and height 95 mm were used as sample bottles. The amino acids were added in excess of the amount required for saturation. 25 ml of water, and the acid or base were added in the sample bottles. The sample bottles were sealed using Parafilm. They were then placed in a thermostatic water bath at 298.2 K. The solution was agitated for 48 hours using teflon-coated magnetic stir bars. The mixing was then stopped and the solutions were allowed to settle for 7 hours.

Samples were taken of the supernatant liquid phase using a plastic syringe and filtered through a polyteraflurorethylene membrane of diameter 25 mm and a 0.22 μm pore size. The equilibrium pH was measured using a Fisher Scientific Accumet pH meter 10 and an Oakton sealed single junction epoxy electrode (Labcor Inc. Montreal, Quebec, Canada). Samples were collected in 40 ml glass beakers, which were weighed empty and with the samples and then dried in an oven for 48 hours at about 308 K. The weight of the dry samples was then taken. As discussed in section 2.3, gravimetric analysis was used to determine the solubility.

The same procedure was used for studying the effect of acids and bases at higher concentrations. The values reported are the average of at least three replicates. In the replicates done, different quantities of amino acids in excess to that at saturation were taken, and the solubility was determined. The relative accuracy of the pH meter was ±0.02 pH. At higher base concentrations, the 95% confidence interval, in molalities, was ±0.01 for DL-alanine-NaOH and KOH; ±0.01 for glycine-NaOH and ±0.02 for glycine-KOH; ±0.01 DL-serine-NaOH and ±0.01 for DL-serine-KOH; ±0.01 for DL-valine-NaOH and ±0.01 for DL-valine-KOH respectively. At higher concentration of the acids, it was ±0.01 for DL-alanine-HCl and ±0.02 DL-

alanine-HNO_3; ±0.01 for glycine-HCl and ±0.01 for glycine-HNO_3; ±0.02 for DL-serine-HCl and HNO_3; ±0.01 for DL-valine-HCl and ±0.01 for DL-valine HNO_3 respectively.

3.3 Results and discussion

Table B.1 (Appendix B) gives the values of the experimental data points at different pH values for DL-alanine using NaOH or HCl and Table B.2 gives the solubility using KOH or HNO_3. Data reported by Hitchcock (1924), Dalton and Schmidt, (1933), Needham et al., (1971) were obtained using NaOH or HCl. No data have been reported using other acids or bases. Figure 3.1 depicts the solubility of DL-alanine at different pH values. The effect of different ions such as Na^+ or K^+ and NO_3^- or Cl^- on the solubility is small in the pH range measured.

For solid liquid equilibrium of an amino acid in an aqueous solution, from equation (1.1) in terms of the zwitterionic form of the amino acid, we obtain,

$$x_{A\pm}\gamma_{A\pm}f^{OL} = f_A^{\ S} \tag{3.5}$$

where f^{OL} is the standard state fugacity and $f_A^{\ S}$ is the fugacity of the solid amino acid in contact with the acid or base. Expressing the ratio of fugacities

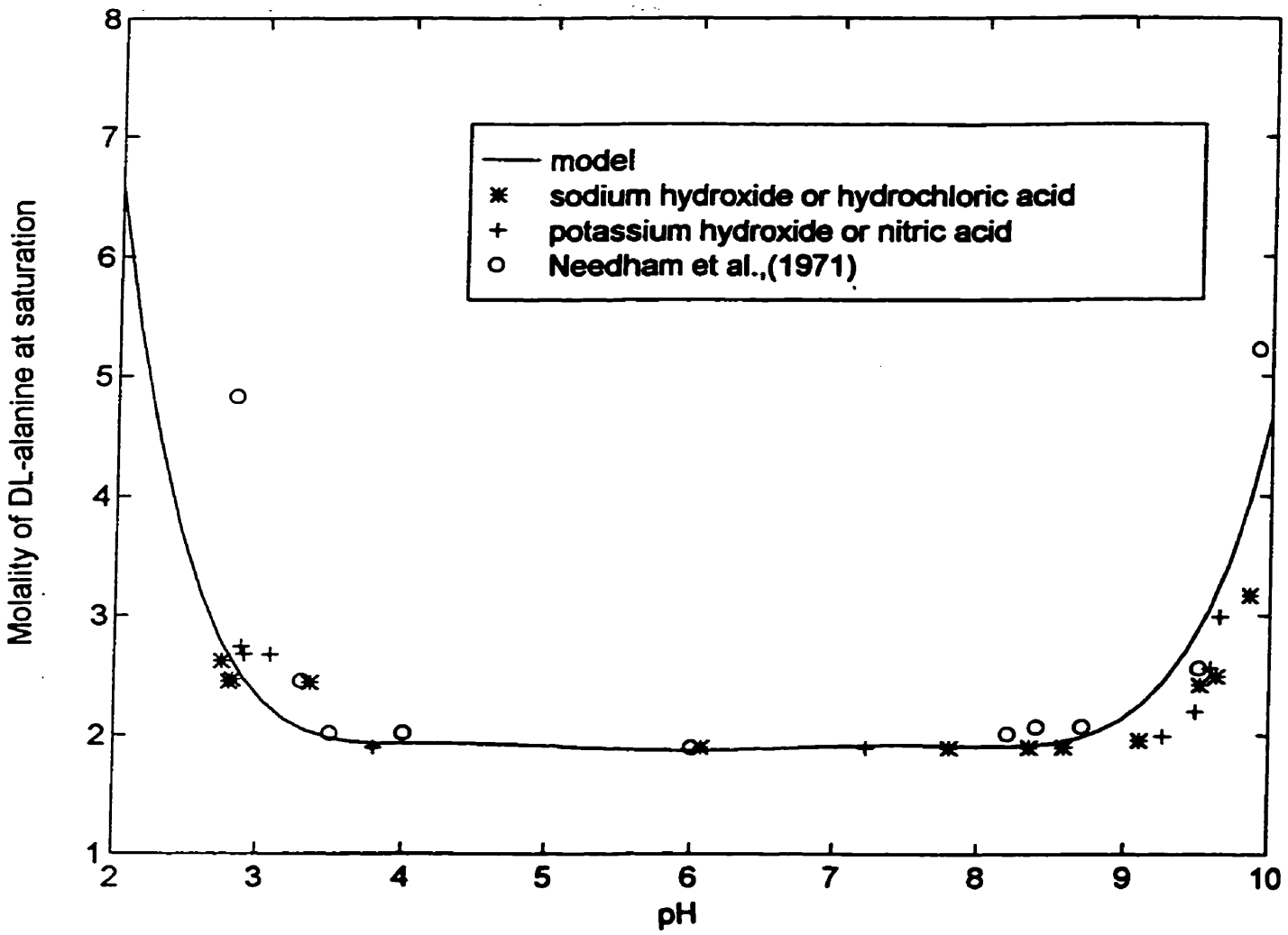


Figure 3.1: Effect of acids and bases in the pH range on the solubility of DL-alanine.

f_A^S / f^{OL} in terms of the molar entropy and enthalpy changes (Khoshkbarchi and Vera, 1996(a)) equation (3.5) takes the form:

$$x_{A\pm}\gamma^x_{A\pm} = \exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \tag{3.6}$$

Using equations (3.2) and (3.3), retaining the activity coefficients of each ionic form we obtain,

$$x_{A+} = \frac{[H^+]}{K_1} x_{A\pm}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A+}}\right) \tag{3.7}$$

$$x_{A-} = \frac{K_2}{[H^+]} x_{A\pm}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A-}}\right) \tag{3.8}$$

the total solubility x_A can be written as,

$$x_A = x_{A\pm} + x_{A+} + x_{A-} \tag{3.9}$$

Thus, substituting (3.7) and (3.8) in (3.9),

$$x_A = x_{A\pm}\left[1 + \frac{[H^+]}{K_1}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A+}}\right) + \frac{K_2}{[H^+]}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A-}}\right)\right] \tag{3.10}$$

Finally, combining equation (3.6) and (3.10), gives the form:

$$x_A\gamma^x_{A\pm} = \left[1 + \frac{[H^+]}{K_1}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A+}}\right) + \frac{K_2}{[H^+]}\left(\frac{\gamma^x_{A\pm}}{\gamma^x_{A-}}\right)\right]\exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \tag{3.11}$$

In the isoelectric region the cationic and anionic forms of the amino acid are at infinite dilution. Thus γ^{x}_{A+} and γ^{x}_{A-} are at unity and $x_A = x_{A\pm}$. Hence, equation (3.11) can be written as:

$$x_{A\pm}\gamma^{x}_{A\pm} = \left[1 + \frac{[H^+]}{K_2}\gamma^{x}_{A\pm} + \frac{K_3}{[H^+]}\gamma^{x}_{A\pm}\right]\exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \qquad (3.12)$$

In Figure 3.1 the solubility at different pH values has been correlated using equation (3.12). For DL-alanine the value of the rational activity coefficient $\gamma^{x}_{A\pm}$ =1.03 was obtained using the NRTL model (Khoshkbarchi and Vera, 1996(b)) at the isoelectric solubility. A value of $\Delta h/R$ =1107.4 (K) was used, as reported in the literature (Fasman, 1976), and a value of $\Delta s/R$ =0.332 was obtained by fitting the isoelectric solubility. The activity of the hydrogen ion $[H^+]$, is obtained using the pH definition:

$$pH = -\log_{10}[H^+] \qquad (3.13)$$

Equation (3.12) represents well the data between pH 3.8 and 8.5. At pH lower than 3.8 or higher than 8.5 equation (3.12) gives the correct qualitative trend. At high or low pH values, the amino acid molecule has a net charge and thus, it is expected to have a value of the activity coefficient different from the isoelectric point. At low pH the cationic form of the amino acid species are

predominant and the activity coefficients of the zwitterionic and anionic forms approach unity at infinite dilution.

Thus equation (3.11) is written as,

$$x_{A+} = \left[1 + \frac{[H^+]}{K_1}\left(\frac{1}{\gamma_{A+}}\right) + \frac{K_2}{[H^+]}\right] \exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right) \tag{3.14}$$

or for the activity coefficient,

$$\gamma_{A+} = \frac{[H^+]\exp\left(\frac{\Delta s}{RT} - \frac{\Delta h}{RT}\right)}{K_1\left\{x_A - \left(1 + \frac{K_2}{[H^+]}\right)\exp\left(\frac{\Delta s}{RT} - \frac{\Delta h}{RT}\right)\right\}} \tag{3.15}$$

Similarly at high pH, the amino acid species are in their anionic form and the zwitterionic and cationic forms are at infinite dilution. Therefore the activity coefficients of these species tend to unity. From equation (3.11) we obtain the following activity coefficient for the anionic form,

$$\gamma^x{}_{A-} = \frac{K_2 \exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right)}{[H^+]\left\{x_A - \left(1 + \frac{[H^+]}{K_1}\right)\exp\left(\frac{\Delta s}{R} - \frac{\Delta h}{RT}\right)\right\}} \tag{3.16}$$

using (3.15) and (3.16) we can calculate the activity coefficient at a known solubility. In fact, from the experimental data shown in Figure 3.1 it is possible

to estimate a value of the activity coefficient in its charged forms. For this purpose we consider the two values reported by Needham et al., (1971). Considering Δh and Δs constant, for a solubility of 4.83 m at a pH of 2.82, the value of the activity coefficient for the positively charged amino acid obtained from equation (3.15) is 0.3. Similarly for a solubility of 2.55 m at a pH of 9.5 the activity coefficient from equation (3.16) is 0.4 for the negatively charged form of the amino acid. This explains the differences between the model using equation (3.12) and the data at high and low pH values. The curve representing the model was drawn using the MATLAB polyfit function.

The molal activity coefficient was calculated using the following equation,

$$\gamma_A = \frac{m_A^{\circ}\gamma_A^{\circ}}{m_A} \qquad (3.17)$$

where m_A° and γ_A° are the solubility and the activity coefficient of the amino acid at saturation in pure water, and m_A is the solubility of the amino acid in aqueous solution of an acid or base. The molality based activity coefficient γ_A in equation (3.17) is converted to the rational activity coefficient by the equation (Khoshkbarchi and Vera, 1996(b)):

$$\gamma^{x}_{A} = \gamma_{A}[1+0.0018(m_{S}+m_{A})] \qquad (3.18)$$

where m_S is the molality of the acid or base. Thus, the value of γ_A° used in the above equation was 1.0. As discussed in detail elsewhere (Soto-Campos et al., 1998) equation (3.17) assumes that the fugacity of the amino acid in a solid phase in equilibrium with an aqueous electrolyte solution saturated in amino acid, is the same as the fugacity of the amino acid in a solid phase in equilibrium with an aqueous solution without an electrolyte. Again considering two values reported by Needham et al., (1971), using equation (3.17) at a pH=2.82 and solubility of 4.83 m we get the molal activity coefficient as 0.4 and at pH=9.5 and solubility of 2.55 m the activity coefficient is 0.8. Comparison of the results obtained from equations (3.15), (3.16) and (3.17) shows that there is agreement at low pH but at high pH the results slightly disagree. The disagreement is probably due to the assumptions implicit in equations (3.15) to (3.17). In fact, equations (3.15) and (3.16) assume that equation (3.6) holds at low and high pH. This is not necessarily true since at these conditions the zwitterionic form is at infinite dilution. On the other hand, equation (3.17) assumes that the fugacity of the solid phase is the same at different concentrations of the counterion of the acid or base. There is

evidence that this is not the case in concentrated ionic solutions (Khoshkbarchi and Vera, 1997).

At higher concentration of the acids and bases equations (3.15), (3.16) are not applicable, thus equations (3.17) and (3.18) are used to calculate the rational activity coefficient of DL-alanine at 2M HCl, the value is 0.7 and decreases as the concentration of the acid increases. At 2M NaOH the value of the rational activity coefficient of DL-alanine is 0.5 and decreases as the concentration of the base increases.

The effect of different cations such as sodium and potassium in aqueous solution of the amino acid on the activity coefficient of the amino acid leading to a salting-in or salting-out effect of the amino acid, has been documented in the literature (Khoshkbarchi and Vera, 1997, Soto-Campos et al., 1998, Pradhan and Vera, 1998). However, according to the results shown in Figure 3.1, there does not seem to be a marked difference in the solubility of the amino acids due to the presence of Cl^- or NO_3^- at low pH, or due to the presence of Na^+ or K^+ at high pH. The reason for this lack of response to the nature of the counterion of the acid or base is the low concentration of the counterion in the range of pH measurements. In fact, a value of pH 2 or of pH 8 requires concentrations less than 2M. Thus a 2M concentration is small

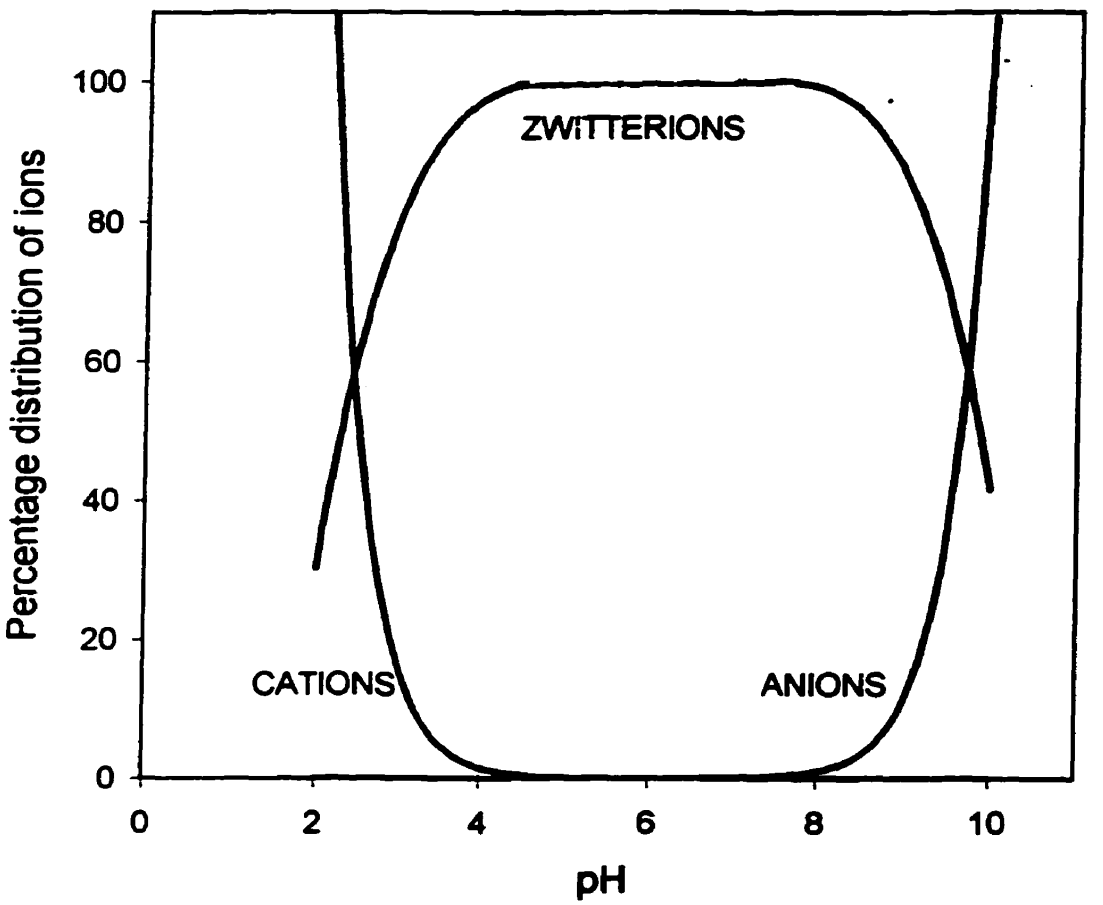


Figure 3.2: Distribution of ionic species at different pH values

compared with the molality of the amino acid at saturation. The trend in the solubility can be explained on the basis of the distribution of the different ionic forms, as shown in Figure 3.2. Figure 3.2 was prepared using equations (3.1), (3.2), (3.3) and the total solubility, to determine the percentage distribution of the ions. Throughout the isoelectric band, i.e., from pH 2 to 9, the concentrations of the cations and anions are low as compared to the zwitterionic species. On either side of the isoelectric band there is an increase in the solubility, at high or low pH due to displacement towards anionic or cationic species of the amino acid.

In order to observe the difference in solubility in the presence of the counterions of different acids and bases, the solubility of the amino acids was measured at higher concentrations of the acid and bases. Table B.3 and B.4 (Appendix B) give the experimental results using the bases and the acids respectively. Figure 3.3 shows the solubility of DL-alanine in NaOH and KOH. As seen from this figure, there is a clear effect of the sodium and potassium on the solubility. The solubility of DL-alanine in potassium is higher as compared to sodium hydroxide. This is probably due to the formation of a complex of the cation with the negatively charged form of the amino acid,

$$AA^- + C^+ \Leftrightarrow (AA^-)C^+ \qquad (3.19)$$

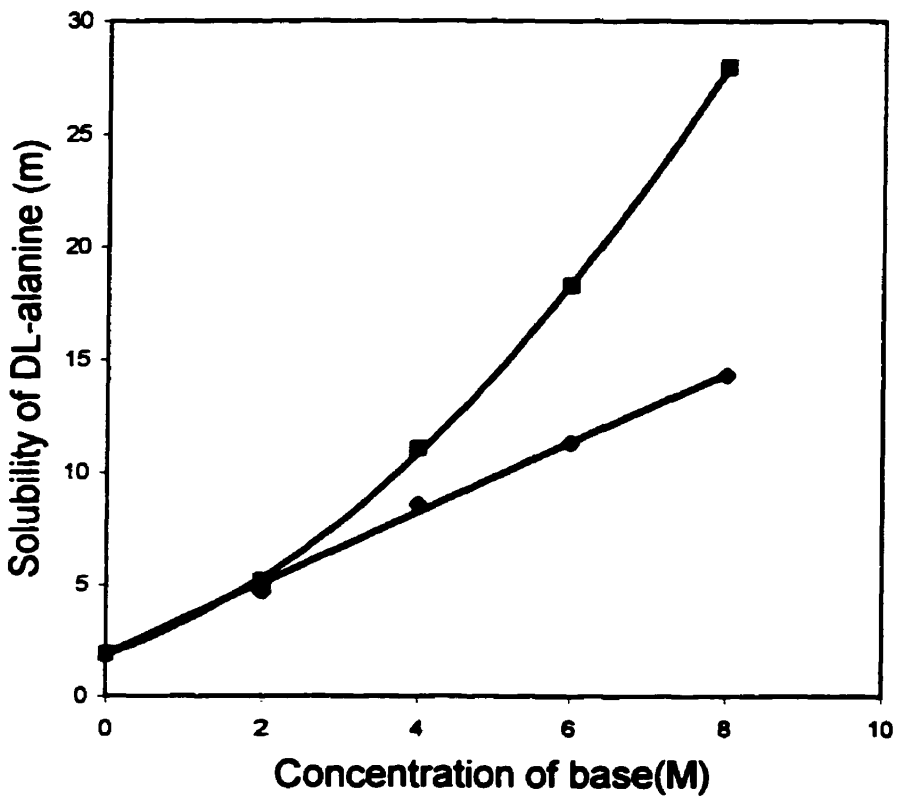


Figure 3.3: Effect of KOH and NaOH on the solubility of DL-alanine

◆ NaOH ■ KOH

Figure 3.4 shows the solubility of DL-alanine in the acids HNO_3 and HCl concentrations up to 10M. The solubility of DL-alanine is higher in HNO_3 as compared with the solubility in HCl. The difference in the solubility increases as the concentration of the acids increase. In this case, the formation of the complex with the anions Cl^- or NO_3^- is of the form:

$$AA^+ + A^- \Leftrightarrow (AA^+)\,A^- \qquad (3.20)$$

The lines joining the experimental points in figures 3.3 and 3.4 show the solubility trend and are not obtained from any model. As shown in Tables B.3 and B.4 (Appendix B), for the other amino acids studied in this work similar trends were seen at higher concentrations of the acids or bases.

3.4 Conclusions

The solubility of four amino acids in the presence of different acids and bases was measured. Comparing figures 3.1 and 3.3 it can be concluded that in the range of pH measurements, the effect of the presence of different ions in the aqueous solution of an amino acid is not significant. At higher concentrations of the ions, an effect is observed in the solubility due to the formation of complexes. The model used to correlate has one adjustable

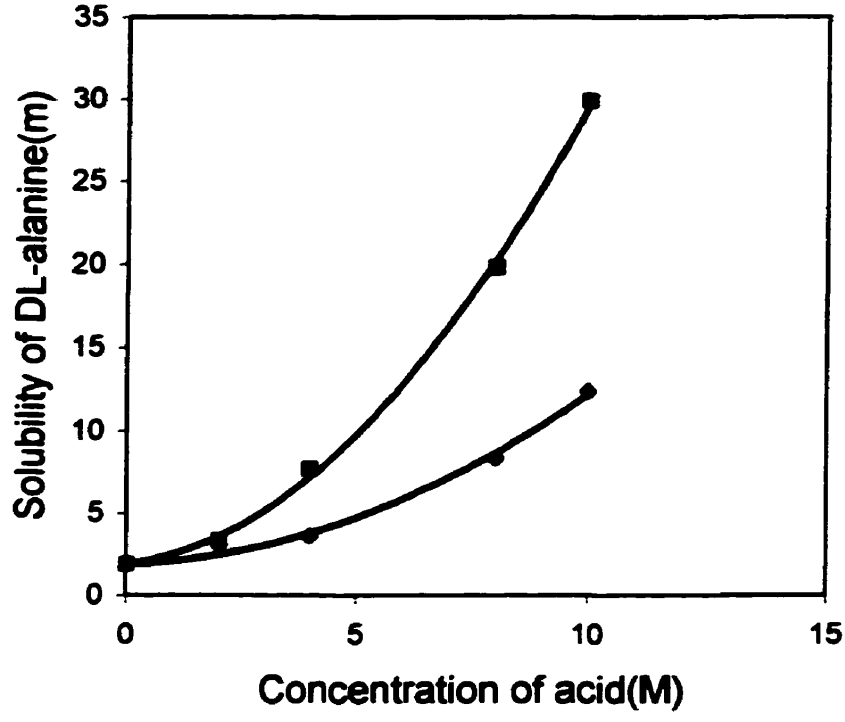


Figure 3.4: Effect of HNO_3 and HCl on the solubility of DL-alanine,

◆ HCl ■ HNO_3

parameter. It accurately represents the data in the isoelectric band producing deviations at low and high pH. In the range of pH measurements the activity coefficients for the complexes of the charged amino acid forms can be estimated assuming that the fugacity of the solid phase remains unchanged.

Chapter 4: Effect of Anions on the Solubility of Amino Acids

4.1 Introduction

The separate effect of different cations and anions is of potential interest for the separation of amino acids. The zwitterionic amino acid molecules have a large dipole moment which gives rise to important interactions with charged ions. In this chapter, we focus our attention on the effect of anions on the solubility behavior of four zwitterionic amino acids: glycine, DL-alanine, DL-valine and DL-serine.

4.2 Experimental Materials and Methods

Glycine, DL-alanine, DL-valine and DL-serine, of 99% purity were obtained from A & C Chemicals Ltd. (Montreal, Quebec, Canada). Sodium Nitrate and Potassium Nitrate were obtained from Anachemia Canada Inc. (Montreal, Quebec, Canada).

The salts were dried for 72 hours and then, prior to use, they were cooled in a dessicator. Electrolyte solutions at five different molalities were prepared

using deionized water, with a conductivity of less than 0.8 $\mu S\ cm^{-1}$, passed through ion exchange columns of East pure RF, (Compact Ultrapure Water System, Barnstead Thermoline, Bubugue, IA). In order to compare the effect of the anion, the electrolyte concentrations used were in the same range as those used by Khoshkbarchi and Vera, (1997) in the study of the effect of the cation using chloride salts. The solutions were prepared on a weight basis.

Vials of outer diameter 24 mm and 95 mm height were used as sample bottles. The amino acids were added in excess of the amount required for saturation. The electrolyte was then added, as a 20 ml solution, into the sample bottles. The sample bottles were sealed using Parafilm and kept in a thermostatic water bath at 298.2 K. The solution was agitated for 48 hours using teflon-coated magnetic stir bars. The mixing was then stopped and the solutions were allowed to settle for 7 hours.

Samples were taken of the supernatant liquid phase using a plastic syringe and filtered through a 0.22 μm, HPLC MSI disposable filter. An aluminum dish was weighed empty and with the filtered solution. The dish was covered with a weighed cap, put into an oven for 48 hours at about 308 K, and weighed again with the dry sample. The solubility of the sample was calculated from knowledge of the weight of the empty dish, weight of the cap, weight of the dish with solution

and cap, the dry weight of the solid and the electrolyte concentration of the sample.

To test the accuracy of the above experimental procedure, the solubility of glycine was measured in pure water and compared with literature values as shown in section 2.3.2. The 95% confidence interval was found to be ±0.009 molal. The values reported are the average of at least three replicates. In the replicates done, different quantities of the amino acid were taken in excess to that at saturation. The results were found to differ by less than 0.009 molal. The 95% confidence intervals in molalities were found to be ±0.003 for DL-alanine+$NaNO_3$ and DL-alanine+KNO_3; ±0.006 for glycine+$NaNO_3$; ±0.008 for glycine+KNO_3; ±0.009 for DL-serine+$NaNO_3$ and DL-serine+KNO_3; ±0.007 for DL-valine+$NaNO_3$ and ±0.009 for DL-valine+KNO_3.

To check the possibility of adsorption or precipitation of $NaNO_3$ on the solid amino acid, atomic absorption was used to analyze sodium in the solution. The concentration of sodium in the electrolyte-water system and in the amino acid-electrolyte-water system was compared. Quantities of amino acid 5%, 10% and 50% in excess to saturation were added for these comparisons, and the sodium concentration was measured in the supernatant phase. The maximum difference in the results was of ±0.009 molal, implying that, even with different

quantities of the amino acid added, no appreciable amount of electrolyte was precipitated or adsorbed on the amino acid in the solid phase.

4.3 Experimental Results and Discussion

Tables C.1 and C.2 (Appendix C) present the experimental data collected in this work. Notably, as shown in Table C.1, DL-alanine for high electrolyte molalities, has a higher solubility in the presence of sodium than in the presence of potassium when nitrate is the anion. As shown in tables C.1 and C.2, the other three amino acids studied here have the opposite behavior. When chloride is the anion, the results reported by Khoshkbarchi and Vera, (1997) show that for all the amino acids considered here the solubility is higher with potassium than with sodium.

Figures 4.1 to 4.8 compare the results of this work with those measured by Khoshkbarchi and Vera, (1997) for the same cations, Na^+ or K^+, with the chloride anion. Figure 4.1 shows that the solubility of glycine in aqueous solutions of sodium nitrate and sodium chloride first decreases and then increases with an increase in the electrolyte concentration. The initial decrease is not followed by the model described in the next section. At high concentration of the electrolyte, there is a marked increase in solubility with sodium nitrate and

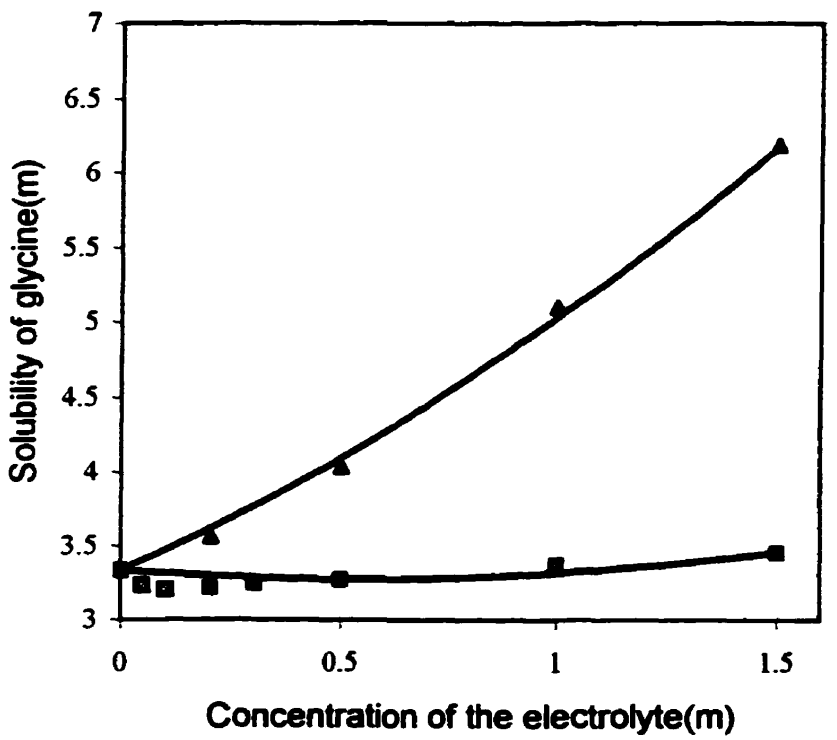


Figure 4.1: Effect of $NaNO_3$ and NaCl on the solubility of glycine, at the same electrolyte molality

▲ $NaNO_3$ ■ NaCl —model

only a slight increase in solubility with sodium chloride. Since the experiments described in the previous section showed that no salt precipitates with the amino acid, one concludes that the nitrate anion retains the amino acid in the aqueous phase. The amino acids, existing as zwitterions in the system, can form complexes with the electrolyte of the form:

$$^{-}AA^{+} + C^{+}A^{-} \Leftrightarrow C^{+}(^{-}AA^{+})A^{-} \qquad (4.3.1)$$

The comparison of results for glycine in potassium nitrate and in potassium chloride aqueous solutions shown in Figure 4.2, confirms the strong effect of the anion on the solubility of this amino acid.

Figure 4.3 shows the solubility of DL-alanine in sodium nitrate and sodium chloride aqueous solutions. A salting-in effect is observed with the nitrate anion and a salting-out effect is seen with the chloride anion. This interesting behavior shows the significant effect of the anion of the electrolyte on the solubility of the amino acid. The difference in the solubility trends is due to the kind of complexes formed in the aqueous phase by the amino acid with the different anions. Figure 4.4 shows the solubility behavior of the same amino acid in aqueous solution with the potassium cation and the two anions. With both potassium chloride and potassium nitrate a salting-in effect is observed.

Figure 4.5 shows the solubility of DL-valine in aqueous solutions

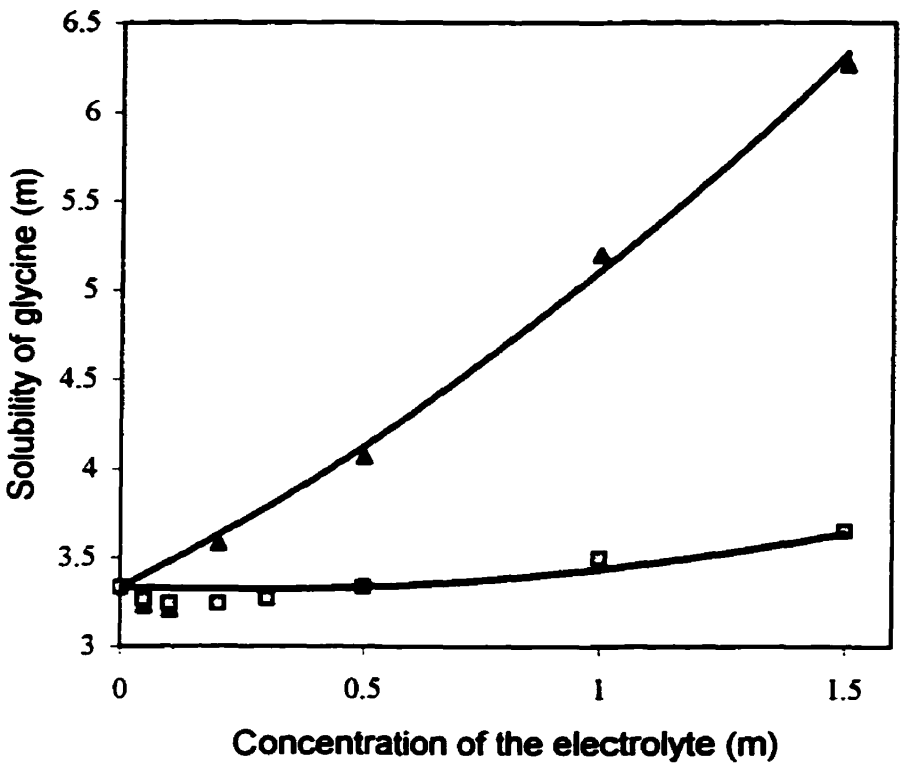


Figure 4.2: Effect of KNO_3 and KCl on the solubility of glycine, at the same electrolyte molality

▲ KNO_3 ▫ KCl —model

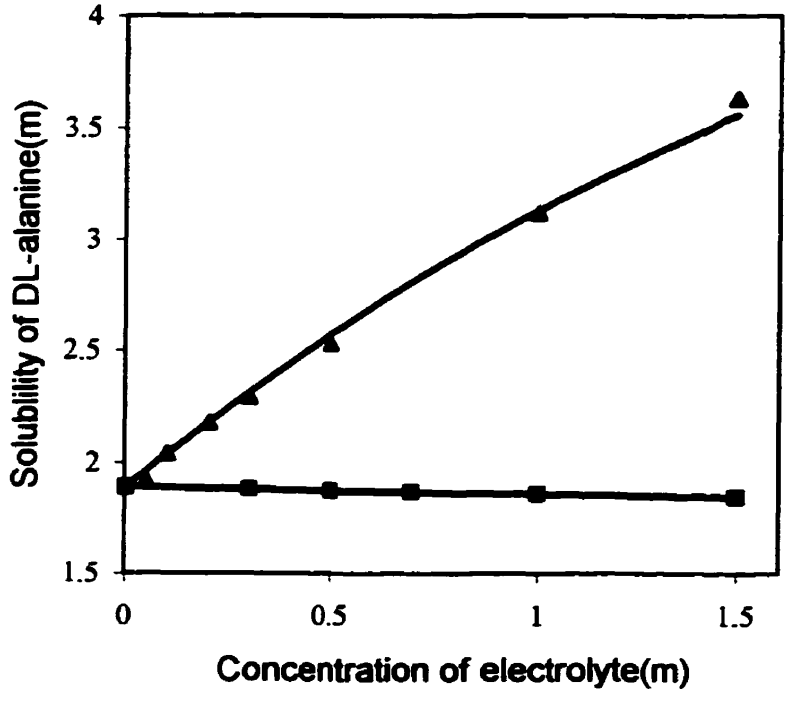


Figure 4.3: Effect of $NaNO_3$ and NaCl on the solubility of DL-alanine, at the same electrolyte molality.

▲ $NaNO_3$ ■ NaCl —model

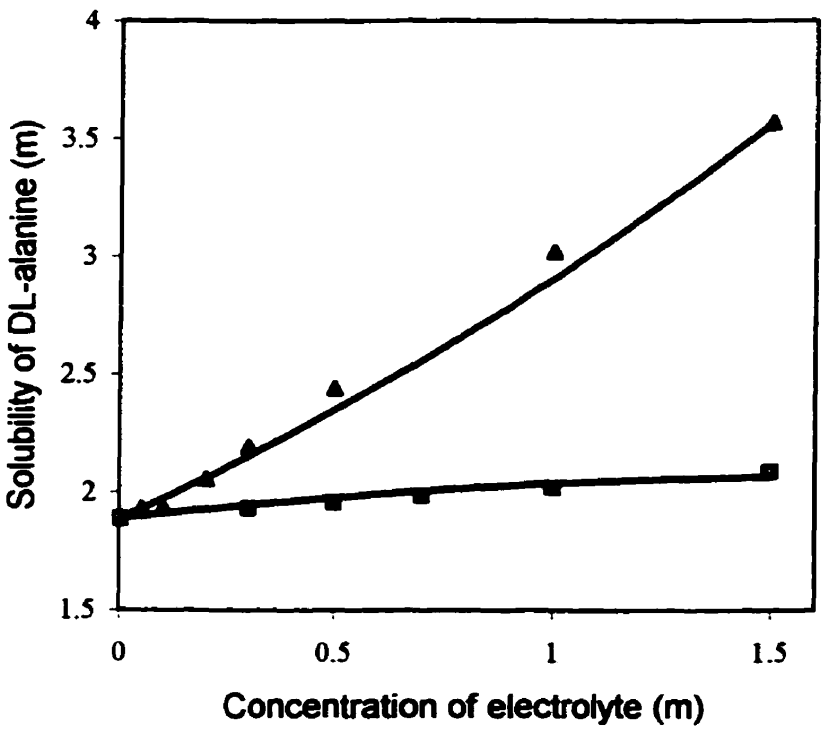


Figure 4.4: Effect of KNO_3 and KCl on the solubility of DL-alanine, at the same electrolyte molality.

▲ KNO_3 ■ KCl — model

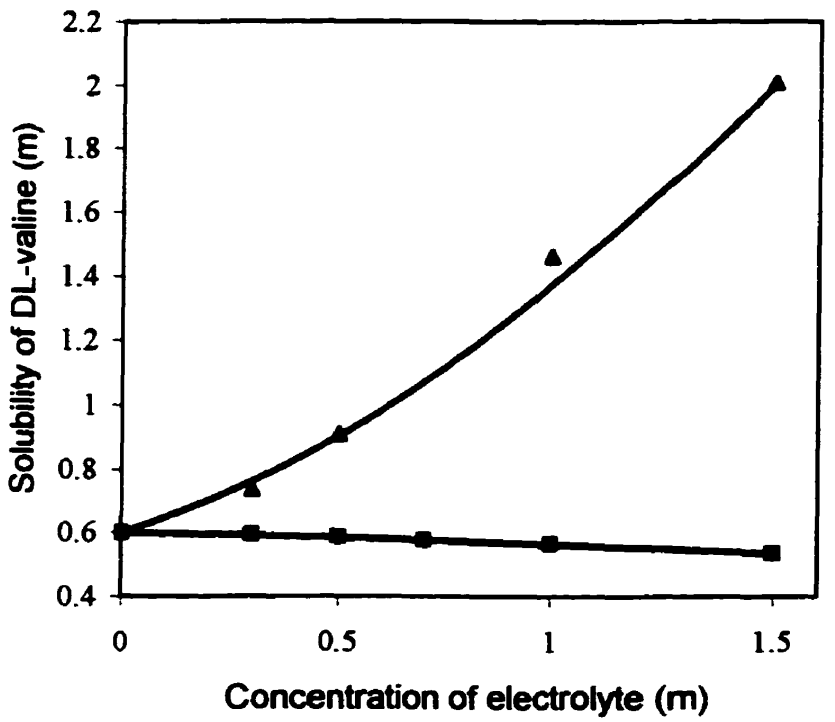


Figure 4.5: Effect of $NaNO_3$ and NaCl on the solubility of DL-valine, at the same electrolyte molality.

▲ $NaNO_3$ ■ NaCl — model

containing sodium nitrate and sodium chloride. The effect of the anion is quite significant in this case, as a salting-in effect is seen with the nitrate anion whereas a salting-out effect is seen with the chloride anion. The difference in the solubility increases as the concentration of the electrolyte increases. This can be due to the type of complexes formed by the amino acid in the presence of the different electrolytes and their tendencies to shield the hydrophobic interactions. The comparison of solubility trends in different anions of DL-valine and DL-alanine is similar; DL-valine has two CH_2 groups more than DL-alanine. Hence in DL-valine and DL-alanine the hydrophobic interactions are larger than in glycine, leading to salting-out effect, even with the chloride anion, over the entire range of concentration studied.

Figure 4.6 shows the solubility of DL-valine in potassium nitrate and potassium chloride aqueous solutions. The chloride and the nitrate anions cause an increase in the solubility of the amino acid. The salting-out effect of chloride ion depicted in Figure 4.5 is not observed for the case of potassium. This difference in effect of cations is also related to the kind of complexes that the cation forms with the amino acid in aqueous solution.

Figure 4.7 shows the solubility of DL-serine in sodium nitrate and sodium chloride aqueous solutions. The solubility increases in both sodium nitrate and

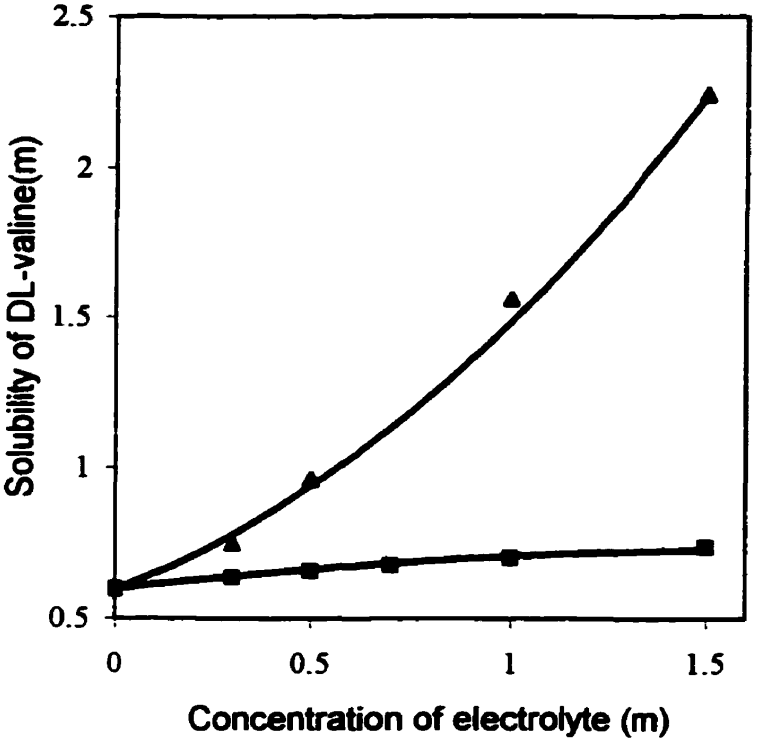


Figure 4.6: Effect of KNO_3 and KCl on the solubility of DL-valine, at the same electrolyte molality.

▲ KNO_3 ■ KCl — model

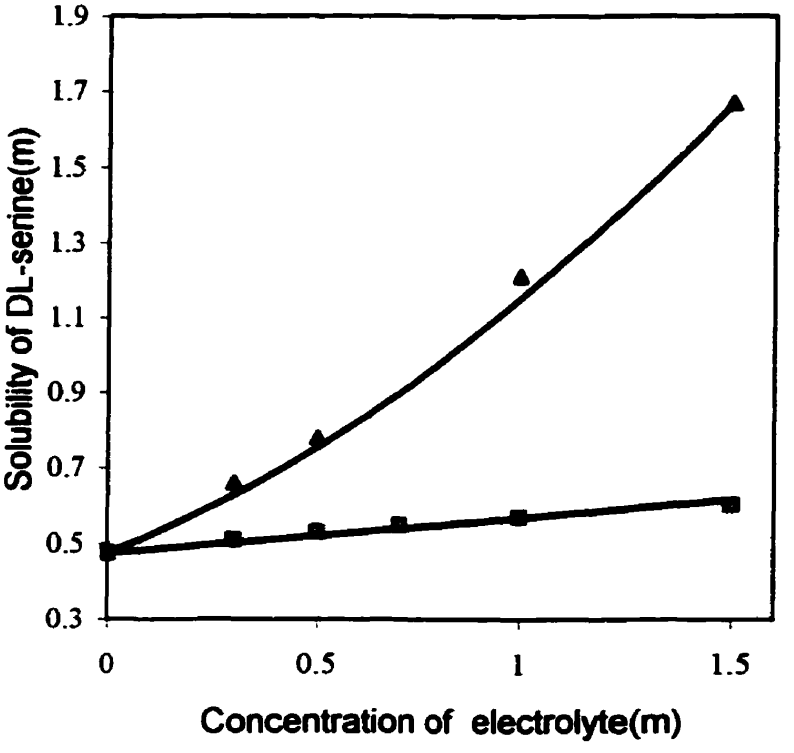


Figure 4.7: Effect of $NaNO_3$ and NaCl on the solubility of DL-serine, at the same electrolyte molality

▲ $NaNO_3$ ■ NaCl —model

sodium chloride solutions. With sodium nitrate, in the range of concentration studied, the solubility increases to almost twice the value of the solubility in pure water. The difference between DL-serine and DL-valine is that DL-serine has an OH group. This group obviously causes a salting-in effect with both anions. Figure 4.8 shows the solubility of DL-serine in potassium chloride and potassium nitrate aqueous solutions. The solubility of DL-serine increases with both the chloride and the nitrate ions when the cation is potassium.

4.4 Modeling

For solid-liquid equilibrium of an amino acid in aqueous solution, without an electrolyte, we can write,

$$m_A^{\circ}\,\gamma_A^{\circ}\,f^{OL} = f_A^{OS} \qquad (4.4.1)$$

where m_A° and γ_A° are the amino acid saturation molality and activity coefficient, respectively. In equation (4.4.1), f^{OL} is the standard state fugacity and f^{OS} is the fugacity of the pure amino acid in the solid phase. For the solid-liquid equilibrium of an amino acid in an aqueous electrolyte solution,

$$m_A\,\gamma_A\,f^{OL} = f_A^{S} \qquad (4.4.2)$$

where m_A and γ_A are the amino acid saturation molality and activity coefficient

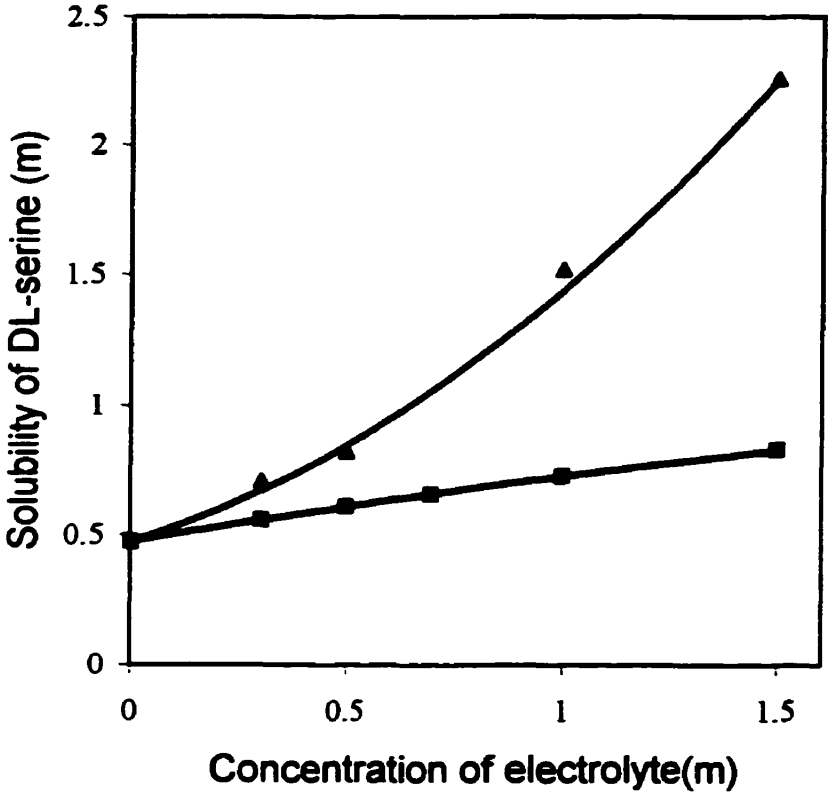


Figure 4.8: Effect of KNO_3 and KCl on the solubility of DL-serine, at the same electrolyte molality.

▲ KNO_3 ■ KCl —model

in the aqueous electrolyte solution. The standard state fugacity f^{OL} of the amino acid is the same in equations (4.4.1) and (4.4.2), while the fugacity of the solid amino acid in contact with the electrolyte solution, f_A^S, is considered to be different from f^{OS}, the fugacity of the solid amino acid in contact with pure water. Although the experiments described in the previous section indicate that the electrolyte does not participate in the solid phase, the amino acid crystals obtained in the presence of the electrolyte are different in appearance to the crystals obtained in pure water. At low salt concentration the crystals are small and as the concentration of electrolyte increases they grow in size and become needle-like. This phenomenon was also observed in a previous study (Khoshkbarchi and Vera, 1997). In this study, we additionally observed that length of the amino acid crystals formed in the presence of potassium nitrate at high concentrations was up to 1mm whereas in the presence of sodium nitrate, at the same molality, the length of the crystals was shorter.

Eliminating f^{OL} from equations (4.4.1) and (4.4.2), and rearranging, we obtain:

$$m_A = m_A^{\,o}\left(\frac{\gamma_A^{\,o}}{\gamma_A}\right)\left[\frac{f^S}{f^{OS}}\right] \qquad (4.4.3)$$

From exact thermodynamics, we write

$$\frac{f^S}{f^{OS}} = \exp\left(\frac{\Delta G_A{}^S}{RT}\right) \qquad (4.4.4)$$

where T is the absolute temperature, R is the universal gas constant, and $\Delta G_A{}^S$ is the difference in the free energy of the solid phase in equilibrium with an aqueous solution of an electrolyte, $G_A{}^S$ and the free energy of the solid phase in contact with a saturated solution without electrolyte, $G_A{}^{OS}$,

$$\Delta G_A^S = G_A^S - G_A^{OS} \qquad (4.4.5)$$

In order to correlate results we have introduced an empirical equation for the Gibbs free energy, as a function of the electrolyte concentration m_S, of the form:

$$\exp\left(\frac{\Delta G_A^S}{RT}\right) = a\ m_S{}^{0.05} + b\ m_S{}^{0.5} \qquad (4.4.6)$$

where a and b are adjustable parameters. The values of a and b were determined by curve fitting of the experimental data. The values of a and b are presented in Table 4.1 along with root mean square deviation of the correlation of the experimental solubility data for each water-electrolyte amino-acid system. If the fugacities of the solid amino acid phases in the aqueous electrolyte-amino acid system and in the water-amino acid system were considered to be the same, we would obtain

Table 4.1. Values of the parameters a and b for the model for the amino-acid electrolyte systems studied.

Amino acid-electrolyte	a	b	r.m.s.d[#]
glycine-NaCl	-0.060	0.032	0.028
glycine-KCl	-0.050	0.020	0.025
glycine-$NaNO_3$	-0.306	0.695	0.136
DL-alanine-NaCl	0.016	-0.053	0.006
DL-alanine-KCl	-0.080	0.188	0.019
DL-alanine-$NaNO_3$	-0.160	0.601	0.040
DL-valine-NaCl	0.010	0.016	0.0031
DL-valine-KCl	-0.108	0.368	0.010
DL-serine-NaCl	0.344	-0.485	0.016
DL-serine-KCl	-0.014	0.226	0.0585

r.m.s.d [#] = root mean square deviation

$$m_A = m_A^o\left(\frac{\gamma_A^o}{\gamma_A}\right) \tag{4.4.7}$$

In equations (4.4.3) and (4.4.7), the activity coefficients were expressed using the NRTL model:

$$\ln \gamma_i^{NRTL} = \frac{\sum_{j=1}\tau_{ji}G_{ji}x_j}{\sum_{j=1}G_{ji}x_j} + \sum_{j=1}\frac{x_jG_{ij}}{\sum_{k=1}x_kG_{kj}}\left(\tau_{ij} - \frac{\sum_{k=1}x_k\tau_{kj}G_{kj}}{\sum_{k=1}x_kG_{kj}}\right) \tag{4.4.8}$$

where the parameter G_{ij} is defined as

$$G_{ij} = \exp(-\alpha\tau_{ij}) \tag{4.4.9}$$

The value of α was fixed at 0.3 for all the cases. As explained elsewhere (Khoshkbarchi and Vera, 1996(b)), the activity coefficient γ_A of the amino acid is given by:

$$\ln \gamma_A = \ln \gamma_A^{NRTL} - \lim_{x_A \to x_S \to 0} \ln \gamma_A^{NRTL} - \ln\left(1 + 0.001M_w(m_A + m_S)\right) \tag{4.4.10}$$

where M_w is the molecular weight of water. The binary form of the NRTL model is recovered as the mole fraction of the electrolyte approaches zero.

Data for activity coefficients for glycine, DL-alanine and DL-valine and DL-serine in NaCl and KCl aqueous solutions and for the systems of glycine and DL-alanine in $NaNO_3$ are reported in the literature (Khoshkbarchi et al., 1997) For the systems of the four amino acids in NaCl and KCl aqueous solutions

the model using equations (4.4.3) to (4.4.6) accurately correlates the solubility data. Also for glycine with $NaNO_3$, and DL-alanine with $NaNO_3$, as seen from figures 4.1 and 4.3, there is good agreement between the model and the data.

For the cases of the four amino acids with KNO_3 solutions and also for DL-valine and DL-serine with $NaNO_3$ solutions, where the experimental data for activity coefficients of the amino acids in the presence of the electrolyte were not available, the assumption was made that the ratio of the fugacities in the presence of the electrolyte to the fugacity in the absence of the electrolyte was unity. Thus, for modeling the solubility, equation (4.4.7) was used with two adjustable parameters $\tau_{A\text{-}S}$ and $\tau_{W\text{-}S}$. The values of these parameters are given in Table 4.2. The results of the correlation for the systems are shown in Figure 4.2 and in figures 4.4 to 4.8.

4.5 Conclusions

Experimental data for the solubility of four amino acids in $NaNO_3$ and KNO_3 solutions were measured. Comparison of these results with data Khoshkbarchi (1996) showed the effect of the two anions Cl^- and NO_3^-. For all cases studied, it was observed that the solubilities are always higher in the presence of NO_3^- than in the presence of Cl^-. These systems also show

Table 4.2: Value of the parameters τ_{A-S} and τ_{W-S} for the following systems with the root mean square deviation.

Amino acid- electrolyte	τ_{A-S}	τ_{W-S}	r.m.s.d#
glycine-KNO_3	-1.984	0.359	0.142
DL-alanine- KNO_3	-3.460	1.275	0.054
DL-valine- KNO_3	-4.754	2.246	0.050
DL-valine -$NaNO_3$	-4.580	2.193	0.051
DL-serine-KNO_3	-2.635	1.008	0.092
DL-serine-$NaNO_3$	-2.132	1.024	0.001

r.m.s.d #= root mean square deviation

difference between solubilities in the presence of sodium and potassium. It can be concluded therefore that the nature of both the cation and the anion seem to affect the solubility of the amino acids in electrolyte solutions. The behavior of DL-alanine with $NaNO_3$ and KNO_3 seems to be anomalous. While for all other amino acids studied here the solubility is higher in the presence of potassium, when the anion is NO_3^-, for DL-alanine the reverse holds. The results were verified repeatedly for this system, since with the Cl^- as anion all amino acids, including DL-alanine, present higher solubility with potassium than with sodium (Khoshkbarchi and Vera, 1997). Recently reported results for aminobutyric acid with $NaNO_3$ and KNO_3, however, also show a higher solubility in the presence of sodium (Soto et al., 1998).

The model using the empirical equation (4.4.6) with the NRTL equation for the activity coefficients correlates well the solubilities of amino acids in electrolyte solutions for systems for which the experimental data of activity coefficients were available.

Chapter 5: Conclusions and Recommendations

From the results obtained in this work the following conclusions were obtained and recommendations can be made.

5.1 Conclusions

The effect of cations and anions on the solubility of the amino acids is not detectable in the pH range. At higher concentrations of the acids and bases, however it was observed that the solubility of all four amino acids was higher in the presence of the nitrate anion than in the presence of the chloride ion and in the case of potassium as cation than sodium. This is probably due to the complex formation of the specific ionic species of the amino acid with the cation of the acid or the anion of the hydroxide, respectively. In the pH range, this effect was not detectable due to the low concentration of the counterion as compared to the solubility of the amino acid. The model, as given by equation (3.12) was used for the correlation of the solubility data in the pH range. It requires a single adjustable parameter, and accurately represents the data in the isoelectric range. Deviations at higher and lower pH

values are due to the assumption that the activity coefficients of all ionic forms are considered equal. This is not the case as proved by the results of the calculations obtained using equations (3.15), (3.16) and (3.17).

In the study of the effect of anions in the aqueous solutions of amino acids at neutral pH, the solubility of all amino acids in the presence of nitrate was higher than in the presence of chloride. Experimental data of the effect of nitrate on the solubility of four amino acids was obtained using sodium or potassium nitrate and compared with that of Khoshkbarchi (1996). The effect of different cations, namely sodium and potassium, was also observed. For DL-alanine, unlike all the other amino acids studied, the solubility was higher in the presence of sodium than in the presence of potassium. However a similar result has been recently reported by Soto et al., 1998 for aminobutyric acid in the presence of sodium or potassium nitrate.

The model, using the empirical equation (4.4.6), gave accurate correlations for the systems where the activity coefficients had been experimentally determined.

5.2 Recommendations

Based on results obtained in this work the following suggestions are made:

- The measurement of the activity coefficients of the amino acids systems in the aqueous solutions of electrolytes, which are presently not available, should be continued. These data could be correlated with the empirical equation (4.4.6) and a study of the effect of the structure of the amino acid should be made.
- The solubility and activity coefficients of other amino acids in aqueous electrolyte solutions should be measured.
- Extend the application of this work, by studying thermodynamic behavior of mixture of amino acids, proteins and peptides.

References

1. Belter, P.A., *Bioseparations: Downstream Processing for Biotechnology,* John Wiley and Sons, New York, 1988.
2. Carta, R. and Giseppe, T., Solubilities of L-Cystine, L-Tyrosine, L-Leucine and Glycine in Aqueous Solutions at Various pH's and NaCl Concentrations, *J. Chem. Eng. Data*, 41, 414-417,1996.
3. Christian, G.D., *Analytical Chemistry*, Third Edition, John Wiley & Sons, New York, 1971.
4. Cohn, E.J. and Edsall, J.T., *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions,* Hafner Publisher Company, New York, 1965.
5. Dalton, J.D. and Schmidt, C.L.A, The Solubility of Certain Amino Acids in Water, The Densities of Their Solutions at Twenty-Five Degrees, and the Calculated Heats of Solution and Partial Molar Volumes, *J. Biol. Chem.* 103, 539-545, 1933.
6. Edwin J.C., Thomas L.M., Edsall J.T. and Weare, J.H., The Solubility of α-Amino Acids in Water and in Alcohol-Water Mixtures, *J. Am. Chem. Soc.*, 56, 2270- 2282, 1934.
7. Eyal, A.M. and E. Bressler, Industrial Separation of Carboxylic and Amino Acids by Liquid Membranes: Applicability, Process Considerations, and

Potential Advantages, *Biotechnology and Bioengineering*, 41, 287-295, 1993.

8. Fasman G.D., *Handbook of Biochemistry and Molecular Biology,* 3rd Ed, *Physical and Chemical Data,* Vol. 1, CRC Press, Cleveland, 1976.
9. Gatewood, M.D. and Rousseau R.W., Effect of Sodium Hydroxide on the Solubilities of L-isoleucine, L-Leucine and L-Valine, *Biotechnol. Prog.*, 10, 253-257, 1994.
10. Greenstein, J.P. and M. Winitz, *Chemistry of Amino Acids*, John Wiley and Sons, New York, Vol.1, 1961.
11. Hitchcock D.I, The Solubility of Tyrosine in Acid and in Alkali, *Physiol. Chem.*, 6, 747-775, 1924.
12. Khoshkbarchi, M.K., Thermodynamics of Amino Acids in Aqueous Electrolyte Solutions, Ph.D. Thesis, 1996.
13. Khoshkbarchi, M.K. and J.H. Vera, A Simplified Perturbed Hard Sphere Model for the Activity Coefficients of Amino Acids and Peptides in Aqueous Solutions, *Ind. Eng. Chem. Res.*, 35, 4319-4327, 1996 (a).
14. Khoshkbarchi M.K and J.H. Vera, Measurement and Modeling of Activities of Amino Acids in Aqueous Salt Systems, *AIChE J.*, 42, 2354-2364, 1996 (b).

15. Khoshkbarchi, M.K. and J.H.Vera, Effect of NaCl and KCl on the Solubility of Amino Acids at 298.2K: Measurement and Modeling, *Ind. Eng. Chem. Res.*, 39, No. 6, 2445-2451, 1997.

16. Khoshkbarchi, M.K, Soto-Campos A.M. and J.H. Vera, Activity Coefficients of the Electrolyte and the Amino Acid in Water + $NaNO_3$ + Glycine and Water +NaCl + DL-methionine Systems at 298.15 K, *Biophysical Chemistry*, 2289-2297, 1997.

17. Kirkwood, J.G., Theory of Solutions of Molecules Containing Widely Separated Charges with Special Applications to Zwitterions, *J. of Chem. Phys.*, 2, 351-360, 1934.

18. Kirkwood, J.G., Theoretical Studies Upon Dipolar Ions, *Chem. Rev*, 5, 233-251, 1962.

19. McCuen and Richard H., Statistical Methods for Engineers, Prentice Hall, 1985.

20. Mackenzie L.S., Amino Acids, Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, Canada.

21. Needham, T.E., Gerraughty, R.J. and A.N. Paruta, Solubility of Amino Acids in Pure Solvent Systems, *J. Pharm. Sci.*, 60, 565-567, 1971.

22. Orella, C.J. and Kirwan, D.J., The Solubility of Amino Acids in Mixtures of

Water and Aliphatic Alcohols, *Biotechnol. Prog.*, 5, 89-91, 1989.

23. Orella, C.J. and Kirwan, D.J., Correlation of Amino Acid Solubilities in Aqueous Aliphatic Alcohol Solutions, *Ind. Eng. Chem. Res.*, 30, 1040-1045, 1991.

24. Pitzer, K.S., *Activity Coefficients in Electrolyte Solutions,* C.R.C. Press: Boca Ranton, Florida, 1991.

25. Pradhan A. A. and J.H. Vera, Effect of Anions on the Solubility of Zwitterionic Amino Acids, submitted to *Ind. Eng. Chem. Res.*, 1998.

26. Pradhan A. A. and J.H. Vera, Effect of Acids and Bases on the Solubility of Amino Acids, submitted to *Fluid. Phase. Equilib.*, 1998.

27. Prausnitz J., Coen, C.J. and Blanch H.W., Salting Out of Aqueous Proteins: Phase Equilibria and Intermolecular Potentials, *AIChE. J.*, 41, 1997-2004, 1995.

28. Robinson, J.W., *Undergraduate Instrumental Analysis*, Fourth Edition, New York, 1987.

29. Robinson, R.A. and R.H. Stokes, *Electrolyte Solutions*, Butterworth Scientific, London, 1970.

30. Roth, M., Fluorescence Reaction for Amino Acids, *Anal. Chem.*, 43, 880-882, 1971.

31. Schreir, E.E. and R.A. Robinson, Free Energy Relationships in Aqueous Amino Acid and Peptide Solutions Containing Sodium Chloride, *J. Solution Chem.*, 3, No.7, 493-501, 1974

32. Sibilia, J.P., Materials Characterization and Chemical Analysis, Second Edition, New York, 1996.

33. Skoog, D.A. and West, D.M., *Principles of Instrumental Analysis*, New York, 1971.

34. Soto-Campos, A., Arce A., Khoshkbarchi, M.K., and J.H. Vera, Effect of the Cation and Anion of an Electrolyte on the Solubility of DL-Aminobutyric Acid in Aqueous Solutions: Measurement and Modeling, *Biophysical Chemistry*, accepted for publication, 1998.

35. Wang W., Reverse Micellar Extraction of Amino Acids Using the Two-Tailed Cationic Surfactant DODMAC, Ph.D. Thesis, 1994.

36. Zumstein R.C. and Rousseau R.W., Solubility of L-isoleucine and Recovery from Neutral and Acidic Solutions, *Ind. Eng. Chem. Res.*, 28, 1226-1231, 1989.

APPENDIX A: EXPERIMENTAL RESULTS OF THE METHODS OF ANALYSIS FOR SOLUBILITY OF AMINO ACIDS

Table A.1: Results of analysis methods

Method of analysis	Confidence interval (95%)	Literature values at 298K (g/1000g H_2O)
UV	241.19 ± 0.90	250.55
CARBON ANALYZER	214.92 ± 1.90	
GRAVIMETRIC	251.10 ± 0.90	
	TRIAL WITH KNOWN CONCENTRATION =0.9(SATURATED CONC.)	KNOWN CONCENTRATION (g/1000g H_2O)
UV	216.94 ± 2.60	
CARBON ANALYSER	219.06 ± 1.00	225.49
GRAVIMETRIC	225.30 ± 0.70	

APPENDIX B: EXPERIMENTAL RESULTS ON THE EFFECT OF ACIDS AND BASES ON THE SOLUBILITY OF AMINO ACIDS

Table B.1: Experimental data of the solubility (in molality) of DL-alanine in the presence of NaOH or HCl at different pH values

pH	Solubility of DL –alanine (m)
	NaOH or HCl
2.75	2.620
2.80	2.449
2.82	2.468
3.36	2.438
6.07	1.891
7.80	1.893
8.35	1.896
8.58	1.896
9.10	1.953
9.51	2.420
9.62	2.488
9.85	3.167

Table B.2: Experimental data of the solubility (in molality) of DL-alanine at different pH values using KOH or HNO_3 to adjust the pH.

pH	Solubility of DL–alanine (m)
	KOH or HNO_3
2.80	2.739
2.90	2.674
3.08	2.672
3.80	1.895
6.07	1.891
7.23	1.894
9.10	1.955
9.26	1.990
9.48	2.194
9.58	2.556
9.64	2.985

Table B.3 (a): Experimental data of the solubility (in molality) of DL-alanine, and DL-valine in concentrated bases.

Concentration (M)	DL-alanine		DL-valine	
	NaOH	KOH	NaOH	KOH
0	1.891	1.891	0.600	0.600
2	4.684	5.157	2.600	3.566
4	8.582	11.030	4.670	8.050
6	11.290	18.273	7.590	13.740
8	14.280	27.951	9.970	24.440

Table B.3 (b): Experimental data of the solubility (in molality) glycine and DL-serine in concentrated bases.

Concentration (M)	glycine		DL-serine	
	NaOH	KOH	NaOH	KOH
0	3.333	3.333	0.476	0.476
2	3.418	4.610	4.000	4.000
4	6.140	10.027	7.647	8.440
6	9.970	18.070	11.560	14.50
8	13.110	32.130	16.110	21.92

Table B.4 (a): Experimental data of the solubility (in molality) of DL-alanine and DL-valine in concentrated acids.

Concentration (M)	DL-alanine		DL-valine	
	HCl	HNO_3	HCl	HNO_3
0	1.891	1.891	0.600	0.600
2	3.020	3.020	0.690	1.570
4	3.600	7.710	2.001	3.600
8	8.398	19.800	5.610	9.300
10	12.400	29.900	8.000	13.780

Table B.4 (b): Experimental data of the solubility (in molality) of glycine and DL-serine in concentrated acids.

Concentration (M)	glycine		DL-serine	
	HCl	HNO_3	HCl	HNO_3
0	3.333	3.333	0.476	0.476
2	4.000	4.000	2.340	3.430
4	6.368	11.350	3.430	6.141
8	12.370	28.001	7.650	16.59
10	15.080	43.120	11.980	22.00

APPENDIX C: EXPERIMENTAL RESULTS OF THE EFFECT OF ANIONS ON THE SOLUBILITY OF AMINO ACIDS

Table C.1. Experimental data of the solubility (in molality) of glycine and DL-alanine in the presence of the electrolytes $NaNO_3$ or KNO_3.

	glycine		DL-alanine	
Electrolyte (m)	$NaNO_3$	KNO_3	$NaNO_3$	KNO_3
0	3.333	3.333	1.891	1.891
0.05	3.229	3.229	1.916	1.931
0.1	3.201	3.202	2.040	1.935
0.2	3.558	3.575	2.175	2.055
0.3	—	—	2.290	2.190
0.5	4.034	4.066	2.530	2.440
1.0	5.097	5.194	3.115	3.020
1.5	6.184	6.263	3.628	3.567

Table C.2. Experimental data of the solubility (in molality) of DL-valine and DL-serine in the presence of the electrolytes $NaNO_3$ or KNO_3.

	DL-valine		DL-serine	
Electrolyte (m)	$NaNO_3$	KNO_3	$NaNO_3$	KNO_3
0	0.600	0.600	0.476	0.476
0.3	0.736	0.750	0.660	0.707
0.5	0.911	0.960	0.780	0.830
1.0	1.460	1.560	1.210	1.520
1.5	2.000	2.240	1.670	2.257