Densities and Speed of Sound in Aqueous Ammonium Sulfate Solutions Containing Glycine or Alanine

Mónia Andreia Rodrigues Martins

Final Report Dissertation presented to the

Escola Superior de Tecnologia e Gestão

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September of 2012

To my Grandparents

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In this short but intense period I learned a lot, personally and professionally. I owe that to some persons, to whom I thank.

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To all, Obrigada!

Abstract

The main objectives of this work are the measurement of the densities and speed of sound in aqueous ammonium sulfate solutions containing glycine or alanine.

The study of mixtures containing charged electric species is of great relevance for the chemical industry. In this work the new experimental information is combined with that collected from the open literature in order to contribute for an understanding of the forces that rule biologically important structures.

A systematic experimental program is implemented to measure the densities and speed of sound in aqueous and in mixed aqueous solutions of ammonium sulfate, (0.1, 0.3, 0.7, 1.0, 1.3, and 2.0) mol·kg^{-1}, containing glycine or alanine, using a digital densimeter at (278.15, 288.15, 298.15, 308.15, and 318.15) K.

Density data have been used to calculate the partial molar volumes at infinite dilution which are evaluated and further used to obtain the corresponding transfer volumes for amino acids. Hydration numbers, temperature dependence, and side chain group contributions of amino acids have also been studied.

The parameters obtained from the volumetric study are used to understand various mixing effects due to the interactions between amino acids and ammonium sulfate in aqueous solutions. The $V_{\emptyset,tr}^{0}$ data suggest that ion charged/hydrophilic group interactions are predominant and applying the McMillan and Mayer formalism it was concluded that they are mainly pair wise. According to Hepler (1969) all amino acids, in water and in aqueous ammonium sulfate solutions, can be considered as a structure breaking solutes. These results represent a step in building up an empirical database of different volumetric parameters of protein functional groups in ammonium sulfate and water.

Keywords: Amino Acid, Electrolyte, Partial Molar Volumes, Adiabatic Compressibilities.

Resumo

Os principais objectivos deste trabalho são a medição da densidade e da velocidade do som em soluções aquosas de sulfato de amónio contendo os aminoácidos glicina ou alanina.

O estudo de misturas contendo espécies eléctricas carregadas é de grande relevância para a indústria química. Neste trabalho a nova informação experimental é combinada com informação recolhida na literatura de forma a contribuir para uma melhor compreensão das forças que regem estruturas biológicas importantes.

Neste trabalho, foi implementado um programa experimental sistemático para medir as densidades e a velocidade do som em água e em soluções aquosas de sulfato de amónio, (0.1, 0.3, 0.7, 1.0, 1.3 e 2.0) mol·kg^{-1}, contendo glicina ou alanina, usando um densímetro digital a (278.15, 288.15, 298.15, 308.15 e 318.15) K.

Os dados da densidade foram utilizados para calcular os volumes molares parciais a diluição infinita, os quais foram avaliados e posteriormente utilizados para obter os correspondentes volumes de transferência para os aminoácidos. Os números de hidratação, a dependência com a temperatura e as contribuições de grupo da cadeia lateral dos aminoácidos foram também estudados.

Os parâmetros obtidos a partir do estudo volumétrico foram utilizados para compreender vários efeitos de mistura devido às interacções entre os aminoácidos e o sulfato de amónio em soluções aquosas. Os dados de $V_{\phi,tr}^{0}$ sugerem que as interacções ião carregado/grupo hidrofílico são predominantes, e aplicando o formalismo de McMillan e Mayer concluiu-se que elas são maioritariamente do tipo dupleto. De acordo com Hepler (1969) ambos os aminoácidos, em água e em soluções aquosas de sulfato de amónio, podem ser considerados como solutos que provocam a rutura da solução. Estes resultados representam um passo na construção de uma base de dados empírica de parâmetros volumétricos de diferentes grupos funcionais das proteínas, em sulfato de amónio e água.

Palavras-chave: Aminoácidos, Electrólito, Volumes Molares Parciais, Compressibilidades Adiabáticas.

Abstrakt

Hlavním cílem této práce je měření hustot a rychlostí zvuku ve vodných roztocích síranu amonného obsahující glycin nebo alanin.

Studium směsí obsahujících nabité elektrické částice je velmi významné pro chemický průmysl. V této práci jsou obsaženy nové informace podložené experimentem a údaji z odborné literatury, které mohou přispět k pochopení sil, které působí na biologicky důležité struktury.

Systematický experimentální program je založen na měření hustoty a rychlosti zvuku vodných a smíšených vodných roztoků síranu amonného (0.1, 0.3, 0.7, 1.0, 1.3, a 2.0) $mol \cdot kg^{-1}$ obsahujících glycin a alanin pomocí vibrační trubice digitálního hustoměru a zvukové cely při teplotách (278.15, 288.15, 298.15, 308.15 a 318.15) K.

Údaje o hustotě byly použity pro výpočet parciálních molárních objemů při nekonečném zředění, které dále slouží k získání odpovídajících přenosových objemů aminokyselin. Dále byla zkoumána hydratační čísla, teplotní závislost a skupinové příspěvky vedlejších řetězců aminokyselin.

Tyto parametry získané na základě studia objemových vlastností jsou použity k pochopení různých směšovacích efektů, které jsou způsobeny interakcemi mezi aminokyselinami a síranem amonným ve vodném prostředí. Tyto údaje o $V_{\emptyset,tr}^{0}$ naznačují, že interakce nabitý ion/hydrofilní skupina mají převládající vliv, a na základě použití formalismu McMiliana a Mayera lze odvodit, že jsou převážně párové. Podle Heplera (1969) mohou být všechny aminokyseliny ve vodě a vodných roztocích síranu považovány za látky rozrušující strukturu. Tyto výsledky představují krok při budování empirické databáze různých objemových parametrů proteinových skupin v síranu amonném a vodě.

Klíčová slova: Aminokyselina, Elektrolyt, Parciální Molární Objemy, Adiabatické Stlačitelnosti.

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a, b, c Constants related with temperature effects

A, B Coefficients of the U-tube

a_k, b_k Empirical parameters

a_v, b_v Empirical parameters

m Molality (mol·kg^{-1} H_20)

M Molar mass (kg·mol^{-1})

m_2^* Number of moles of amino acid per kilogram of binary solvent

n Number of moles

n_c Number of carbon atoms in the alkyl chain of the amino acids

n_H Hydration number

S_v Experimental slope

u Speed of sound

V Total volume (cm^3)

V_b^0 Partial molar volume of water in the hydration shell (cm^3·mol^{-1})

V_e^0 Partial molar volume of water in the bulk state (cm^3·mol^{-1})

V_{in}^0 Intrinsic volume of a solute molecule (cm^3·mol^{-1})

V_w Van der Waals volumes (cm^3·mol^{-1})

V_{XY} Pair interaction coefficient

V_{XYY} Triplet interaction coefficient

$V_\emptyset$ Apparent molar volume (cm^3·mol^{-1})

$V_{\emptyset}^{0}$ Standard partial molar volume ($cm^3 \cdot mol^{-1}$)

$V_{\emptyset,tr}^{0}$ Standard partial molar volume of transfer ($cm^3 \cdot mol^{-1}$)

w_i Mass of the specie i (kg)

Greek Letters

K_s^0 Partial molar adiabatic compressibilities ($cm^3 \cdot mol^{-1} \cdot GPa^{-1}$)

ρ_r Densities of dry amino acids ($g \cdot cm^{-3}$)

$\Delta\rho$ Change in density ($g \cdot cm^{-3}$)

ρ Density ($g \cdot cm^{-3}$)

τ Oscillation period

Subscripts

B Binary

T Ternary

1 Water

2 Amino acid

3 Electrolyte

Abbreviations

AA Amino Acid

1. Introduction

1.1 Importance and Motivation

Amino acids are the main components of proteins, its building blocks, and became a very important subject due to their biological and industrial importance. Amino acids are used in a large variety of applications, mainly in food and chemical industries. Due to their importance in biochemistry, the amino acids also strongly attracted the attention of researchers to understand and describe their physical-chemical properties.

For researchers in biochemistry and biophysics the knowledge of thermodynamics properties of molecules of biological interest is frequently of importance, both for planning experiments and understanding molecular interactions. Among the various thermodynamics properties, density and speed of sound measurements of high precision have become significant toward investigating molecular interactions in solutions. In this work, the new experimental information will contribute to better understand the forces that rule biological important structures.

1.2 Objectives

The main objective of this thesis is to measure density and speed of sound of aqueous ammonium sulfate solutions containing the amino acids glycine or alanine at five different temperatures, using salt molalities up to two molal. Following, derived properties such as partial molar volumes and hydration numbers are calculated to obtain information concerning the interactions in solution.

In chapter 2 an introduction to the thermodynamics of amino acids solutions is given, as well as their chemistry, production and main uses. A critical review of the available information from the open literature, concerning experimental methods and data, is also addressed. The derived properties, partial molar volumes and partial molar adiabatic compressibilities are after discussed.

Chapter 3 deals with the experimental measurements of density and speed of sound. The techniques chosen to perform the experimental measurements are presented and details about the procedure as well.

The experimental results obtained for the density and speed of sound in water or in aqueous ammonium sulfate solutions containing the amino acids glycine or alanine in the temperature range between 278.15 and 318.15 are presented, from which the partial molar volumes are calculated. A critical analysis of the data is also displayed.

The theoretical analysis is presented in chapter 4. The density data obtained in this work were used to obtain the hydration numbers and the groups contribution. The Co-sphere Overlap Model, the McMillan and Mayer formalism and the temperature effects are also discussed.

In chapter 5 are presented the main conclusions derived from the present work and suggestions for future work.

2. Thermodynamics of Amino Acids Solutions

2.1 Introduction

Amino acids are found in all living organisms on earth. Because of their biological and industrial importance, their physical and chemical properties became a very important studied subject.

The main reasons to study the thermodynamics properties of solutions containing amino acids are directed towards problems in protein chemistry. Thermodynamic data on proteins are generally provided by calorimetric and volumetric methods (Chalikian et al., 1994). The volumetric approach is based on evaluation of the partial molar volumes, expansibilities and adiabatic compressibilities of protein systems. For the researchers in biochemistry and biophysics the knowledge of thermodynamics properties of molecular of biological interest is frequently of importance, both in the planning of experiments and in understanding molecular interactions.

In this chapter, some aspects about the production of amino acids are firstly presented; since its discovery in animal and plant proteins in the nineteenth century, most amino acids have been produced by extraction, after protein hydrolysis, enzymatic reaction or fermentation (Kirk-Othmer Encyclopedia). It is also showed that amino acids are widely used in industrial processes, particularly in food, feeds, chemical, medical, pharmaceutical, and cosmetics industries. The chemistry of amino acids will be considered due to the relationship between their properties, structure and biological functions. In this concern, a review about intermolecular forces in biological systems is also addressed. Finally, experimental techniques applied for the determinations of the density and speed of sound, experimental data collected from the open literature and theoretical analysis of derived properties (hydration numbers and transfer volumes) concerning aqueous amino acids solutions, with or without ammonium sulfate, are overviewed.

2.2 Production and Use of Amino Acids

All amino acids possible to be found in proteins can be produced and are currently available commercially. Their uses are growing as they have own characteristic effects in flavoring, nutrition and pharmacology.

The amino acid business is a multi-billion dollar enterprise. In 2003, the total annual worldwide consumption of amino acids was estimated to be over 2 million tons (Hermann, 2003). All amino acids are sold, albeit each in greatly different quantities. Glutamic acid, lysine and methionine account for the majority, by weight, of amino acids sold. The major producers of amino acids are based in Japan, the United States of America, South Korea, China and Europe.

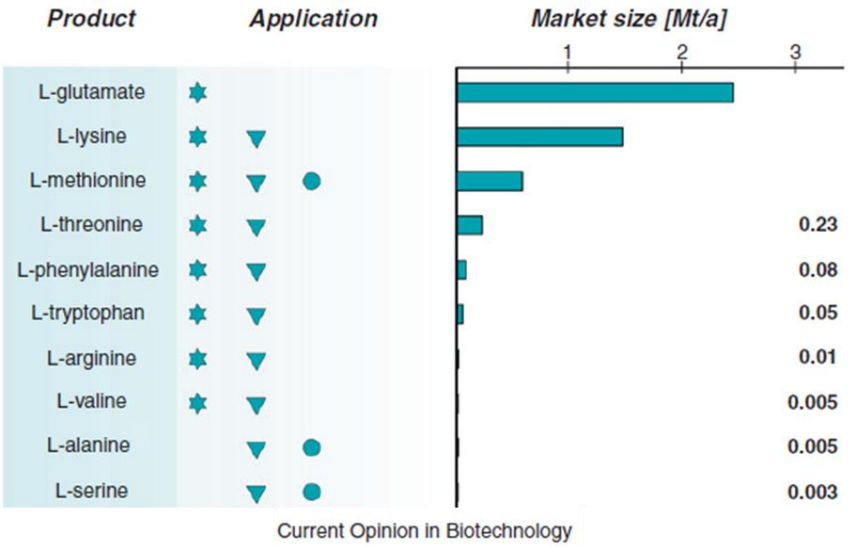

Figure 2.1. Major application (food and feed ✹; health and hygiene ▼; agriculture and technical application ● and textiles, packaging and housing ▲) and current market size of some L-amino acids, adapted from Becker and Wittmann (2012).

Currently, the amino acids are mainly manufactured by the fermentation method using natural materials, similar to yogurt, beer, vinegar, soy sauce, etc. In this method the microorganisms convert nutrients to various vital components necessary to themselves. With the fermentation method, raw materials such as syrups are added to microorganism culture media, and the proliferating microorganisms are allowed to produce amino acids. Consecutive reactions by 10 to 30 kinds of enzymes are involved in the process of fermentation, and various amino acids are produced as a result of these reactions (Shiio and Nakamori, 1989). Glutamic acid and lysine are made by fermentation. The amino acids which are produced by this method are mostly of the L-

form. The fermentation method has the advantage of mass production at low cost, which was the great impetus for expanding the amino acid market (Kirk-Othmer Encyclopedia).

In addition to the fermentation method, the enzymatic reaction and extraction methods are used for producing amino acids. Some amino acids like DL-alanine, glycine and DL-methionine are produced by chemical synthesis, of which, DL-alanine was the first (Matoba et al., 1982 and Strecker, 1850). With the enzymatic reaction method, an amino acid precursor is converted to the target amino acid using 1 or 2 enzymes. This enzyme method allows the conversion to a specific amino acid without microbial growth, thus eliminating the long process from glucose. This method comes into its own when the amino acid precursor is supplied at low prices. L-alanine was produced from the substrate L-aspartic acid through this method. With the extraction method, natural proteins are degraded to various amino acids, but the amount of each amino acid contained in the raw material proteins naturally restricts the yield and there are many problems in the efficient isolations of the desired amino acid in the pure form. Choosing between processes depends on available technology, costs of raw material, market prices and sizes, and the environmental impact of the process itself.

Amino acids are used for a variety of applications in industry, but their main use is as additives to animal feed (lysine, methionine, and threonine), flavor enhancers (monosodium glutamic, serine, and aspartic acid) and as specialty nutrients in the medical field. Aspects about the utilization and production of amino acids were surveyed by Izumi et al. (1978). According to these authors, in the seventies, the most important applications of amino acids included the fortification of plant food and feeds by supplementation of the deficient essential amino acid(s). Apart from their uses in the food industry, medical applications of amino acids (nutritional preparations and therapeutic agents) were becoming increasingly important.

Nowadays the amino acids have an application area much more extensive, they are used in feeds, food, medicine, cosmetics and in chemical industries. In animal nutrition, amino acids are added to the feeds, when they are lacking, to improve the economical growth of the animals without affecting their growth response. In Western Europe, for

example, L-threonine and L-lysine are usually added to the wheat and barley (Kirk-Othmer Encyclopedia).

The food industry is also a major consumer of amino acids. Beyond their nutritive value, each amino acid has its characteristic taste of sweetness, sourness, saltiness, bitterness or *umami*, which is related to their structure (Schallenberger et al., 1973). Glycine and alanine, the amino acids focused in this study, are slightly sweet. Glycine is used for sweet jams and salted vegetables, sauce, vinegar and fruit juice. In foods for humans, the flavor uses of amino acids, specially the artificial sweetener, represent the dominant factor in total market value. Similar technology to that used for animal nutrition is employed in the human nutrition industry to alleviate symptoms of mineral deficiencies, such as anemia. Amino acids are major additives in dairy industry. The other major use of amino acids is as buffers or acid correctors. Glycine is used as such in wine and soft beverage. Likewise glycine also finds use as an anti-oxidant in e.g. cream and cheese. Because glycine also retains the reproduction of bacteria, e.g. *E. coli.*, is used as an antiseptic agent for fish flakes (Kirk-Othmer Encyclopedia).

In the medicine field many amino acids have been used or studied for pharmaceutical purposes, such as treating type I diabetes, lesions and wounds. They are used too in pharmaceutical industry for the treatment of Parkinson's disease and depression related disorders. Glycine is medically used in amino acid injection solution as nutritional infusion and as a raw material for making L-Dopa, a pharmaceutical for treating Parkinson's disease. Amino acids are also widely used in cosmetics because they and their derivatives exhibit a controlling or buffering effect of pH variation in skin and a bactericidal effect. Serine, for example, is one component of skin care cream or lotion.

Recently, as some amino acids (eg, L-glutamic acid, L-lysine, glycine, DL-alanine, DL-methionine) have become less expensive chemical materials, they have been employed in various application fields of industrial chemical. Poly(amino acids) as potential source for biodegradable plastics is under research. The chelating ability of amino acids has been used in fertilizers for agriculture to facilitate the delivery of minerals to plants in order to correct mineral deficiencies, such as iron chlorosis. These fertilizers are also used to prevent deficiencies from occurring and improving the overall health of the

plants (Ashmead, 1986). In the fertilizer industry glycine is used as a solvent for removing CO_2, and is also an intermediate in the production of pesticides.

2.3 Amino Acids Chemistry

Amino acids (AA) are defined as organic substances containing both amino and acid groups. Among more than 300 AA in nature, only 20 of them (α-AA) serve as building blocks of protein, of which 19 are α-amino acids and one is a cyclic α-amino acid (proline). Contrary to plants and some microorganisms, animals and humans are only capable of synthesizing 10 of the 20 naturally occurring amino acids. The rest must be included in the diet; these amino acids are classified as *essential*. Because of variations in their side chains, amino acids have remarkably different biochemical properties and functions (Wu et al., 2007).

From a chemical viewpoint an amino acid is a base as well as an acid; i.e. it consists both of an amino group and a carboxylic group. The amino acid is therefore an ampholyte since it can react both as a base and as an acid. The most common amino acids are the α-amino acids, which are amino acids where the amino group is located at the α-carbon atom of the carboxylic group as shown in Figure 2.2. The α-carbon atom (usually) has hydrogen and a side chain at the last two sites.

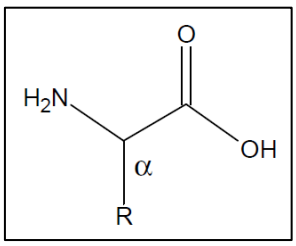

Figure 2.2. Basic structure of α-amino acids.

If the side chain is also an hydrogen, the compound is the simplest amino acid glycine, as presented in Figure 2.3.

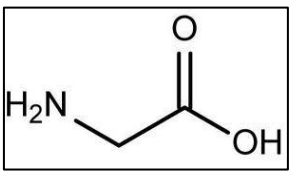

Figure 2.3. Glycine, the simplest α-amino acid.

Because of the chirality of the α-carbon atom the amino acids exist in two enantiomers. They can be characterized by their ability to rotate light to the right (+) or to the left (-), depending on the solvent and the degree of ionization. The DL-notation is experimentally based, only. Except for glycine, all AA can have L- and D-isoforms.

When two amino acids are linked together by a peptide bond are called a dipeptide. Continuing this process will eventually lead to the formation of protein (Morrison and Boyd, 1992). Amino acids have a higher solubility in polar solvents (e.g. water, ammonia) than in less polar solvents (e.g. ethanol, methanol, acetone). They are crystalline solids with relatively high melting points. In aqueous solutions, the amino acids are generally stable, at physiological pH, and they exist as neutral dipolar ions, i.e., due to physiological conditions, the two terminals of amino acids are both charged; positive charge (amino group NH_3^+) and negative charge (carboxyl group, COO^-), therefore the molecules have the properties of zwitterion (Venkatesu et al., 2007). The pH at which the dipolar ions do not migrate in an electric field is called the isoelectric point, pI. It is the point at which the acid and the base action of the ampholyte is equal. At this pH the concentration of zwitterion is maximum and those of cations and anions are minimum and equal. Each amino acid has a particular isoelectric point. The twenty amino acids, along with the respective structures, common names, and the customary three-codes that abbreviate their names can be consulted in Table A.1.

2.4 Review of Intermolecular Forces

When molecules, atoms or ions approaching each other two phenomena may occur, reaction or interaction. In chemical interactions molecules attract or repel each other, without the occurrence of breakage and/or forming new chemical bonds such as in reactions. The intermolecular interactions arise due to intermolecular forces, which are essentially electrical and they make a molecule influence the behavior of other molecules in their vicinity. The behavior of the intermolecular forces varies with the inverse of the distance separating the molecules involved, i.e., interactions are stronger for smaller separation distances between the molecules (Leckband and Israelachvili, 2001).

The intermolecular interactions and their understanding have a crucial importance in biological systems and are closely related to the thermodynamic properties of liquids,

solids and gases. The molecules of life (DNA, RNA, proteins) are held in three-dimensional structures through the intra and intermolecular interactions. Since the three-dimensional molecular structure is responsible for specific biological activities of these molecules, it is then clear the importance of understanding these interactions (Chimankar et al., 2011).

A *biological interaction* is often very different from a chemical reaction or physical change in a system. This is due, in part, to the high complexity of biological macromolecules. Furthermore, the interactions between these compounds do not occur linearly and they are dynamic because biological systems are never in thermodynamic equilibrium. The complex biological interactions that often involve different molecules or aggregate structures occur over long distances, and evolve over time which prevents its rigorous understanding in terms of the fundamental laws of physics or even chemistry (Leckband and Israelachvili, 2001).

The forces and interactions may be specific or non-specific. The non-specific forces are those that arise from many different types of atoms, molecules, molecular groups or surfaces, and which can be generally described in terms of a potential of generic interaction or a force. The main physical forces that fall into this category are listed in Table 2.1. The specific interactions arise when a unique combination of physical forces or bonds between two molecules act together cooperatively in the space to originate a connection (generally) strong, but non-covalent. These interactions usually arise from a synergy of various geometries and bonds steric, ionic and directional (Leckband and Israelachvili, 2001). The forces that stabilize protein structures are predominantly of a non-covalent nature (Tanford, 1968).

Table 2.1. Non-specific physical interactions, adapted from Leckband and Israelachvili (2001).

Name/type of interaction or bond	Special features
van der Waals	A force that exists between all bodies. Usually attractive, but can be repulsive.
Electrostatic (also Coulomb, ionic, double-layer)	A force that exists only between charged molecules (ions) or surfaces. Can be attractive or repulsive.
Hydrogen bonding	A special electrostatic binding (attractive) interaction involving positively charged H atoms covalently bound to electronegative atoms. Directional.
Hydrophobic	A special attractive interaction in water between inert, non-polar molecules or surfaces.

A dependence on pH and/or ionic strength is the signature of electrostatic interaction. The dominant element of a hydrogen bond is electrostatic interaction between hydrogen atoms and highly electronegative atoms such as N, O and occasionally S. It is stronger than van der Waals interactions but weaker than charge-charge interactions. Arguably, hydrogen bonds are one of the major contributors to protein folding. It is difficult to account for other interactions such as van der Waals that contribute significantly to measure thermodynamic properties. The hydrophobic effect is another force that is driven by solute solvent interactions and plays important role in protein stability. This effect occurs because of the unfavorable interactions between water molecules and the nonpolar groups of a protein (Leckband and Israelachvili, 2001).

In order to contribute to the understanding of molecular interactions in systems containing biological molecules two thermodynamic properties were selected; partial molar volumes and adiabatic compressibilities, whose relevance is presented in this work.

2.5 State of the Art

The aim of this thesis is to study the thermodynamics properties of aqueous amino acids solutions with or without a salt, because of their particular importance in biology.

Before start any experimental work is fundamental carry out a detailed literature review. In this case it will help deciding the systems and conditions to perform the experimental measurements, the type of experimental methods and analytical techniques to implement. A literature search in terms of the experimental available data is needed, in order to establish the experimental program. It is also necessary to review theoretical interpretations already proposed and accepted by the scientific community.

2.5.1 Experimental Methods

In the following sections critical evaluation of the different measuring techniques of density and sound speed is presented.

2.5.1.1 Density

For the density determination there are several experimental methods and devices used like hydrodensitometry, pycnometry, hydrometer and digital density meters (hydrostatic pressure-based instruments, vibrating element transducers, ultrasonic transducer, radiation-based gauge). But the most widely used are pycnometry and densimetry (Assael et al., 2011).

The hydrodensitometry is a method that measures the density of a solid sample by solid volume determinations. Using a spring scale, the sample is weighed first in air and then in water. This technique cannot easily be used to measure relative densities less than one, because the sample will then float (Brožek et al., 1963).

Pycnometry technique, widely used due to its low cost, is currently in disuse because is a somewhat imprecise method. In this technique is used a pycnometer to measure the density. The pycnometer is a glass flask with a close-fitting ground glass stopper with a capillary hole through it. This fine hole releases a spare liquid after closing a top-filled pycnometer and allows for obtaining a given volume of measured and/or working liquid with a low accuracy (Dhir, 2011). Before being used is calibrated using a working liquid with well-known density, usually, double distilled water. To maintain the temperature of the water uniform a thermostatic bath is used. The pycnometer is held approximately 30 minutes in a water bath, for minimize the thermal fluctuation of the density. The density of water at different temperatures required for the calibration can be obtained using, for example, the standard equation proposed by Islam and Waris (2004).

The relative density of a liquid can be measured using a hydrometer. This consists of a bulb attached to a stalk of constant cross-sectional area. First the hydrometer is floated in the reference liquid, and the displacement (the level of the liquid on the stalk) is marked. The reference could be any liquid, but in practice it is usually water. The hydrometer is then floated in a liquid of unknown density. The change in displacement is noted. It is necessary that the hydrometer floats in both liquids. The application of simple physical principles allows the relative density of the unknown liquid to be calculated from the change in displacement (Ashwortha et al., 2001). In practice the stalk of the hydrometer is pre-marked with graduations to facilitate this measurement.

Hydrometers may be calibrated for different uses, such as a lactometer for measuring the density (creaminess) of milk, a saccharometer for measuring the density of sugar in a liquid, or an alcoholometer for measuring higher levels of alcohol in spirits (Lorefice and Malengo, 2006).

In the technique of densimetry is usual to use a densimeter digital commercialized by the brands such as Anton PAAR, Metler-Toledo, Inter Alia, that can be calibrated with n-heptane, aqueous solutions of sodium chloride, dry air, distilled water, etc. To perform a measurement of density, accurately and reproducibly, it is necessary to perform a water check every day before measurements, if it fail an adjustment of the instrument constants is compulsory. The sample preparation should be done with care, and the same way every time, and the filling of the measuring cell must be made carefully and without bubbles. The use of automatic sample filling devices eliminates filling errors due to the operator. After the measurement, the sample should be immediately remove from the measuring cell. Cleaning the instrument regularly is also an essential condition for a good measurement. The digital densimeters may have implemented different technologies and methodologies. Some of them will be presented below.

The hydrostatic pressure-based instruments technology relies upon the Pascal Principle which states that the pressure difference between two points within a vertical column of fluid is dependent upon the vertical distance between the two points, the density of the fluid and the gravitational force. This technology is often used for tank gaging applications as a convenient means of liquid level and density measure (Lipták, 2003).

The vibrating element transducers are instruments that require a vibrating element to be placed in contact with the fluid of interest. The resonant frequency of the element is measured and is related to the density of the fluid by a characterization that is dependent upon the design of the element. One of the methodologies most used in this case is the so called oscillating U-tube. In this method, the sample is introduced into a U-shaped tube which is electronically excited to oscillate at a characteristic frequency. The frequency characteristic changes depending on the density of the sample. Through a precise determination of the characteristic frequency and a proper fit, can be determined the density of the sample. Due to the great dependence of the density with temperature, the measuring cell has to be thermostatically controlled with high precision. The

vibrating fork immersion probe is another good example of this technology. These instruments are capable of measurement to 5 to 6 places beyond the decimal point at temperatures between 0 and 80 °C and are used in the brewing, distilling, pharmaceutical, petroleum and other industries (Lipták, 2003).

In ultrasonic transducer, ultrasonic waves are passed from a source, through the fluid of interest, and into a detector which measures the acoustic spectroscopy of the waves. Density can be inferred from the spectrum (Lipták, 2003).

In radiation-based gauge, radiation is passed from a source, through the fluid of interest, and into a scintillation detector, or counter. As the fluid density increases, the detected radiation *counts* will decrease. The source is typically the radioactive isotope cesium-137, with a half-life of about 30 years. A key advantage for this technology is that the instrument is not required to be in contact with the fluid—typically the source and detector are mounted on the outside of tanks or piping (Lipták, 2003).

The high precision densimeters are able to correct viscosity and have a reference oscillator which is very useful because it allows accurate results in a wide range of temperatures using only one adjustment.

2.5.1.2 Speed of Sound

The most common techniques for measuring the speed of sound can be categorized as variable-frequency fixed-cavity resonators, variable path-length fixed-frequency interferometers, and time-of-flight methods. These methods can be selected given a knowledge of the phase of the material and the geometry that can be employed (Assael et al., 2011).

In principle, the measurements of a single resonance frequency of a known mode of oscillation within a cavity of known dimension, or of a single time-of-flight over a known distance, is sufficient to determine the speed of sound. To determine the speed of sound from standing-wave measurements in either a cavity or interferometer requires efficient reflection of sound at the interface between the medium and the wall of the container and this is necessarily the case when the acoustic impedances (the product of density and sound speed) of the medium differs greatly from that of the wall (Assael et al., 2011).

For liquids there are essentially two methods that are used to determine the speed of sound and these are variable path-length fixed-frequency interferometry and time-of-flight measurements. The time-of-flight methods can be divided into single and multiple path-length devices. A single path pulse echo apparatus that was modified by a fractional uncertainty of < ±0.5 % typically operate at frequencies on the order of 10 MHz and can be operated at temperatures up to 2100 K and pressures up to 200 MPa, although more typically at temperatures of less than 500 K. The path length is determined by calibration measurement with water for which the sound speed is known with sufficient precision. Time-of-flight measurements are often used for solids albeit with methods, which differ from those adopted for liquids (Assael et al., 2011).

2.5.2 Experimental Data

Experimental data are essential in the development, design, and modeling of separation process, in the following sections the information collected concerning the properties of interest is briefly presented.

2.5.2.1 Water/Amino Acid

The study of density and speed of sound in aqueous solution of amino acids is of paramount importance, as it provides information about the nature of molecular interactions. Another important aim of such studies lies on the effects of amino acids on the structure of water. The interactions of water with the various functional groups of amino acids play a crucial role in determining the conformational stability of proteins (Dhir, 2011).

In Table B.1 is presented a literature review of those properties in amino acids aqueous solutions, highlighting the properties measured and the respective temperature ranges. Some of these studies are focused below.

Rao et al. (1984) have described a method of estimating partial molar volumes of α-amino acids in water, starting from the partial molar volume of glycine. For eleven zwitterionic amino acids the estimated partial molar volumes are found to be in very good agreement with the experimental values given in the literature. The method has

given good results for the ionic species of lysine and arginine but not for all species of aspartic and glutamic acids.

Apparent molar volumes, expansibilities, and adiabatic compressibilities of a homologous series of eight α,ω-aminocarboxylic acids within the temperature range 18-55 ºC were measured by Chalikian et al. (1993). They found that at low temperatures, water that hydrates CH_2 groups in α,ω-amino acids is less compressible than bulk water, while at high temperatures the opposite is true, and the water in the hydration shells of aliphatic groups differs from water that hydrates charged groups, not only in the absolute values of the density and the coefficient of adiabatic compressibility, but also in the temperature dependences of these characteristics.

The densities and speeds of sound in dilute aqueous solutions of some the L-amino acids, were measured at (5, 15, 25, 35, and 45) °C by Yasuda et al. (1998). Partial molar volumes and partial molar isentropic compressibilities at infinite dilution were evaluated and discussed. The partial molar volumes of all the amino acids studied increase with increasing temperature and, furthermore, their curves obtained are always concave downward. This feature is typical of aqueous electrolyte or hydrophilic nonelectrolyte solutions (Hepler, 1969). In this work the authors also showed that compressibility decreases steeply decreasing temperature; that is characteristic for dilute aqueous mixtures, regardless of whether they are hydrophilic or hydrophobic solutes.

Recently, Zhao (2006) collected published standard molar volumes for a large number of aqueous amino acids under atmospheric pressure. The consistency of different sources was checked and a value is proposed at 298.15 K. However, for other temperatures the information is scarce and present large variations. Nevertheless, his review allowed to conclude that partial molar volumes of amino acids can be used to calculate the volumes of amino acids residues. In its turn, these can be used to calculate the partial molar volumes of peptides and proteins based on the group contribution methods.

In 2010, Cibulka et al., published density in aqueous solutions of glycine and L-alanine, at temperatures from 298 up to 443 K and at pressures in the range from (15 to 17) MPa, and at 30 MPa. The partial molar volumes at infinite dilution were calculated from these data. They conclude that zwitterions are by far the dominant solute species

and the change of the measured partial molar volumes due to the presence of other species can be considered as negligible.

2.5.2.2 Water/Electrolyte/Amino Acid

The addition of electrolytes, to solutions of biologically important molecules such as proteins, is known to influence their stability and affect their structure and configuration (Singh and Banipal, 2008). A better understanding of the effect of electrolytes on the thermodynamic properties of amino acids in aqueous solution is of vital importance because such studies give useful information regarding protein unfolding and the extent of hydrophobic interactions of nonpolar side chains (Enea and Jolicoeur, 1982). Salt-protein interaction induced electrostatic forces are used to play a very important role in modifying the protein structure by affecting properties like solubility, denaturation and activity of enzymes (Chimankar et al., 2011).

The salt chosen to perform this work is ammonium sulfate. It is commonly used as its solubility is so high that salt solutions with high ionic strength are allowed. The solubility of proteins varies according to the ionic strength of the solution, and hence according to the salt concentration. Since proteins differ markedly in their solubilities at high ionic strength, salting-out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate, as it is very water soluble, forms two ions high in the Hofmeister series, and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution).

The volumetric and other thermochemical properties of aqueous solutions of salts containing amino acids (e.g. densities, viscosity, apparent molar volumes, apparent molar heat capacities, partial molar volumes and compressibility) are important tools to investigate the interaction between ionic salts and amino acids, and have also been measured and reviewed by several researchers. These results lead to the conclusion that some of the electrolytes can stabilize the biological important molecule i.e. proteins (Chimankar et al., 2011). A compilation of some of these experimental works is presented in Table B.2. A literature survey shows a considerable lack of information

and the majority of the data available is at 298.15 K only. Some of the most important works are shown, focusing those involving the ions (NH_4^+ or SO_4^{2-}) studied in this work.

Ogawa et al. (1984) measured the density, speed of sound and viscosity of glycine, DL-alanine, β-alanine, α-aminoisobutyric acid, L-serine and L-threonine in lithium, sodium and potassium chlorides at 298.15 K. In this study was verified that threonine and serine had unusual compressibilities. This was attributed to the fact that this both amino acids have one OH group and consequently hydrogen-bond with water.

Wadi and Ramasami (1997) measured the density and the speed of sound of glycine and DL-alanine in aqueous solutions of sodium sulfate at 288.15, 298.15 and 308.15 K. From the analysis of the experimental data, it is found that the introduction of a methyl group in glycine introduces hydrophobic hydrations in DL-alanine and decreases the former *pure* hydrophilic hydrations. This is supported by the fact that the calculated hydration numbers for DL-alanine are lower than the corresponding ones for glycine. The dehydrating effect on the amino acids confirms the structure-making ability of sodium sulfate.

Apparent molar volumes and adiabatic compressibilities of glycine, L-alanine and L-valine in binary aqueous solutions of $MgCl_2$ have been determined at 298.15 K from precise density and sound speed measurements by Pal and Kumar (2005a). The results have been used to estimate the number of water molecules hydrated to the amino acids. This approach of relating the volume and compressibility behavior seems successful in obtaining credible values for apparent hydration numbers when applied to amino acids in aqueous salt solutions.

Concerning the measurements of amino acids density and speed of sound in water / ammonium sulfate, no literature data was found, but electrolytes involving one of the cation or anion with glycine or alanine are possible to find in the workers by Natarajan et al. (1990), Wadi and Ramasami (1997), Islam and Wadi (2003), Singh and Kishore (2003), Mallick and Kishore (2006), Sadeghi and Gholamireza (2011) and Sinha et al. (2011).

2.5.3 Modeling and Derived Properties

Thermodynamic properties of amino acids, i.e., proteins in electrolyte solution, provide information about solute-solvent interactions, those are important in understanding the stability of proteins. Some of these interactions are found applicable in several biochemical and physiological processes in a living cell (Millero et al., 1978). Traditionally, thermodynamic models have not been widely used in the biotechnological industry as is the case in the chemical industry, but it is becoming more prevalent because of the increasing demand for computer aided design and optimization of processes (Chimankar et al., 2011).

Understanding the various types of interactions operating between amino acids to protein folding is an extremely difficult task that has been tried by number of theoretical and experimental studies. Partial molar volumes derived from density and adiabatic compressibilities derived from speed of sound, are examples of thermodynamic properties that contribute to the understanding of molecular interactions in systems containing biological molecules.

2.5.3.1 Partial Molar Volumes

Density is an important physical characteristic of matter and for pure substances it depends on the temperature and pressure. Many applications or processes require a precise knowledge of this property. As a curiosity it is added that the density has a long history of application in fiscal measures related to quality control, particularly in the oil and gas industries. Partial molar volume is a thermodynamic property that can be derived from density. Information about partial molar volume has proved very useful in the study of solute-solvent interactions in solutions. The concentration dependence of volume reflects information about solute-solute interactions, which is helpful in understanding complex biochemical systems.

The standart partial molar volume, V_{ϕ}^{0}, of a solute in solution can be defined as the apparent volume occupied by one mole of a solute at infinite dilution. The partial molar volume of a solute can be regarded as the sum of intrinsic volume plus volumetric effects of solute-solvent interactions. The latter can be divided into different contributions such as volume due to ion-solvent interaction (in case of ionic solutes) or

hydrogen bonding between solute and solvent molecules (in case of H-bonded solute-solvent systems) or hydrophobic hydration (in case of hydrophobic solutes). The group partial molar volume is also well suited for predicting partial molar volume of different solutes.

Knowledge of partial molar volume of small molecules like salts, allows rough estimate of how bonding or unspecific interaction would influence the overall partial molar volume. This consideration does not influence complicated solvent interaction, structural changes, etc. Characterization of volume data of a larger number of compounds studied in the past have shown that hydrophilic group generally decrease the molar volume because polar groups disrupt the water structure around a hydrophilic group. It seems that several neighboring hydrophilic groups strengthen this volume contraction.

Various concepts regarding molecular processes in solutions (solvation, electrostriction, hydrophobic hydration, micellization and co-sphere overlap during solute-solute interactions) to a large extent have been derived and interpreted from the partial molar volume data of many compounds.

From the density measurements, the partial molar volume at infinite dilution ($m_2 \rightarrow 0$) of the amino acids, $V_\emptyset^0$ ($cm^3 \cdot mol^{-1}$) in pure water can be calculated by:

$$V_\emptyset^0 = \frac{1}{\rho_1}\left(M_2 - \frac{a_v}{\rho_1}\right) \tag{2.1}$$

$$\frac{\Delta\rho}{m_2} = \frac{\rho - \rho_1}{m_2} = a_v + b_v m_2 \tag{2.2}$$

where ρ and ρ_1 are the densities of the solution and pure water, M_2 and $m_2$are the molar mass and the molality of the amino acid, respectively, and a_v and b_v are empirical constants obtained by fitting the experimental values in the form $\Delta\rho/m_2$.

In ternary systems, AA + salt + water, partial molar volume at infinite dilution are calculated using:

$$V_\emptyset^0 = \frac{1}{\rho_B}\left[M_2 - (1 + m_3 M_3)\frac{a_v}{\rho_B}\right] \tag{2.3}$$

$$\frac{\rho_T - \rho_B}{m_2} = a_v + b_v m_2 \quad (2.4)$$

where ρ_T and ρ_B are densities of the ternary and binary solutions (water/electrolyte) and M_3 and m_3 are the molar mass and the molality of the salt, respectively. The demonstration of the equation 2.3 is presented in Appendix C.

2.5.3.2 Partial Molar Adiabatic Compressibilities

Ultrasonic is a versatile non-destructive technique and highly useful for the investigation of various physical properties such as residual stress, hardness, grain size micro structure, elastic constant, etc. Recent developments have found use of ultrasonic energy in medicine, engineering and agriculture. Ultrasonic study on the amino acids with aqueous solution of electrolytes and non-electrolytes provides useful information in understanding the behavior of liquid systems (Millero et al., 1978).

The compressibility is a volumetric property, derived from the speed of sound, that is known to be a sensitive indicator of molecular interactions and can provide useful information about these phenomena, particularly in cases when partial molar volume data alone fail to provide an unequivocal interpretation of the results. The partial molar adiabatic compressibility of a solute is a linear function of the first derivative of the partial molar volume with pressure (Katriňák, 2012). It represents the apparent compressibility of one mole of a solute at infinite dilution. Analogous to partial molar volume, the partial molar adiabatic compressibility can be presented as the sum of the intrinsic and hydration contributions. The intrinsic compressibility of a solute is a measure of intramolecular interactions, while the hydration induced change in the solvent compressibility, reflects the influence of the solute molecules on the solvent.

The most important and serious work is to determine the intrinsic compressibility of globular proteins which is the most significant parameter to study the physical state of the central of a protein molecule (Leyendekkers, 1986). There are three factors which are contributing the overall partial compressibility of proteins in solution (Leyendekkers, 1986): intrinsic, from the residue-residue interaction in the globule interior; hydration, from surface atomic group-water interaction, and relaxational, from the structural transformations accompanied by volume changes. Compressibility effect

of temperature on the various intermolecular interactions cannot be ignored which ultimately effect the compressibilities (Makhatadze and Privalov, 1992).

The partial molar adiabatic compressibilities, K_S^0 ($cm^3 \cdot mol^{-1} \cdot GPa^{-1}$), of the amino acids solutions are determined using the following equations for binary systems:

$$K_S^0 = \frac{1}{(\rho_1 u_1)^2}\left(M_2 - \frac{a_k}{(\rho_1 u_1)^2}\right) \tag{2.5}$$

$$\frac{\Delta[(\rho u)^2]}{m_2} = \frac{(\rho u)^2 - (\rho_1 u_1)^2}{m_2} = a_k + b_k m_2 \tag{2.6}$$

where u and u_1 are the speed of sound of the solution and the solvent, respectively, and a_k and b_k are adjustable parameters fitted from experimental data.

For ternary systems the partial molar adiabatic compressibilities are calculated using:

$$K_S^0 = \frac{1}{(\rho_B u_B)^2}\left[M_2 - (1 + m_3 M_3)\frac{a_k}{(\rho_B u_B)^2}\right] \tag{2.7}$$

$$\frac{\Delta[(\rho u)^2]}{m_2} = \frac{(\rho u)_T^2 - (\rho u)_B^2}{m_2} = a_k + b_k m_2 \tag{2.8}$$

where u_T and u_B are the speed of sound of the ternary and binary solutions (water/electrolyte).

2.5.3.3 Interpretation of Intermolecular Interactions

The stabilization of biological macromolecules is commonly related to several non-covalent interactions including hydrogen bonding, electrostatic and hydrophobic interactions. These interactions are affected by the surrounding solutes and solvent of macromolecules (Zhao, 2006). Especially, volumetric properties (such as standard partial molar volumes and partial molar adiabatic compressibilities) as well as changes in enthalpy and free energy in water and solutions of organic solvents or salts can provide valuable clues for comprehending the protein unfolding and the hydrophobic interactions of non-polar side chains.

The standard partial molar volumes of amino acids in solutions containing salts allow to understand the effect of salt on the hydration of amino acids. These data are often

embedded with important information of solute hydrophobicity, hydration properties and solute–solvent interactions (Romero and Negrete, 2004).

The criteria proposed by Hepler (1969), called hydrophobicity criteria, uses the partial molar volume derivatives with temperature to reflect about the hydrophobicity of the solute:

If $(\partial V_{\emptyset}^{0}/\partial T)_P > 0$ and $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P < 0$, the solute is hydrophilic;

If $(\partial V_{\emptyset}^{0}/\partial T)_P < 0$ and $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P > 0$, the solute is hydrophobic.

where $(\partial V_{\emptyset}^{0}/\partial T)_P$ is the so-called partial molar expansibility.

Taking into account these criteria, studies presented by Romero and Negrete (2004), have suggested that hydrophilic interactions between water and amino acids are stronger than the hydrophobic interactions, but they decrease with increasing length of the hydrophobic chains. Meanwhile, since $(\partial C_p^o/\partial P)_T = -T(\partial^2 V_{\emptyset}^{o}/\partial T^2)_P$, a negative value of $(\partial^2 V_{\emptyset}^{o}/\partial T^2)_P$ is associate with a structure-breaking solute and a positive one is associated with a structure-making solute (Hepler, 1969).

The hydration numbers, n_H, explicitly reveal the hydration degree of a solute in water. Usually increases with the size of the amino acid in water, or solutions, and can be calculated from the volumetric properties directly or from the second derivative of the partial molar volume, or partial molar compressibility, with temperature (Zhao, 2006). The hydration number can be calculated using the following equation:

$$n_H = (V_{\emptyset}^{0} - V_{in}^{0})/(V_e^{0} - V_b^{0}) \quad (2.9)$$

where V_{in}^{0} is the intrinsic volume of a solute molecule, V_e^{0} and V_b^{0} are the partial molar volumes of water in the bulk state and in the hydration shell of a solution. According to Yan et al. (2004), $(V_e^{0} - V_b^{0}) = -2.6, -2.9, -3.3, -4.0$ $cm^3.mol^{-1}$ at 278.15, 288.15, 298.15 and 308.15 K, respectively. The value at 318.15 K can be obtained by polynomial regression of order two, being -4.85 $cm^3 \cdot mol^{-1}$. The intrinsic volumes of amino acids are calculated through several methods: converting the densities of dry amino acids (ρ_r) using $V_{in}^{0} = (0.7/0.634)(M_2/\rho_r)$, where 0.7 is the packing density

for the molecule in an organic crystal and 0.634 is the packing density for the random packed spheres, assuming they are equal to the partial molar volumes of equivalent amides or substituting with van der Waals volumes (V_w), which can be estimated from the group contribution methods (Zhao, 2006). The densities of dry amino acids can be found in Berlin and Pallansch (1968).

Another important parameter usually used in the analysis of the data is the standard partial molar volume of transfer, $V^0_{\emptyset,tr}$, which is defined as the difference between the standard partial molar volume in solution to that in pure water.

$$V^0_{\emptyset,tr} = V^0_{\emptyset}(solution) - V^0_{\emptyset}(water) \tag{2.10}$$

Strongly hydrated ions have small values of $V^0_{\emptyset,tr}$ (may even be negative). If the volume of transfer is a positive value indicates that the hydration number of the amino acid is reduced by the addition of cosolutes (Ogawa et al., 1984). Therefore, a high value of $V^0_{\emptyset,tr}$ means that the amino acid is more dehydrated in solution. Generally, the volume of transfer increases with the salt concentration indicating that the high ionic strength dehydrates amino acids (Yan et al., 2005). Zhao et al. (2006) noted that the volumes of transfer are typically high in solutions containing strong ions (such as $SO_4^{2-}, Ca^{2+}, CH_3COO^-$) and usually low in solutions of weakly hydrated ions (such as Cl^-, Br^-, NH_4^+). Therefore, there are indications that suggest that the amino acids are less hydrated in solutions containing strongly hydrated ions because these ions tend to take more molecules of water to hydrate, leaving less water available to the amino acids. In fact, the effect of dehydration of ions of amino acid is considered as one of the most important reasons of salting-out of amino acids (Wang et al., 2000). Badarayani and Kumar (2004a) suggested that ions experiencing hydrophilic hydration have stronger effect on the amino acid hydration than those ions experiencing hydrophobic hydration.

The zwitterionic heads of amino acids are hydrated by hydrophilic hydration whereas its apolar part is hydrated by hydrophobic hydration. Overlap of different hydrated spheres of amino acids and cosolutes take place. As a result a water molecule mainly from the hydrophobic hydration sphere gets out. The volume change during transfer is a good indicator of these released water molecules due to different types of overlap among hydration spheres (Das et al., 2004). Therefore, the co-sphere overlap model can be

used to rationalize the $V^0_{\emptyset,tr}$ values in terms of solute-cosolute interactions (Siddique and Naqvi, 2010). According to this model, when two solute particles come sufficiently close together so that their co-spheres overlap, some co-sphere material is displaced (Figure 2.4) and this is accompanied by the change in thermodynamic parameters (Banipal et al., 2007a).

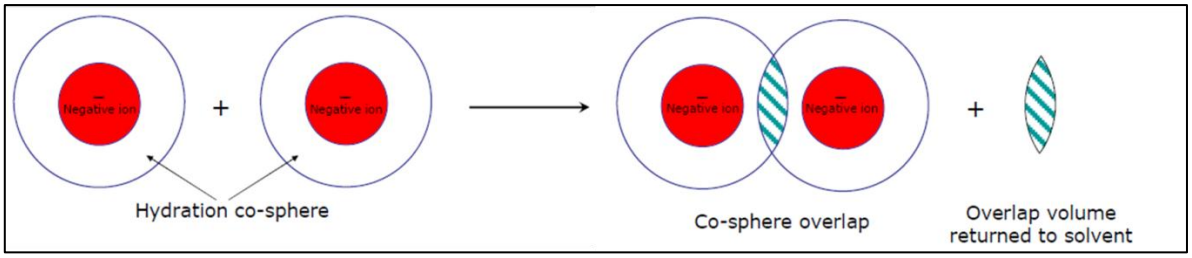

Figure 2.4. Ion hydration co-sphere overlap, adapted from Krishnan et al. (2009).

In ternary systems (amino acids + salt + water), the overlap of cosolute ions and amino acids comes into play because of interactions between: (i) the ($-NH_3^+$, $-COO^-$) charged ends of amino acids and ions of the cosolute, called ion-charged/hydrophilic group or ion-ion interactions; (ii) the hydrophobic parts of the amino acids and cosolute ions or the charged ends/hydrophilic parts of amino acids and the hydrophobic parts of the cosolutes, called ion-hydrophobic group interactions; and (iii) the hydrophobic parts of the amino acids and hydrophobic parts of ions of cosolutes, called hydrophobic-hydrophobic group interactions (Siddique and Naqvi, 2010).

Thermodynamic investigations of amino acids in aqueous salt solutions identified the significant hydration characteristics of the solutes such as (Aktar, 2007): (i) NH_3^+ and COO^- terminals in these solutes are hydrated in an electrostatic manner and the intervening backbone is hydrated, but depending on its nature; (ii) electrostriction of the NH_3^+ group is greater than that of the COO^- group by a factor of 10; and (iii) the overlap of hydration co-spheres of terminal groups adjacent to the core results in a volume change. Thus, thermodynamic properties of amino acids in aqueous electrolyte solutions provide valuable information about solute–solvent and solute–solute interactions. The changes in volume due to the various types of interactions above are summarized in Figure 2.5.

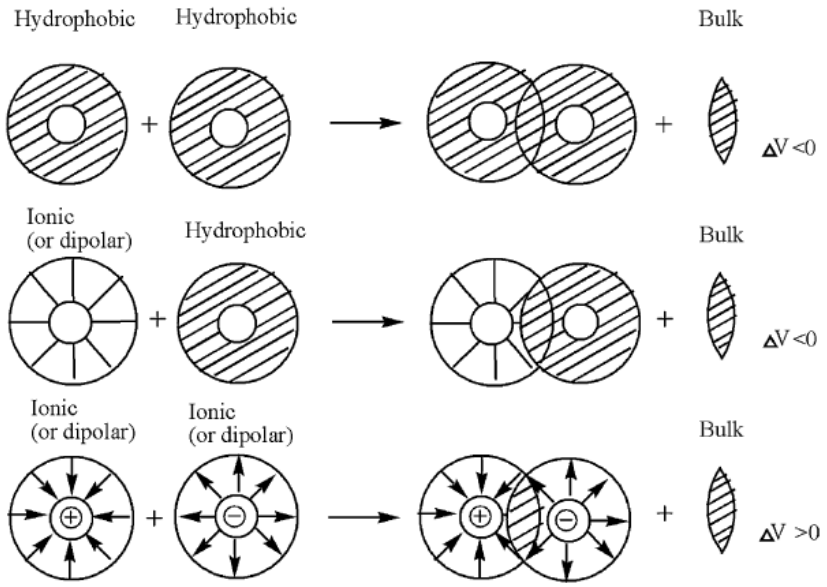

Figure 2.5. The structural interaction of two cospheres, adapted from Lin et al. (2006).

According to this model, the ion-charged/hydrophilic group interactions result in positive $V^0_{\emptyset,tr}$ values, whereas ion-hydrophobic and hydrophobic-hydrophobic group interactions result in negative $V^0_{\emptyset,tr}$ values. If it is observed a positive $V^0_{\emptyset,tr}$ value for amino acid throughout the concentration range of salt then the ion charged/ hydrophilic group interactions are much stronger and dominate over the ion-hydrophobic and hydrophobic-hydrophobic group interactions (Banipal et al., 2007a).

Zhao et al. (2006) proposed some explanations based on the co-sphere overlap model in terms of solute-cosolute interactions, and suggested that strongly hydrated ions (such as sulfate, acetate, butyrate, caproate and caprylate) have stronger interactions with polar groups of (NH_3^+ and COO^-) amino acids causing dehydration of amino acids, implying that weakly hydrated ions have weaker interactions with amino acids. However, it is known that kosmotropic anions (such as SO_4^{2-}) do not salt-in the polar peptide group, but rather salt out nonpolar groups of proteins.

In order to establish group-contribution methods to calculate thermodynamics properties in amino acids aqueous solutions several authors present values for the contribution of each group in the amino acids. In that regard the individual contributions of charged groups (NH_3^+, COO^-) and hydrophobic group CH_2 to the standard partial molar volume can be identified through the following linear relationship:

$$V^0_\emptyset = V^0_\emptyset(NH_3^+, COO^-) + n_c V^0_\emptyset(CH_2) \quad (2.11)$$

where n_c is the number of carbon atoms in the alkyl chain of the amino acids.

Through linear regression calculations, the polar group contribution and hydrophobic group contribution are obtained to analyze the interactions of (amino acid)–water and (amino acid)–water–(other solute). Naturally the occurrence of groups such as OH in serine or threonine can be incorporate in expressions of the same type as eq. 2.11.

Chalikian et al. (1993) also showed that the partial molar adiabatic compressibility K_s^0, depends on the number of methylene groups, CH_2, present in the molecule. The hydrated methylene groups of substances which belong to classes with different structures contribute equally to K_s^0. These contributions are negative at low temperatures and positive at temperatures above 30 º C. A negative value of K_s^0 at low temperatures means that the water in the vicinity of hydration of the CH_2 group is less compressible than the bulk water. Rather, at high temperature bulk water compressibility is less than the hydration shell of the methylene group.

2.6 Conclusions

The amino acid industry has grown to its present size as a result of technical innovations in manufacturing methods and in applications. Questions about the physical principles that control the biological mechanisms of association and the organization and stability of biological structures led to the rapid development of various techniques of analyzing intermolecular forces.

An extensive literature search on the available density and speed of sound data and experimental methods was essential to establish the systematic experimental program, and to choose the most appropriated techniques to measure the density and the speed of sound of the amino acids in aqueous solutions containing electrolytes.

Some aqueous salt solutions with relevance in biochemical studies have been identified in lacking enough experimental thermodynamic data. Furthermore, the available data are scarce, in terms of the number of studied systems, and some rather old leaving doubts about their quality. So, contributing to the very recent efforts within the scientific community to extend the experimental database already available, systems containing ammonium sulfate were chosen to perform new measurements.

Therefore, in this work measurements of density and speed of sound in aqueous solutions of ammonium sulfate containing the amino acid glycine or alanine will be performed, at five different temperatures. These data will allow the interpretation of the physical-chemical of those solutions, using hydration numbers and transfer volumes, among others. The experimental information obtained will also be combined with that from the open literature in order to compare and understand the forces that rule biologically important structures.

3. Experimental Results

3.1 Introduction

The development of more sophisticated and efficient processes for separation, concentration and purification of valuable biomolecules such as peptides and proteins have been a subject of main interest for the biochemical industry. Thermodynamic studies of these compounds are of great interest to biochemist and biotechnologists, and are needed for increasing the understanding of their phase equilibria and separation behavior.

The extensive literature search on the available density and speed of sound data and experimental methods (Chapter 2), was fundamental to establish a systematic experimental program and to implement the most appropriated techniques to determine the density and the speed of sound of the amino acids in aqueous solutions containing electrolytes.

In this chapter, details of the experimental techniques, the analytical methods, the procedure, and the experimental data are given, as well as a critical analysis of the obtained data. Finally, some conclusions are drawn concerning this experimental study. The experimental results are presented divided into two parts: density of the different amino acids in pure water and in aqueous electrolyte solutions.

Density and speed of sound data was measured in a temperature range between 278.15 K and 318.15 K, at ambient pressure, and without pH adjustment. In this study the amino acids used were alanine and glycine, because they differ in one methyl group. The salt chosen was ammonium sulfate because it is one of the mostly used for protein precipitation and curiously hardly ever studied (Yu and Ito, 2004), and a better knowledge of its interactions with different amino acid functional groups is certainly an interesting point for further studies on protein purification and precipitation. This project was carried out at in the laboratories of the Institute of Chemical Technology - Prague.

3.2 Experimental Methodology

In the following sections detailed information is given about the experimental procedure. The apparatus chosen to perform the density and speed of sound measurements was a digital density and sound velocity meter. This apparatus uses the oscillating U-tube methodology and one sound-cell to measure both properties simultaneously. This has been the apparatus most generally presented in the literature to determine the density and the speed of sound since it is simple, accurate and presents generally high reproducibility.

3.2.1 Substances

Glycine (99.7% purity) and DL-alanine (99% purity) were purchased from Merck, kept at room temperature, and were used without further purification. Ammonium sulfate [$(NH_4)_2SO_4$, ≥ 99% purity] were obtained from Sigma Aldrich and was used without further purification. However, it was oven-dried (T = 378.15 K) during at least 24h and used after cooling at room temperature. The distilled water was cleaned in a milli-Q ultra-pure water system from Millipore and was degassed before use by boiling for 60 minutes to avoid micro air bubbles in the solutions.

3.2.2 Equipment Description

The density and the speed of sound measurements in aqueous ammonium sulfate solutions containing amino acids were performed by the Density and Sound Velocity Analyzer DSA 5000 (Anton Paar, Graz, Austria), Figure 3.1.

A vibrating-tube densimeter with glass U-shaped tube and an additional cell (sound-cell) for measurements of speed of sound are the main parts of the instrument. The apparatus is equipped with a built-in thermostat allowing measurement in the temperature range from 0 to 70 °C, at ambient pressure, and with a sample changer which allows automatic loading up to 24 samples. The measurement uncertainty is stated for both properties as 0.01% to 0.1%, with repeatabilities of 0.001 $kg \cdot m^{-3}$ in the density, and 0.1 $m \cdot s^{-1}$ in the speed of sound measurement, and 0.001 ºC for temperature. The principle of measurement by a vibrating-tube densimeter is based on the fact that the frequency (period) of oscillations of a U-shaped tube fixed in a heavy base depends

on the total mass of the tube. It means that the frequency is a function of density of the fluid inside the tube.

Figure 3.1. Experimental apparatus: density and sound velocity meter DSA 5000 from Anton Paar.

Schematically, in Figure 3.2 it is shown that continuous oscillations of the tube are maintained by means of a driver D which electromagnetically forces the tube to oscillate and which is controlled by an electronics E. At the same time the electronics reprocesses the alternated electric signal which is produced by the moving tube in a pick-up device P operating on either photoelectric or electromagnetic principle. In other words, the vibrating-tube densimeter is the oscillator the frequency (period) of which is determined by the mechanical element – the U-tube. The oscillation period is measured by an electronic counter C.

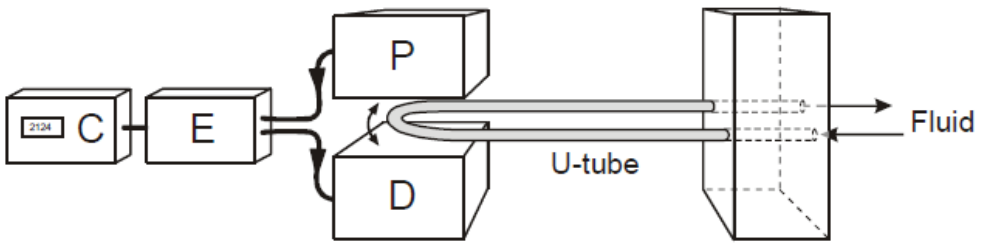

Figure 3.2. Schematic representation of DSA 5000.

Assuming the harmonic oscillator model, a simple relation between density, ρ, and oscillation period, τ, can be derived,

$$\rho = A\tau^2 + B \tag{3.1}$$

where *A* and *B* are coefficients (constants for given temperature and pressure), which are related to the mechanical and geometrical properties of the U-tube. The values of these coefficients must be determined by a calibration procedure.

The calibration of the instrument is based on the measurement of oscillation periods for two fluids with known densities. Most frequently, the fluids are water and air. Density of liquid water (as a function of temperature), density of dry air (as a function of temperature and pressure) and speed of sound in liquid water (as a function of temperature) are stored in an internal memory of the instrument. All values required for the calibration are automatically loaded from the memory according to the set temperature and atmospheric pressure. Calibration is always performed at 20 °C. The U-tube is cleaned, dried and filled with air and then with water. During this procedure all necessary data are acquired and results of the calibration (coefficients *A* and *B*, and data for speed of sound calibration) are stored in the instrument internal memory until the next calibration is performed. For measurement at other temperatures, all calibration parameters are automatically recalculated.

The following apparatus and instruments were used too in this study: electric oven supplied by Binder, ultrasonic bath, Precisa 40SM-200A balance (resolution = 10^{-2} mg, uncertainty = ±0.1 mg), Precisa 2200C SCS balance (resolution = 10 mg, estimated uncertainty = ±2.10^{-2} %), stir plate with 12 positions, milli-Q ultra-pure water system and a degassing system.

3.2.3 Procedure

The following section gives information about the solutions preparation and the procedure to performance the measurements of density and speed of sound of aqueous ammonium sulfate solutions containing amino acids.

3.2.3.1 Solutions Preparation

All the solutions were made on weight basis and prepared using degassed water or, at least, partially degassed, otherwise bubbles of air can form in the liquid at elevated temperatures. The materials were weighed on a Precisa 40SM-200A balance and on a Precisa 2200C SCS balance. The solutions were prepared in standard flasks (1000 cm^3 and 2000 cm^3 for ammonium sulfate solutions). The flasks and all the material used were cleaned with ultra-pure water and after that were dried in oven or at room temperature. Magnetic stirrers are used in the mixing process.

Firstly the ammonium sulfate solutions were prepared using the Precisa 2200C SCS balance. The weight of a cleaned and dried flask of 2000 cm^3 was noted and the balance tared; the required amount of the ammonium sulfate was added into it and the exact weighed of the solute noted. Balance was tared again and the readjusted amount of degassed water was then added and its exact weight was noted. The molality of the resulting solution was calculated. The solution was placed on a stir plate until complete dissolution of the ammonium sulfate. Ammonium sulfate solutions were prepared at the molalities of 0.1, 0.3, 0.7, 1.0, 1.3 and 2.0.

For the preparation of ternary solutions the cleaned and dried flasks of 1000 cm^3 were placed in the Precisa 2200C SCS balance. The weight was noted and the balance tared. The required amount of the amino acid was weighed in Precisa 40SM-200A balance, to obtain a higher accuracy, for difference between the weight of the goblet with the amino acid and the weight after dumping it in to the flask. Readjusted amount of ammonium sulfate solutions was then added and the total weight noted. The molality of the resulting solution was calculated. The solution is stirred to promote complete mixing. In binary systems ammonium sulfate solution is replaced by degassed water. The molality of amino acid varies between 0.05 and 0.5.

It is necessary to be very careful with the evaporation in solutions of ammonium sulfate, keeping bottles well capped.

3.2.3.2 Experimental Procedure

The prepared mixtures are placed in an ultrasonic bath for 10 minutes and filled into glass vials equipped with caps and silicone septa. Before that, the sample vials are

washed several times with the solution or water. These vials, together with vials containing pure solvent and/or pure water, are inserted into the sample changer in series. In Figure 3.3 is displayed one example of the distribution of the vials for glycine in aqueous solutions of 0.3 and 0.7 mol·kg^{-1} ammonium sulfate.

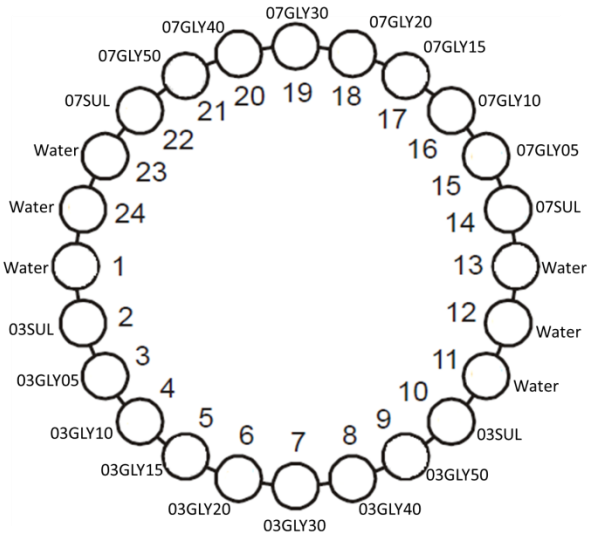

Figure 3.3. Distribution of the vials in carousel for 0.3 and 0.7 mol·kg^{-1} ammonium sulfate containing glycine (XXGLYZZ where XX is salt molality and ZZ is amino acid molality).

Before start the measurements, the instrument is switched on for about 30 minutes. Then if necessary, calibrate of the instrument according to the following procedure and dry the measuring unit.

1. Fill a glass vial with acetone (approximately half of its volume) and put it into the sample changer (carousel).

2. On the sample changer, press the <START> button. The feeder will start filling the measuring cell.

3. When the filling is finished, press the <STOP> and then <0> button, and remove the vial from the sample changer.

4. Attach the hose leading from the air pump to the lower end of the filling needle.

5. By turning in a counterclockwise direction, release the handle of the peristaltic pump.

6. Press the <PUMP> button on the front panel of the DSA 5000. The air pump will blow air through the measuring cell and after 15 min will stop automatically. During pumping “Pump” flashes on the instrument display.

7. After the cell is dry, wait until the display shows current density of air (its value should be close to 0.0011 $g \cdot cm^{-3}$).

8. Activate the calibration procedure using keys and menus: {Menu} [↲]; {adjustment} [↲]; {adjust} [↲]; {d+vos(air,water)} [↲]; {OK}.

9. Enter current atmospheric pressure in mbar.

10. Wait until temperature is stabilized (“valid“ is displayed as the condition) and then press the {OK} soft key.

11. Put a vial with demineralized water into the sample changer. Disconnect the air hose from the filling needle, return back the handle of the peristaltic pump and press the <START> button on the filling unit. The measuring cell will be filled with water. Caution: Leave the air hose on the left side of the instrument; otherwise it could get into the mechanical gear of the sample changer.

12. Wait until temperature is reached and then press the {OK} key for measurement of the oscillation period.

13. Save the result of calibration by the {Save} soft key. Choose {NO} as response to Print?

14. Finish the calibration procedure by the {OK} soft key.

After the calibration procedure is necessary to check the validity of the calibration by the “water check” function throwing the following procedure:

1. Put a vial with demineralized water into the sample changer.

2. Activate the “water check” procedure using {Menu} and keys: {adjustment} [↲]; {watercheck} [↲]; {start watercheck}[↲].

3. Press the <START> button on the filling unit. After filling the cell wait until temperature is reached.

4. If the measurement of density and speed of sound is within the permitted range the display shows “water check: OK”. Save the result by the [Save] soft key. Choose [NO] as response to Print?

5. Terminate the procedure by pressing the <STOP> and <0> buttons on the filling unit.

Densities and speed of sound of the mixtures are then measured by the instrument DSA 5000 at temperatures between 278.15 and 318.15 K according to the following procedure.

1. Put the empty sample changer to its initial position by pressing the <STOP> and <0> buttons.

2. Insert the vials with samples into the sample changer.

3. Use soft keys and the Enter key [↲] and from the instrument {Menu} on its display select the following: {measurements settings} [↲]; {general settings} [↲]; {temp scan} [↲]; {off} [↲]. Terminate the setting by the {Esc} soft key. Response {Yes} to the question Save changes?

4. Return to the primary menu and set the experimental temperature {Menu} [↲]; {temperature setting} [↲]; {set temperature °C} [↲]; [↲]. Using the soft keys {Left, Right} and arrows {Up, Down} set the desired temperature. Terminate the setting by the {Esc} soft key. Response {Yes} to the question Save changes?

5. Finish the selection by the {Esc} soft key.

6. Press the <START> button on the sample changer to start measurements.

7. When the measurement cycle is finished and the sample changer returns to the initial position, press the <STOP> button. Transfer the collected experimental data stored in the instrument memory into the PC by activating the memory read-out program installed on the PC.

This procedure is isothermal because different compositions are measured at the same temperature. The measurements of pure water serve for the check of a possible instrument drift. Values measured for water should not differ significantly (within few units of the order 10^{-6} g·cm^{-3}).

During the experimental work another type of procedure was used in which the solutions were injected continuously into the lower opening of the U-shaped vibrating tube, until the excess liquid flowed out of the upper part. Liquid was filled in the tube very slowly to enable a liquid to properly wet the walls of the sample tube. Care was taken to avoid trapping of micro air bubbles. Disposable, polyethylene syringe of about 20 cm^3 in volume was used for injecting the liquid into the U-tube. In the apparatus was used the option "temperature scan" in which the temperatures are measured sequentially without replacing the sample. This procedure is phetostatic because different temperatures are measured at the same composition.

To prevent air-humidity condensation on densimeter parts when cooled down to low temperatures, 278.15 and 288.15 K, the nitrogen at low flow rate was introduced into the interior of the apparatus cover box.

pH measurements were also carried out during the measurements, using a digital pH meter PHI 04, in order to evaluate possible chemical phenomena in the solutions.

3.3 Experimental Measured Data

The data of measured density are presented in appendix D and the calculated partial molar volumes are reported in the following sections. Adiabatic compressibilities are not shown for reasons explained in section 3.4. However, the speed of sound data is compiled in Appendix E. The studied temperature range is between 278.15 and 318.15 K. The results are divided in binary and ternary aqueous amino acid systems.

3.3.1 Water/Amino Acids Systems

The measured values of the density of AA solutions, between 278.15 and 318.15 K, expressed in grams per cubic centimeter, are reported in Table D.1. Concerning that table is possible to observe that, for both amino acids the density decreases with the increasing temperature and, at the same temperature and molality, the density of the

glycine solutions is larger than alanine solutions. Increasing the concentration of amino acid, the density increases too. It is important to refer that, the values of the density for all solutions are the result of an average of three independent measurements. Through density measured values, partial molar volume ($\mathbf{V^0_{\emptyset,this\ work}}$), expressed in cubic centimeters per mole, of pure solutions were calculated using equations 2.1 and 2.2. These values are reported in Table 3.1 and presented in the Figure 3.4. In the case of partial molar volume, it increases with temperature for both amino acids and, at the same temperature the partial molar volume of alanine is, as expected, bigger than that of glycine. The trend is similar like show in Figure 3.4.

Table 3.1. Comparison of the partial molar volumes of amino acids in pure water.

Amino Acid	Temperature (K)	$V^0_\emptyset$ (Zhao, 2006) ($cm^3 \cdot mol^{-1}$)	$V^0_{\emptyset,average}$ ($cm^3 \cdot mol^{-1}$)	$V^0_{\emptyset,this\ work}$ ($cm^3 \cdot mol^{-1}$)	$\Delta V^0_\emptyset / V^0_{\emptyset,average}$ (%)
Glycine	278.15	41.1, 41.9, 41.07, 41.25	41.33	41.18	-0.36
	288.15	42.3, 42.5, 42.6, 42.29, 42.54, 42.4, 42.35, 42.48, 42.37	42.41	42.38	-0.07
	298.15	42.9, 43.5, 43.19, 43.2, 43.25, 43.3, 43.18, 42.89, 43.16, 43.14, 43.26, 43.217, 43.199, 43.15, 42.54, 43.2, 43.27, 43.23, 43.24, 43.3, 43.2, 43.22, 43.62, 42.54, 43.12, 42.48	43.18	43.20	0.04
	308.15	44.2, 43.81, 43.85, 43.8, 44.12, 43.9, 43.98, 41.7, 43.79, 44.95, 43.69, 44.52, 43.87	43.87	43.76	-0.25
	318.15	44, 44.17, 44.15	44.11	44.15	0.09
Alanine	278.15	59.4, 58.64, 58.81	58.95	58.87	-0.14
	288.15	60, 59.73, 60.1, 59.9, 59.89, 59.67, 59.77	59.85	59.79	-0.09
	298.15	60.7, 60.52, 60.3, 60.6, 60.4, 60.23, 60.41, 60.5, 60.53, 60.609, 60.47, 60.36, 60.3, 60.45, 60.47, 60.43, 60.42, 60.19, 60.5, 60.49, 60.71, 60.8, 60.68, 60.74, 60.19, 60.4, 60.35, 60.37, 60.43, 60.92	60.48	60.46	-0.03
	308.15	61.4, 60.96, 61.06, 60.9, 61.27, 60.44, 60.88, 61.01, 61, 61.99, 61.16	61.12	60.93	-0.32
	318.15	61.46, 61.31, 61.46	61.41	61.29	-0.19

$\Delta V^0_\emptyset = V^0_{\emptyset,this\ work} - V^0_{\emptyset,average}$

The amino acids studied have in common an amino and a carboxyl group but their side chain is different. The chemical structure of the amino acids is depicted in Table A.1. Glycine is the simplest amino acid with only two carbon atoms. Alanine has one $–CH_3$ group more than glycine. A comparison of the densities of glycine and alanine shows that the density in water decreases as the size of the hydrocarbon backbone increases. For partial molar volumes, they increase with the size of the hydrocarbon backbone.

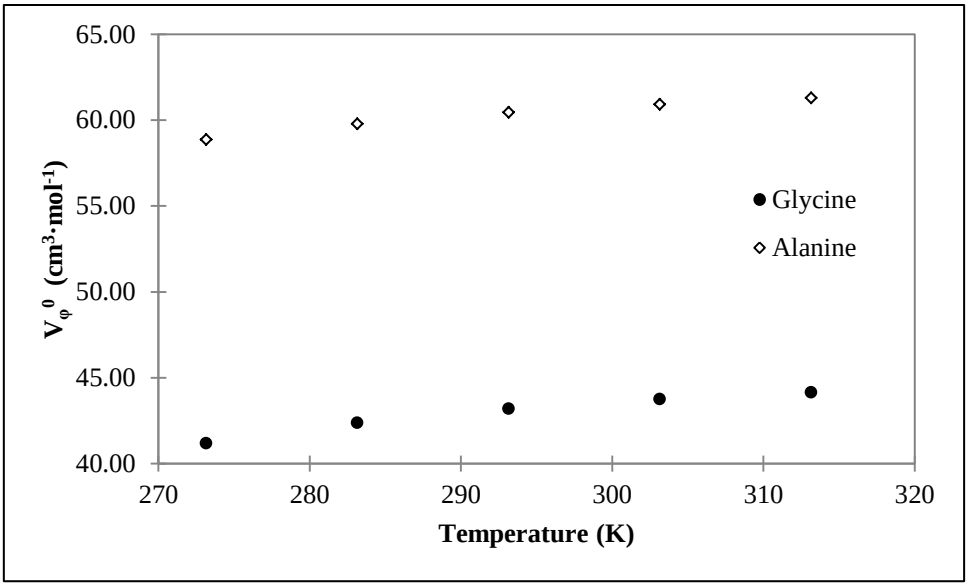

Figure 3.4. Partial molar volumes of amino acids in water a different temperatures.

3.3.2 Ternary Systems

In this section, the experimental measured density data of solutions containing the amino acids, glycine and alanine, in aqueous systems of the electrolyte ammonium sulfate (molality ranging from 0.1 to 2.0) at five temperatures, from 278.15 to 318.15 K, is discussed and are presented in Table D.2. Like in the binary systems, the values of the density are the result of an average of three independent measurements. The partial molar volumes were calculated through density using the equations 2.3 and 2.4 and are shown in Table 3.2 together with the parameter a_v (equation 2.4) and a measure of the linear regression quality (r^2).

Figure 3.5 and 3.6 show the change of partial molar volume at different temperatures for glycine and alanine, respectively. Examining the figures it is possible to observe a more pronounced increase of the glycine and alanine partial molar volumes at low electrolyte molality. Despite the alanine have one more hydrophobic -CH_3 group than glycine the behavior is similar. Comparing the results obtained at five different temperatures, a similar effect of the ammonium sulfate concentration on the partial molar volume of glycine and alanine is found, the partial molar volume increases with temperature increasing. It is important to mention however the unexpected change observed in Figure 3.6 at the salt molality near one. In fact, a small decrease is observed which must be related with some unforeseen experimental deficiency. Due to the lack of time it was not possible to repeat all the measurements at 1.0 molal ammonium sulfate solutions containing alanine, but that will, for sure, be carried out in the near future. Figure 3.6

also demonstrate the difficulty to achieve consistent results as it is needed to approach $\Delta\rho/m_2$ to infinite dilution which is, from a theoretical point of view, a mathematical indetermination.

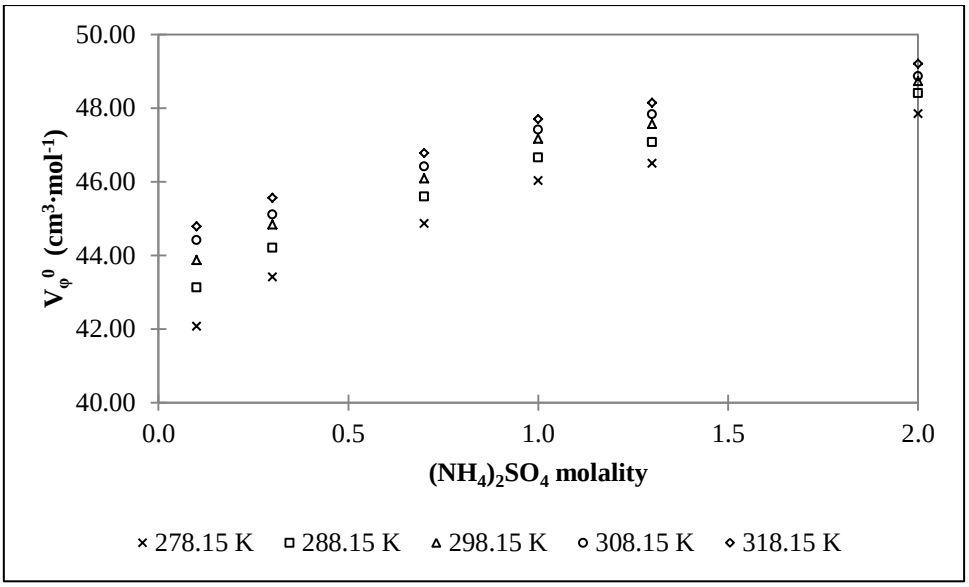

Figure 3.5. Partial molar volumes of glycine in water/$(NH_4)_2SO_4$ solutions, depending on salt molality, at different temperatures.

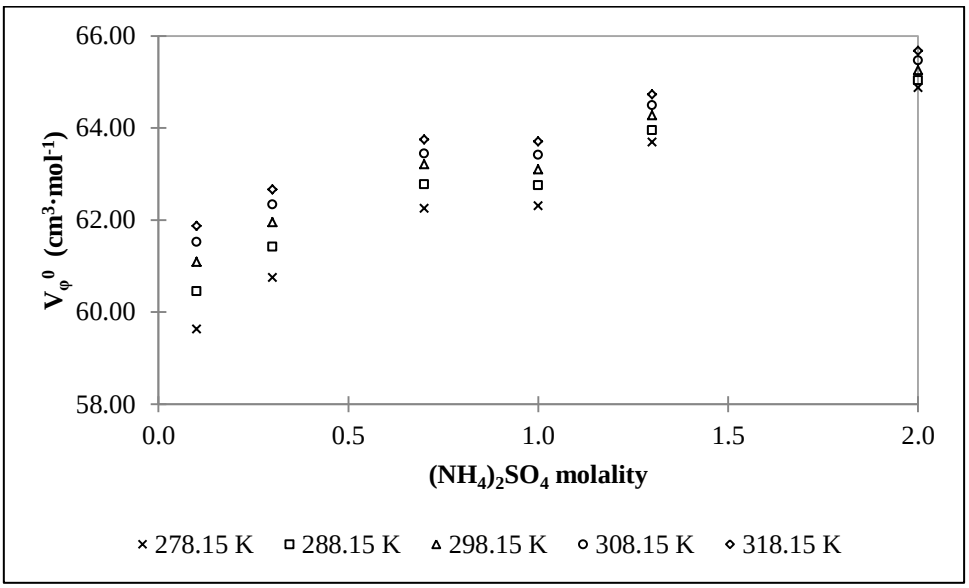

Figure 3.6. Partial molar volumes of alanine in water/$(NH_4)_2SO_4$ solutions, depending on salt molality, at different temperatures.

Table 3.2. Partial molar volume, a_V ($kg^2 \cdot m^{-3} \cdot mol^{-1}$) and r^2 for glycine and alanine in water/ammonium sulfate solutions at different temperatures.

	278.15 K			288.15 K			298.15 K			308.15 K			318.15 K		
Amino acid	**V_φ^0 ($cm^3 \cdot mol^{-1}$)**	**a_V**	**r^2**	**V_φ^0 ($cm^3 \cdot mol^{-1}$)**	**a_V**	**r^2**	**V_φ^0 ($cm^3 \cdot mol^{-1}$)**	**a_V**	**r^2**	**V_φ^0 ($cm^3 \cdot mol^{-1}$)**	**a_V**	**r^2**	**V_φ^0 ($cm^3 \cdot mol^{-1}$)**	**a_V**	**r^2**
0.1 mol·kg^{-1} ammonium sulfate															
Glycine	42.07	32.49	0.9991	43.13	31.44	0.9995	43.87	30.73	0.9992	44.41	30.24	0.9968	44.78	29.92	0.9925
Alanine	59.63	28.83	0.9923	60.45	28.04	0.9942	61.09	27.48	0.9925	61.52	27.16	0.9900	61.87	26.94	0.9915
0.3 mol·kg^{-1} ammonium sulfate															
Glycine	43.41	30.16	0.9844	44.20	29.38	0.9969	44.84	28.79	0.9888	45.11	28.57	0.9626	45.56	28.18	0.9773
Alanine	60.75	26.50	0.9902	61.42	25.88	0.9920	61.95	25.43	0.9932	62.33	25.17	0.9862	62.66	24.99	0.9965
0.7 mol·kg^{-1} ammonium sulfate															
Glycine	44.87	26.85	0.9604	45.59	26.15	0.9617	46.09	25.71	0.9640	46.41	25.46	0.9567	46.77	25.18	0.9294
Alanine	62.25	22.76	0.9899	62.77	22.31	0.9920	63.22	21.98	0.9969	63.44	21.89	0.8607	63.75	21.75	0.9945
1.0 mol·kg^{-1} ammonium sulfate															
Glycine	46.02	24.40	0.9723	46.66	23.81	0.9769	47.17	23.37	0.9230	47.41	23.21	0.9147	47.70	23.01	0.9047
Alanine	62.31	21.18	0.9118	62.75	20.83	0.9064	63.10	20.60	0.9075	63.41	20.44	0.9112	63.71	20.31	0.9157
1.3 mol·kg^{-1} ammonium sulfate															
Glycine	46.50	22.73	0.9300	47.08	22.21	0.9481	47.57	21.79	0.9563	47.83	21.62	0.9394	48.14	21.41	0.9212
Alanine	63.70	18.38	0.9837	63.95	18.24	0.9355	64.28	18.05	0.9543	64.49	18.00	0.9470	64.73	17.94	0.9657
2.0 mol·kg^{-1} ammonium sulfate															
Glycine	47.85	18.91	0.9070	48.40	18.44	0.9177	48.73	18.21	0.7983	48.86	18.19	0.8338	49.20	17.97	0.8017
Alanine	64.88	14.36	0.8182	65.03	14.35	0.9395	65.25	14.29	0.8951	65.47	14.25	0.9450	65.67	14.24	0.9462

In a similar way, Figure 3.7 and 3.8 show the change of the partial molar volume with the temperature, at different molalities of ammonium sulfate either, for glycine and alanine, respectively. At all molalities, for both amino acids, the trend is the same. The partial molar volume increases with electrolyte molality and with temperature.

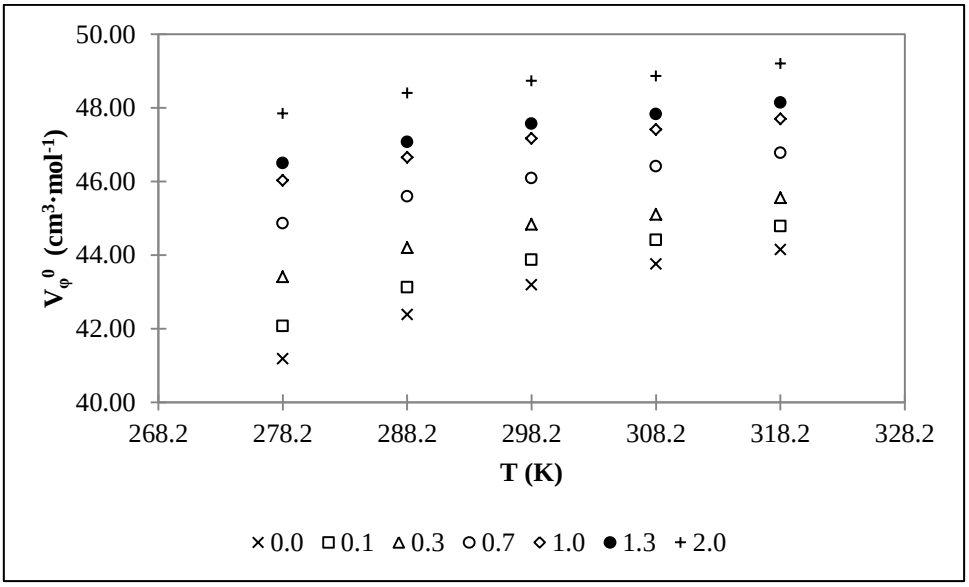

Figure 3.7. Partial molar volumes of glycine in water/$(NH_4)_2SO_4$ solutions, depending on the temperature, at different molalities.

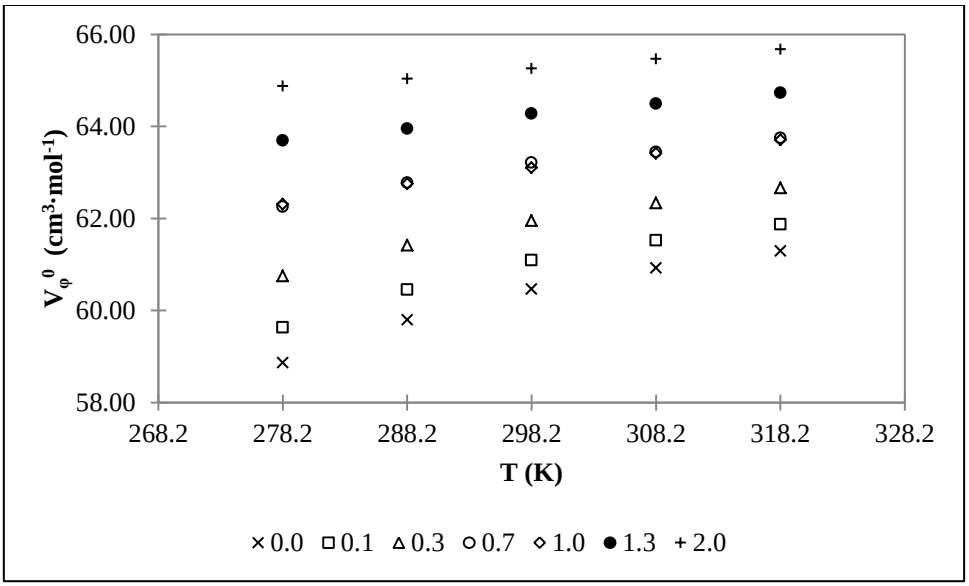

Figure 3.8. Partial molar volumes of alanine in water/$(NH_4)_2SO_4$ solutions, depending on the temperature, at different molalities.

3.4 Critical Analysis of the Results

After an extensive analysis of all data measured and that obtained by other authors it was possible to draw conclusions regarding the substances and procedures, the measurements and the results obtained.

A very small concentration range was used for AA's (ranging up to 0.5 molal). Amino acids are usually recommended in small quantities since it is intended to get close to infinite dilution. At higher concentration non-ideal behavior of solutions would dominate.

During the measurements of the solutions, two different procedures were applied in order to try get better results. The first and most used, isothermal, wherein the solutions were inserted into the sample changer in series and the second, phetostatic, in which the solutions were injected continuously into the lower opening of the densimeter. The data analysis revealed that the second procedure is not valid since the equipment is very sensitive to changes in the pressure exerted on the syringe by the operator. It is also more propitious to bubbles formation and takes much more time.

It is necessary to take into account that possible errors in density data may arise from temperature fluctuations, presence of air bubbles in the solution, insufficient dialysis time, high sample viscosity, high sample concentration, among others. In the measurements carried out in this work the main difficulties were related with bubbles formation and evaporation.

Additionally, in order to evaluate possible chemical phenomena in the solutions, pH measurements were carried out in some solutions. The results are presented in Figure 3.9. The pH values range from 5.17 to 6.12. As the electrical point of glycine and alanine in water are 5.97 and 6.00 (Greenstein and Winitz, 1961), respectively, it can be concluded that the addition of salt does not significantly alter the pH, so it was not possible to identify any chemical processes in solution.

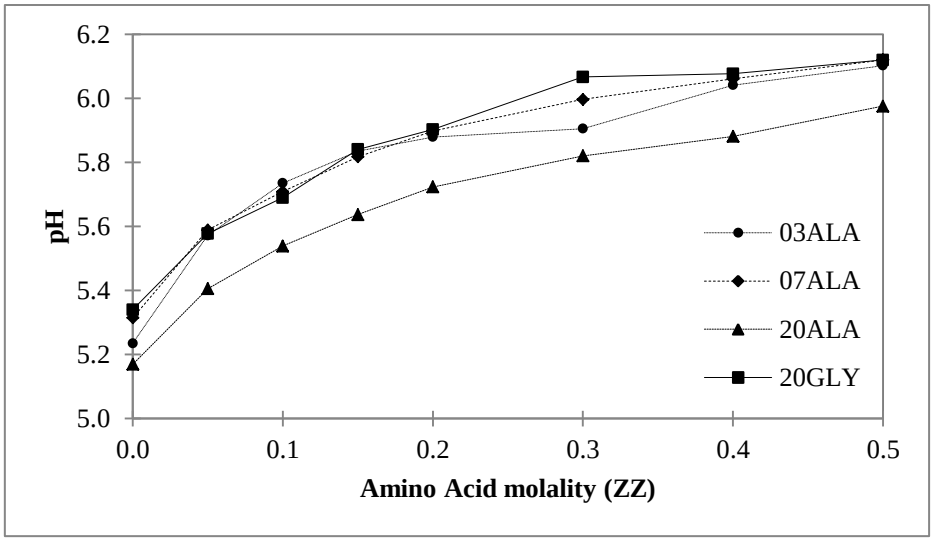

Figure 3.9. pH as function of amino acid and ammonium sulfate molalities (XXAAZZ where XX is salt molality, ZZ is amino acid molality and AA the amino acid).

The partial molar volumes of the amino acids in pure water reported in the literature was reviewed and compared with the new data. Table 3.1 lists the information compiled, the corresponding measured value from this work as well as the deviation to the average. For both amino acids in this study, the deviation to the average is not considerable. For alanine, at higher temperatures, the deviation to the average is more evident, especially at 308.15 K; however the data available in literature is more limited. The same happens for glycine at 278.15 K.

One schematically comparison between the partial molar volume measured in this work and that one from references is shown in Figure 3.10. A highly satisfactory agreement is found.

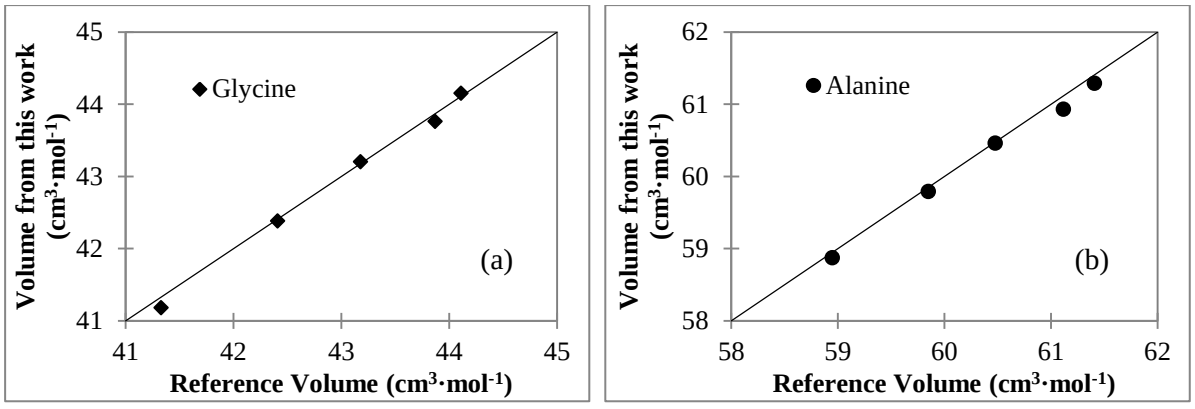

Figure 3.10. Comparison for the partial molar volumes of (a) glycine and (b) alanine in water (reference values from Zhao, 2006).

In conclusion, generally, for binary systems (water + amino acid), a very satisfactory agreement is found; with the few exceptions previously mentioned. For both amino acids the number of data available in the literature decreases at temperatures above and below 298.15 K and some are rather old. In those cases, the chemical purity and accuracy of the experimental technique must have special awareness.

During the experimental work the density of ammonium sulfate solutions at different temperatures and molalities was measured several times, as shown in Appendix F. However in the data analysis were used the mean values, Table 3.3.

Table 3.3. Ammonium sulfate densities ($kg \cdot m^{-3}$) at different temperatures and molalities.

	T (K)				
$m_{[(NH_4)_2SO_4]}$	**278.15**	**288.15**	**298.15**	**308.15**	**318.15**
0.1	1.008181	1.007059	1.004848	1.001746	0.997886
0.3	1.023476	1.021948	1.019470	1.016209	1.012266
0.7	1.051323	1.049183	1.046298	1.042798	1.038728
1.0	1.070293	1.067792	1.064697	1.061062	1.056925
1.3	1.087949	1.085179	1.081890	1.078139	1.073974
2.0	1.124496	1.121319	1.117777	1.113895	1.109681

In Figure 3.11 and 3.12 four typical examples of $\Delta\rho/m_2$ in function of the molality of the amino acid at five different temperatures are represented. Through this figures it is possible to calculate the a_v values required for calculating the partial molar volume. Based on the coefficient of determination (r^2), it can be concluded that by increasing the salt concentration the quality of data worsen. The same applies, generally, to the temperatures 278.15 and 318.15 K, since it is more difficult to stabilize properly the temperature in the densimeter.

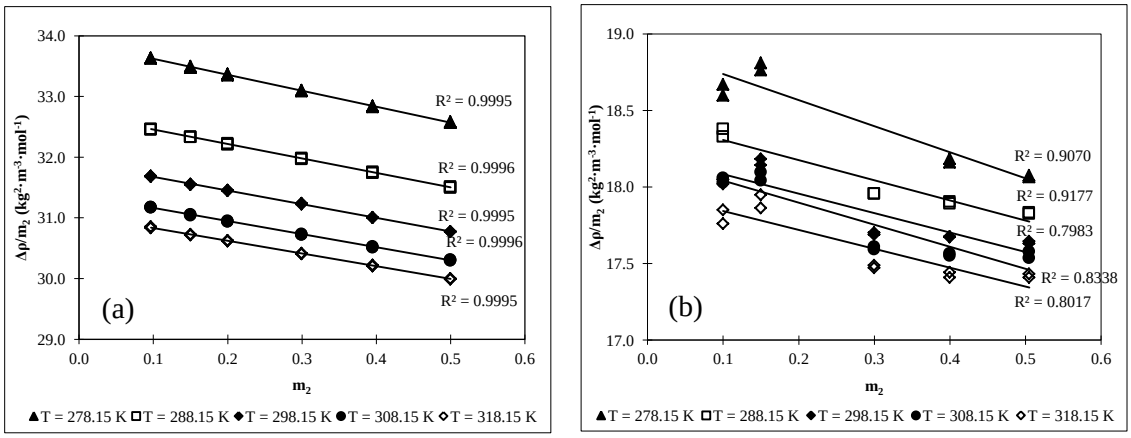

Figure 3.11. $\Delta\rho/m_2$ of glycine at different temperatures and molalities in: (a) water and (b) 2.0 ammonium sulfate.

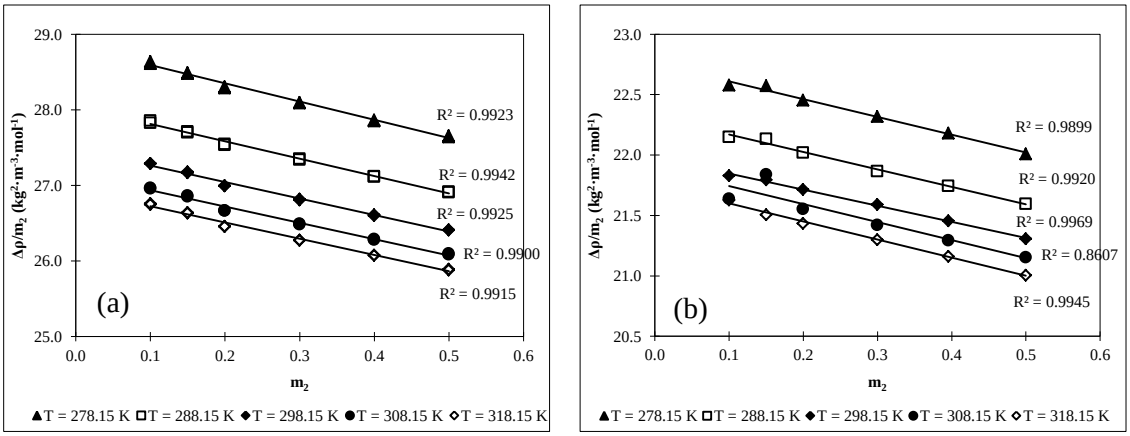

Figure 3.12. $\Delta\rho/m_2$ of alanine at different temperatures and molalities in: (a) 0.1 ammonium sulfate and (b) 0.7 ammonium sulfate.

It is interesting to note that in the representation of density values as a function of the molality of the amino acid the fit obtained is always almost perfect. However, and in accordance to equations 2.2 and 2.4, in the representation of $\Delta\rho/m_2$ as a function of the molality the trend obtained worsens, being even necessary to remove some experimental points, as shown in Figure 3.13 and 3.14. It must be mentioned that, for all systems and conditions, the density value measured for AA molality 0.05 was not considered in the calculations. However, the situation show in Figure 3.14 is an unique case where at 278.15 K was necessary to eliminate the density measured at AA molalities of 0.10, 0.15 and 0.30.

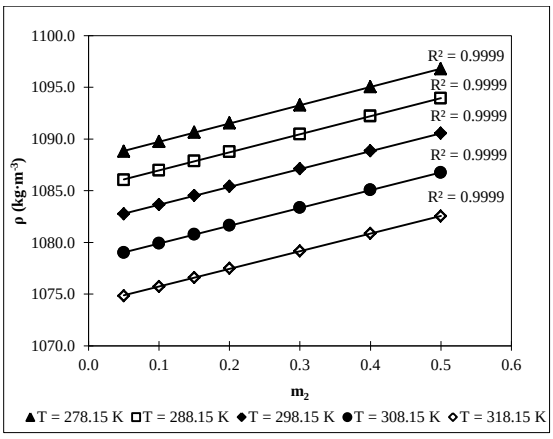

Figure 3.13. ρ of alanine in 1.3 ammonium sulfate at different temperatures and molalities.

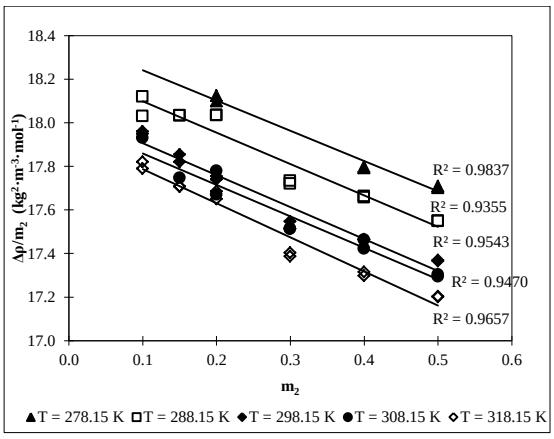

Figure 3.14. $\Delta\rho/m_2$ for alanine aqueous solutions in 1.3 ammonium sulfate molality at different temperatures.

The present study observed higher V_ϕ^0 values for amino acids in ammonium sulfate as compared with their values in water, which suggests that ion-ion interactions dominate the ion-hydrophobic and hydrophobic-hydrophobic interactions. It is also found that V_ϕ^0 increases with increase in size of the alkyl chain of the amino acid.

The values of the speed of sound were also measured for all solutions at all temperatures, as mentioned above, and are presented in Appendix E even if their quality does not allow the calculation of reliable compressibilities. Nevertheless this situation can be accepted as normal since in the majority of the works researchers need to make nine measurements to obtain one single valid point with very large standard deviation. In this study, this procedure was impossible due to time constraints. In the Appendix G one example of the procedure for calculating the adiabatic compressibilities through the values of speed of sound is shown, even if none of those values are explored after.

3.5 Conclusions

The density and speed of sound in solutions containing the amino acids glycine and alanine have been measured in water and in the system water/electrolyte [$(NH_4)_2SO_4$] in the temperature range 278.15 K - 318.15 K.

Concerning the measurement of densities and speed of sound in water and in aqueous ammonium sulfate solutions containing amino acids one density and sound velocity analyzer was used and two procedures were applied. The experimental technique in which the solutions were injected continuously into the lower opening of the densimeter proved to be less accurate.

The experimental results were compared with literature data, when possible. Generally, the experimental results are in good agreement with the published data.

The new experimental data and all the experimental information concerning different thermodynamic properties, compiled from the open literature, allowed the establishment of a more extensive and reliable database, that is very important and crucial for the improvement and development of the thermodynamic analysis. In the following chapter some analysis derived from theoretical models will be presented.

4. Theoretical Analysis

4.1 Introduction

In the following sections results are analyzed through the co-sphere overlap model, the hydration number, temperature effects, and group contribution approach. The solute-solute and solute-solvent interactions are also discussed. The structure of the amino acids support the results found. The data of the system alanine in ammonium sulfate 1.0 molal is not included in the analysis for reasons explained before.

4.2 Co-sphere Overlap Model

The co-sphere overlap model can be utilized to rationalize the standard partial volumes of transfer in terms of solute-cosolute interactions. Since $V^0_{\emptyset,tr}$ is, by definition, free of solute–solute interactions, it provides information regarding solute–solvent interactions. The $V^0_{\emptyset,tr}$ values were calculated through equation 2.10, and are illustrated in Figure 4.1 and presented in Table 4.1.

Interaction coefficients have also been calculated on the basis of formalism proposed by McMillan and Mayer (1945), which permits the formal separation of the effects due to interactions between the pairs of solute molecules and those due to its interactions between two or more solute molecules. According to this theory, the transfer parameter $V^0_{\emptyset,tr}$ can be expressed as:

$$V^0_{\emptyset,tr} = 2V_{XY}m_3 + 3V_{XYY}m_3^2 + \cdots \quad (4.1)$$

where X stands for amino acids and Y stands for the cosolute. The V_{XY} and V_{XYY} are the pair and triplet interaction coefficients from volumetric data which are given in Table 4.2.

As can be seen in Figure 4.1, the values of $V^0_{\emptyset,tr}$ for the both amino acids increase monotonically with the molality of ammonium sulfate, indicating that high ionic strength dehydrates amino acids, and decreases with temperature. An exception occurs for glycine at 0.3 molal of ammonium sulfate solution. In fact, the change with temperature is not monotonic, but is easy to realize that for low electrolyte molalities $V^0_{\emptyset,tr}$ lends to a *plateau* when increasing the temperature. Therefore, it can be within the

uncertainly of the calculated values. It is also possible to state that standard partial volumes of transfer are higher in glycine that in alanine. The trend is more evident when the electrolyte molality is higher.

Table 4.1. Standard partial volumes of transfer, $V^0_{\emptyset,tr}$ /cm^3·mol^{-1}, for the amino acids from water to different ammonium sulfate solutions at various temperatures.

Molality	**0.1**	**0.3**	**0.7**	**1.0**	**1.3**	**2.0**
T (K)			**Glycine**			
278.15	0.89	2.23	3.69	4.84	5.32	6.67
288.15	0.74	1.82	3.21	4.27	4.69	6.02
298.15	0.68	1.64	2.89	3.97	4.37	5.53
308.15	0.65	1.35	2.65	3.65	4.07	5.10
318.15	0.64	1.41	2.62	3.55	3.99	5.05
T (K)			**Alanine**			
278.15	0.76	1.88	3.38	-	4.83	6.01
288.15	0.66	1.62	2.98	-	4.16	5.24
298.15	0.63	1.49	2.75	-	3.82	4.79
308.15	0.59	1.41	2.51	-	3.56	4.54
318.15	0.58	1.37	2.45	-	3.44	4.38

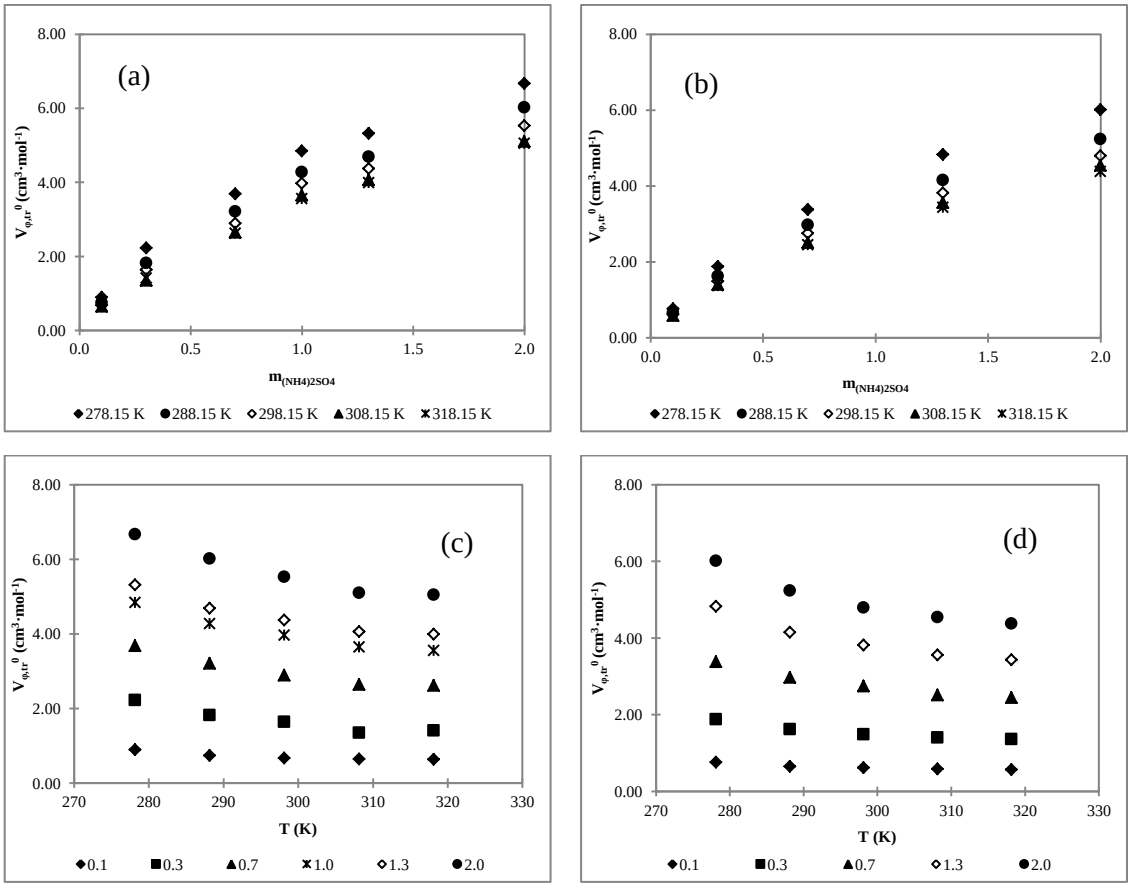

Figure 4.1. Standard partial volumes of transfer at different molalities of ammonium sulfate solution and temperatures in (a), (c) glycine and (b), (d) alanine.

Table 4.2. Pair and triplet interactions coefficients, VXY, and VXYY, of glycine and alanine in aqueous ammonium sulfate solutions.

	Glycine		Alanine	
T (K)	V_{XY} ($cm^3 \cdot kg \cdot mol^{-2}$)	V_{XYY} ($cm^3 \cdot kg^2 \cdot mol^{-3}$)	V_{XY} ($cm^3 \cdot kg \cdot mol^{-2}$)	V_{XYY} ($cm^3 \cdot kg^2 \cdot mol^{-2}$)
278.15	3.1773	-0.5139	2.8551	-0.4589
288.15	2.7251	-0.4148	2.4752	-0.3965
298.15	2.5089	-0.3812	2.2891	-0.3712
308.15	2.2824	-0.3392	2.0981	-0.3279
318.15	2.2447	-0.3317	2.0418	-0.3227

Applying the McMillan and Mayer formalism to the data measured in this work, it was always possible to find good correlation results ($r^2 > 0.982$). The V_{XY} parameter is always positive, showing consistency with the previous analysis since V_{XY} follows glycine > alanine. V_{XYY} is always negative for the both amino acids studied. The higher V_{XY} values than V_{XYY} show that interactions between ammonium sulfate and amino acids (glycine and alanine) are mainly pair wise, even if the magnitude of the triple interaction can not be neglected.

4.3 Hydration Number

The hydration numbers, n_H, which reveal the hydration degree of a solute in water, were calculated through equation 2.9 and are presented in the Table 4.3 and Figure 4.2. The intrinsic volume was calculated using the method of converting the densities of dry amino acids.

Table 4.3. Hydration numbers for the amino acids at different molalities of ammonium sulfate solution at several temperatures.

Molality	0.0	0.1	0.3	0.7	1.0	1.3	2.0
T (K)				Glycine			
278.15	4.11	3.77	3.25	2.69	2.25	2.06	1.54
288.15	3.27	3.01	2.64	2.16	1.80	1.65	1.19
298.15	2.63	2.42	2.13	1.75	1.42	1.30	0.95
308.15	2.03	1.86	1.69	1.36	1.11	1.01	0.75
318.15	1.59	1.46	1.30	1.05	0.86	0.77	0.55
T (K)				Alanine			
278.15	4.95	4.66	4.23	3.65	-	3.10	2.64
288.15	4.12	3.90	3.56	3.10	-	2.69	2.32
298.15	3.42	3.23	2.97	2.59	-	2.26	1.97
308.15	2.71	2.56	2.35	2.08	-	1.82	1.57
318.15	2.16	2.04	1.87	1.65	-	1.45	1.25

The hydration number increases with the size of the amino acid (alanine > glycine) in water or in salt solutions, which is not in accordance with the results from Wadi and Ramasami (1997) that studying the effect of sodium sulfate in the hydration of glycine and alanine. The hydration number decreases with the increasing of concentration of salt and temperature, which is a very expected result in agreement with most theories. The calculated values for the number of hydration show larger differences at low temperatures and low concentrations of ammonium sulfate.

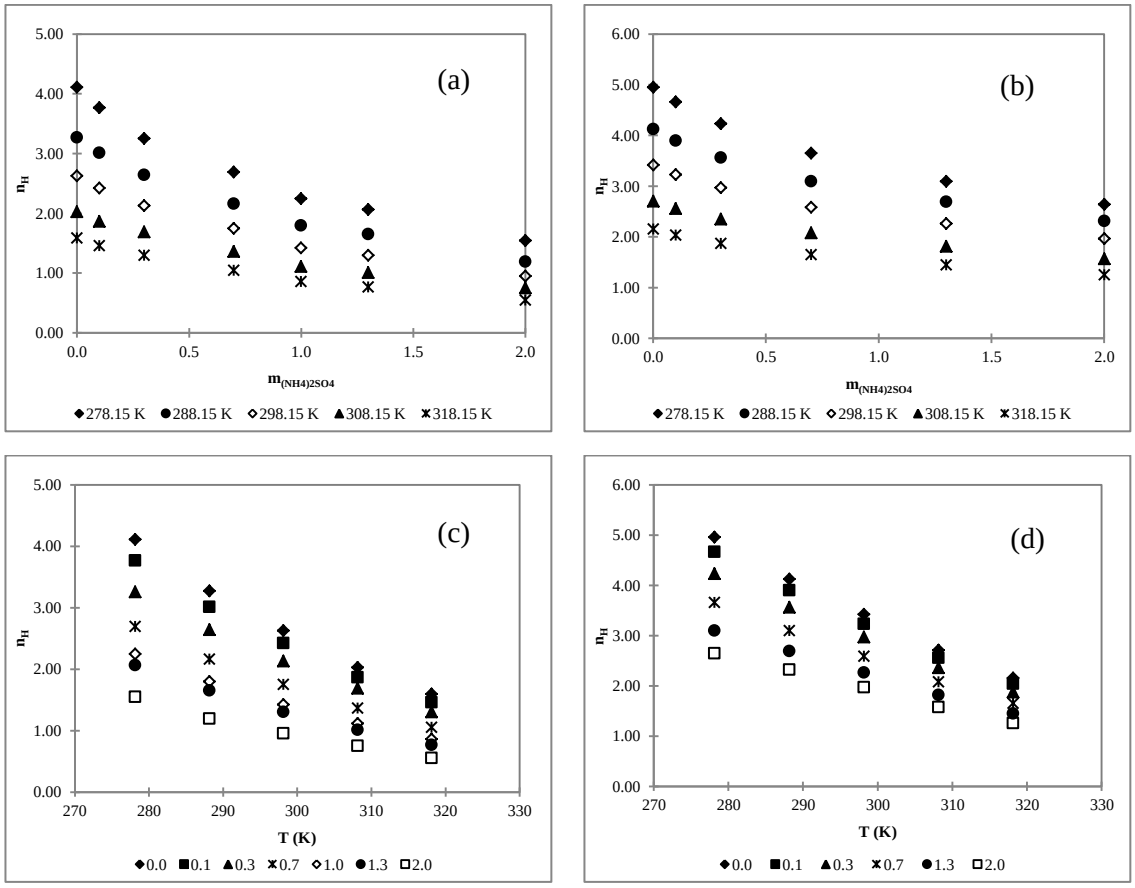

Figure 4.2. Hydration numbers, for different molalities of ammonium sulfate solution at various temperatures in (a), (c) glycine and (b), (d) alanine.

4.4 Temperature Effects

In this work the variation of V_{ϕ}^{0} with temperature can be expressed as:

$$V_{\phi}^{0} = a + bT + cT^{2} \tag{4.2}$$

where T is the temperature in Kelvin, and a, b, and c are constants.

The partial molar expansibility at infinite dilution can be obtained by differentiating equation (4.2) with respect to temperature:

$$(\partial V_{\emptyset}^{0}/\partial T)_P = b + 2cT \quad (4.3)$$

and the derivative from the partial molar expansibility at infinite dilution in order to temperature is given by:

$$(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P = 2c \quad (4.4)$$

The $(\partial V_{\emptyset}^{0}/\partial T)_P$ and $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$ values for both the amino acids at different temperatures and salt molalities were determined using equations 4.3 and 4.4. The determination coefficient (r^2) is always higher that 0.9797.

Table 4.4. Constants a, b and c of glycine and alanine in aqueous ammonium sulfate solutions at different temperatures.

$m_{(NH4)2SO4}$	0.0	0.1	0.3	0.7	1.0	1.3	2.0
Glycine							
a	-1.342E-03	-1.120E-03	-7.512E-04	-6.456E-04	-6.830E-04	-5.437E-04	-4.445E-04
b	8.736E-01	7.350E-01	5.001E-01	4.312E-01	4.483E-01	3.646E-01	2.966E-01
c	-9.792E+01	-7.568E+01	-3.756E+01	-2.510E+01	-2.582E+01	-1.283E+01	-2.263E-01
Alanine							
a	-9.462E-04	-8.194E-04	-5.968E-04	-4.631E-04	-	-1.054E-04	6.651E-05
b	6.241E-01	5.441E-01	4.034E-01	3.128E-01	-	8.888E-02	-1.938E-02
c	-4.150E+01	-2.831E+01	-5.271E+00	1.109E+01	-	4.712E+01	6.511E+01

Analyzing Figure 4.3 it may be noted that the values of $(\partial V_{\emptyset}^{0}/\partial T)_P$ of glycine and alanine in aqueous ammonium sulfate solutions decrease regularly with rising temperature, except for alanine in 2.0 molal of ammonium sulfate where the expansibilities increase regularly with temperature showing a change on the behavior of the solute in solution. The effect of concentration of ammonium sulfate on $(\partial V_{\emptyset}^{0}/\partial T)_P$ values does not follow a well defined trend. From the Figure 4.4 it is possible to verify that the values of $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$ increase with increasing aqueous ammonium sulfate solutions molality except for glycine at 1.0 molal. The $(\partial V_{\emptyset}^{0}/\partial T)_P$ is higher for glycine than for alanine, although for $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$ happens the opposite.

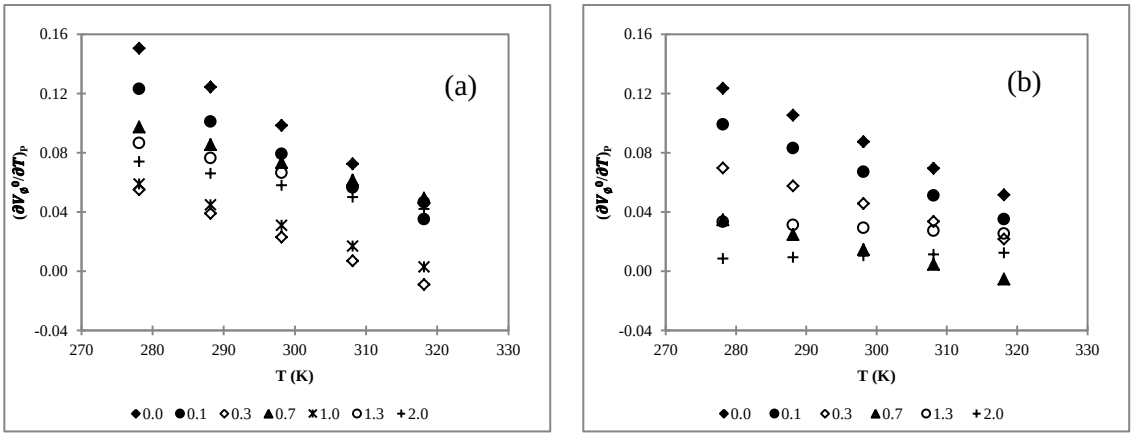

Figure 4.3. $(\partial V_{\emptyset}^{0}/\partial T)_P$/cm^3·mol^{-1}·K^{-1}, in aqueous ammonium sulfate solutions at different temperatures and molalities in (a) glycine and (b) alanine.

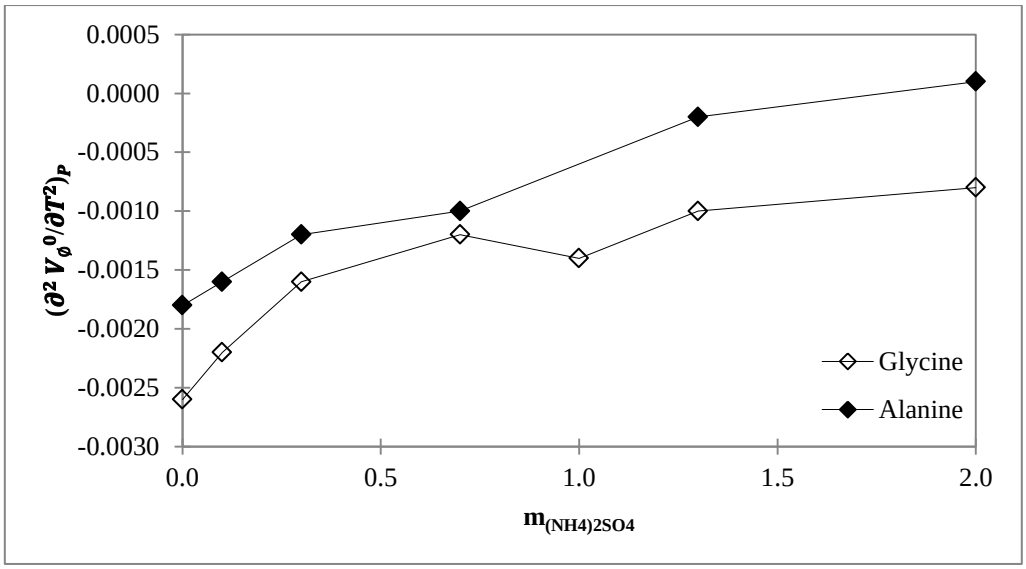

Figure 4.4. $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$/cm^3·mol^{-1}·K^{-2}, in aqueous ammonium sulfate solutions in glycine and alanine.

4.5 Group Contribution

Group-contribution methods are also commonly used to calculate thermodynamics properties in amino acids aqueous solutions. For that is necessary to present values for the contribution of each group in the amino acids. In this case the individual contributions of charged groups (NH_3^+, COO^-) and hydrophobic group CH_2-(Gly) are presented in the Table 4.5. The contribution of the hydrophobic group $CHCH_3$-(Ala) is twice the contribution of CH_2-(Gly), according with the relations proposed by Hakin et al. (1994):

$$V_{2,\emptyset}^{0}(CH_3) = 1.5V_{2,\emptyset}^{0}(CH_2) \quad (4.5)$$

$$V_{2,\emptyset}^{0}(CH) = 0.5V_{2,\emptyset}^{0}(CH_2) \quad (4.6)$$

Table 4.5. Contribution to the partial molar volumes from zwitterionic end groups (NH_3^+, COO^-) and -CH_2- in water and in aqueous ammonium sulfate solutions from T = (278.15 to 318.15) K.

	0.0	0.1	0.3	0.7	1.3	2.0
T(K)	NH_3^+, COO^-					
278.15	23.50	24.52	26.07	27.48	29.30	30.82
288.15	24.97	25.81	26.99	28.42	30.20	31.78
298.15	25.93	26.66	27.72	28.96	30.86	32.20
308.15	26.59	27.30	27.89	29.38	31.16	32.25
318.15	27.00	27.70	28.46	29.80	31.55	32.73
	-CH_2-					
278.15	17.68	17.56	17.34	17.38	17.20	17.03
288.15	17.41	17.32	17.21	17.18	16.88	16.63
298.15	17.26	17.21	17.11	17.13	16.71	16.53
308.15	17.17	17.11	17.22	17.03	16.66	16.61
318.15	17.15	17.09	17.10	16.98	16.59	16.47

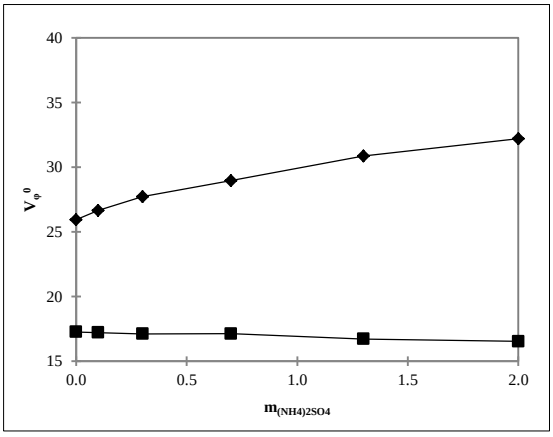

Figure 4.5. $V_{\emptyset}^{0}$ (◆NH_3^+,COO^-; ■ -CH_2-)/cm^3·mol^{-1}, in function of the molality of ammonium sulfate at 298.15 K.

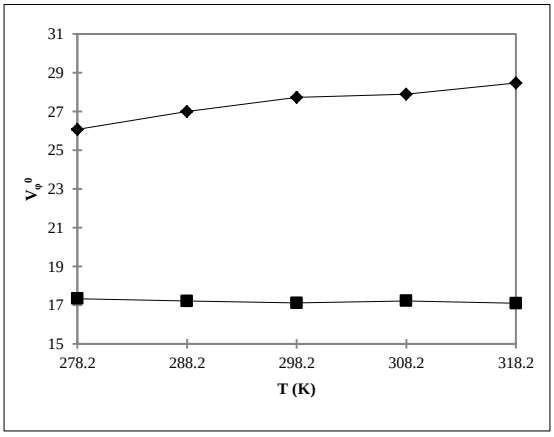

Figure 4.6. $V_{\emptyset}^{0}$ (◆NH_3^+,COO^-; ■ -CH_2-)/cm^3·mol^{-1}, in function of the temperature in 0.3 ammonium sulfate.

As can be seen in Figure 4.5 and 4.6, $V_{\emptyset}^{0}$ followed the order (NH_3^+, COO^-) > (-CH_2-). The $V_{\emptyset}^{0}$ (NH_3^+, COO^-) group contribution for amino acids increases with an increase in the concentration of ammonium sulfate and in temperature, while the (-CH_2-) contribution remains approximately constant. This means that (NH_3^+, COO^-) group contribution is more dependent on the molality, and temperature, and has a higher contribution. Banerjee and Kishore (2005) and Islam and Wadi (2003) obtained the

same results in the measurements of glycine and alanine in aqueous tetraethylammonium bromide and sodium sulfate solutions, respectively. Generally the group contribution V_{ϕ}^{0} (-CH_2-) decrease with an increase in the temperature.

4.6 Discussion and Interpretation

In the ternary solutions evaluated the ions present in solution are SO_4^{2-} and NH_4^+ from ammonium sulfate, while AA's present the charged groups NH_3^+ and COO^-.

Based on the previously discussed theoretical review is possible to obtain some important conclusions for the systems under study.

In solution, the salt used in this work, dissociates into the anion sulfate that is a strong ion and into the cation ammonium that is a weakly hydrated. It is know that strongly hydrated ions have stronger interactions with polar groups of amino acids (NH_3^+ and COO^-) causing dehydration of amino acids, implying that weakly hydrated ions have weaker interactions with amino acids. According to Zhao et al. (2006) volumes of transfer are typically high in solutions containing strong ions and usually low in solutions of weakly hydrated ions. In this case the strong ions dominate, that present higher volumes of transfer. These are all positive which indicates that the hydration number of the amino acid is reduced upon the addition of cosolutes, according with Ogawa et al. (1984). At higher concentrations of ammonium sulfate the $V_{\phi,tr}^{0}$ are more pronounced, confirming the dehydration effect of the salt.

According to the co-sphere overlap model, if the $V_{\phi,tr}^{0}$ are positive values the ion charged/ hydrophilic group interactions are much stronger and dominate over the ion-hydrophobic and hydrophobic-hydrophobic group interactions. So, in this ternary system, the overlap of cosolute ions and amino acids comes into play because of interactions between the (-NH_3^+, -COO^-) charged ends of amino acids and ions of the cosolute. Naturally, like found by Aktar (2007) the interactions between NH_3^+ and the ion SO_4^{2-} are expected to predominate even if a more definitive conclusion can be checked by molecular dynamics. In the case of the amino acids in study, glycine and alanine, the charged head groups of COO^- and NH_3^+ dominate the interactions. The hydration spheres of glycine and alanine are mainly composed of charge-dipole forces. Therefore, an overlap of co-spheres of glycine or alanine with that of exposed nonpolar

groups would squeeze out some water mainly from the hydrophobic hydration spheres of these nonpolar groups.

The $V_{\emptyset,tr}^{0}$ values for glycine are higher than for alanine which indicates that replacement of one –H atom of glycine with $–CH_3$ group provides an increase tendency of hydrophilic–hydrophobic and hydrophobic–hydrophobic group interactions, and as a result, greater electrostriction of the solvent water is produced, leading to smaller values of $V_{\emptyset,tr}^{0}$. In other words, the absence of hydrophobic hydration in glycine due to absence of any methyl group (methyl group tightens the water molecules around itself) causes glycine to be under a higher electrostriction effect than alanine and other amino acids containing methyl groups.

The solution of amino acid in water shows an overall decrease in the volume of water. This is due to the electrostriction of water by charged end groups of amino acids. Addition of $(NH_4)_2SO_4$ will affect the hydration spheres of charged end groups. As a result of NH_4^+ - COO^- and SO_4^{2-}- NH_3^+ interactions, the hydrated water molecules are allowed to relax to the bulk state and cause an increase in the volume. The increase in the temperature favors the relaxation of water, increasing the volume. Some preliminary studies of molecular dynamics suggest that SO_4^{2-}- NH_3^+ interaction is more intense than NH_4^+ - COO^- interaction.

The hydration number decreases with the increasing of concentration of aqueous solutions of ammonium sulfate. This means that the number of water molecules hydrating amino acids decreases, further strengthening the predominance of ionic/hydrophilic interactions in this system, i.e., due to interaction of charged end groups and ions, water is relaxed in the bulk state.

At higher concentration of ammonium sulfate and at low temperature, the $(\partial V_{\emptyset}^{0}/\partial T)_P$ values of alanine are low, favoring solute–solvent interactions. The effect is that charged end-group of alanine influences electrostatically surrounding water molecules (so-called electrostriction). As a result, the electrostricted water may be released from the loose solvation layers of alanine by the increasing the temperature. The effect is that the removal of water molecules favors alanine–ammonium sulfate interactions,

indicating the structure-breaking effect of alanine in the high concentration region of ammonium sulfate and at low temperature.

According to Hepler (1969), and his hydrophobicity criteria it is possible to reflect about the hydrophobicity of the solute. For the both amino acids and almost all concentrations and temperatures $(\partial V_{\emptyset}^{0}/\partial T)_P > 0$ and $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P < 0$, so the solute is hydrophilic. There are only few exceptions which are not defined by this criterion. The obtained $(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$ values are negative for both amino acids, except for alanine at 318.15 K and 2.0 molal of aqueous ammonium sulfate solutions, which suggests that studied amino acids are structure breakers in water as well as in aqueous ammonium sulfate solutions. It is also possible to realize that as the concentration of ammonium sulfate increases, the hydrophilic character of the solute is somehow modified ($(\partial^2 V_{\emptyset}^{0}/\partial T^2)_P$ becomes positive).

Confirming the findings presented so far, the larger value of $V_{\emptyset}^{0}(NH_3^+, COO^-)$ in $(NH_4)_2SO_4$ as compared to the value in water indicates that the interactions of the ions of $(NH_4)_2SO_4$ with the zwitterionic end groups (NH_3^+, COO^-) of the amino acids are stronger and increase with the concentration of $(NH_4)_2SO_4$.

Larger values of $V_{\emptyset}^{0}(NH_3^+, COO^-)$ than $V_{\emptyset}^{0}(CH_2)$ indicate that the interactions of the ions of ammonium sulfate with the zwitterionic groups of the amino acids dominate compared to those of the hydrophobic group–$(NH_4)_2SO_4$ interactions. In addition, the difference in the molar masses of the (NH_3^+, COO^-) and CH_2 groups also accounts for the larger $V_{\emptyset}^{0}$ values of the former.

In the literature there are no published studies involving measurements of density in aqueous ammonium sulfate solutions containing the amino acids glycine or alanine. However there are some studies involving the ions ammonium or sulfate. Based on these it is possible to make some comparisons.

The densities of glycine and alanine were measured by Islam and Wadi (2003) in aqueous sodium sulfate solutions, Mallick and Kishore (2006) in aqueous magnesium sulfate solutions, Natarajan et al. (1990) in aqueous ammonium chloride solutions, Sadeghi and Gholamireza (2011) in aqueous di-ammonium hydrogen citrate solutions

and Sinha et al. (2011) measured densities of aqueous silver sulfate solutions containing alanine. In all of these works the transfer volumes are in general positive, indicating the predominance of the interactions of zwitterionic/hydrophilic groups of amino acids with ions of the salt, and increase with the concentration of salt whereas increased temperature has the opposite effect. According with Natarajan et al. (1990) an increase in the volume of transfer with electrolyte concentration has been explained due to strong interactions of NH_4^+ and Cl^- with the charged centers of the zwitterions compared to ion-nonpolar-group interaction. The interaction of NH_3^+ and Cl^- ions with the amino acids is located at the head groups (COO^- and NH_3^+). Due to these interactions, the electrostriction of water caused by the charge centers of the amino acid will be reduced, which results in an increase in volume. The decrease of the volume of transfer with the temperature rise was explained by Sinha et al. (2011), due to more thermal agitation and less electrostriction at higher temperatures. The hydration numbers decreases with increasing concentration of salt. The number of water molecules hydrated to amino acids decreases, further strengthening the predominance of ionic/hydrophilic interactions in the systems. These results are in agreement with those obtained in this work. An attempt was also tried to relate $V_{\emptyset,tr}^0$ with the ionic radius of the cation in sulfate solutions 1 molal at 298.15 K. Even if the number of systems is still small, Figure 4.7 suggests that the transfer volume decreases with the radius of the cation.

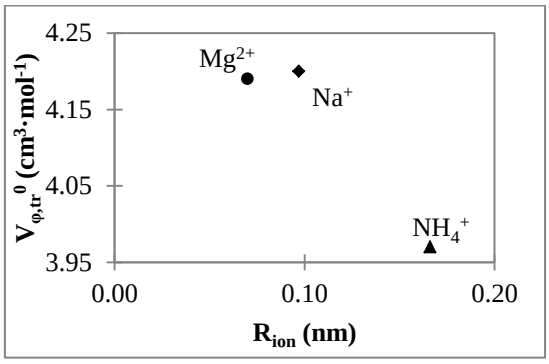

Figure 4.7. Volume of transfer at 1.0 molal of $XXSO_4$ [XX=Mg, Na, $(NH_4)_2$] and 298.15 K in function of the cation radius (Marcus, 1988) [● Mallick and Kishore (2006), ♦ Islam and Wadi (2003) and ▲ this work].

5. Conclusions

5.1 Main Conclusions

A biotechnological production of amino acids is nowadays a prospect market due to major successes in cost effective production and isolation of amino acids products. For the design, optimization and scale-up of the separation processes the knowledge of thermodynamics data is of extreme importance. Although it is possible to find in literature a considerable number of both experimental and modeling work concerning amino acid studies in pure water, the situation is not the same for aqueous amino acid solutions with a salt where there is still a great lack of information. It was then evident the absolute need to carry out further measurements in order to extend the experimental database already available.

The density and the speed of sound of aqueous ammonium sulfate solutions containing the amino acids glycine and alanine, has been measured in water and in the systems water/electrolyte [$(NH_4)_2SO_4$] in the temperature range between 278.15 K and 318.15 K. These values were measured using a digital densimeter (Anton Paar DSA 5000). The solutions were prepared by mass (±0.1 mg) using distilled and degassed water. For each electrolyte molality, the measurements were made sequentially, from the binary solution to the highest amino acid concentration (up to 0.5 molal) at five different temperatures (±0.1 K). The reproducibility of the measurements is estimated to be ±0.01 $kg \cdot m^{-3}$, and concerning the binary solutions the values agree well with literature values. A set of accurate data, presenting around one thousand six hundred new density values were measured from which seventy values of partial molar volumes were obtained. The systematized experimental study developed in this work contributed, to study new systems, while the temperature and composition ranges usually studied were extended.

In the present work, the partial molar volumes, volume of transfer, hydration numbers, partial molar expansibilities and group contributions for glycine and alanine in aqueous solutions of ammonium sulfate were calculated by density measurements at 278.15, 288.15, 298.15, 308.15 and 318.15 K.

Higher $V_{\emptyset}^{0}$ values observed for amino acids in the studied cosolute suggest that ion-charged group interactions dominate over ion-non-polar group interactions. The

volumes of transfer data suggest that ion charged/ hydrophilic group interactions are predominant in the case of glycine and alanine. Applying the McMillan and Mayer formalism was found that interactions between ammonium sulfate and amino acids are mainly pair wise. The hydration numbers of the amino acids under investigation decrease with temperature and this cause a dehydration effect on the amino acids. The negative $(\partial^2 V_\phi^0/\partial T^2)_P$ values indicate that, for both amino acids in water and in ammonium sulfate, solutes are structure breakers. The contribution of the zwitterionic (NH_3^+, COO^-) group to the value of the standard partial molar volume increases with increasing concentration of ammonium sulfate. In general, the contribution of -CH_2- and other alkyl groups has a weak decreasing trend.

The satisfactory experimental results obtained in this work undoubtedly contributed for the understanding of these complex systems, and also to those containing proteins, peptides or antibiotics which certainly provide new insights for applications in the industry.

5.2 Suggestions for Future Work

A systematic experimental study on the density and speed of sound of two amino acids in aqueous solution with or without a salt, was been developed in order to contribute for the fulfillment of the lack of information found in these fields. Naturally, the database is far from being completed so it is fundamental to continue the experimental work increasing the number of studied systems. Specifically, a huge lack of accurate information still remains for the speed of sound of amino acids in aqueous electrolyte solutions, which deserves an important effort due to its importance and contribute to check the consistency of the results found from density data.

One particular suggestion for future work is the study of more systems under exactly the same conditions but involving homo and heteropeptides of different size. Also, the volumetric information for aqueous ammonium sulfate solution 1 molal, containing alanine, should be checked.

From the theoretical point of view it will be important to study the data of different systems in order to search for relations between the partial molar volumes and the properties of the electrolyte.

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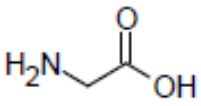
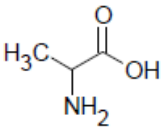
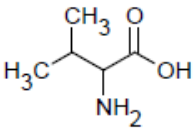
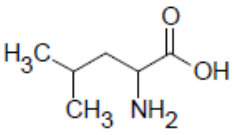
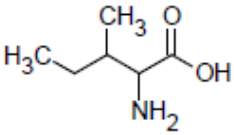
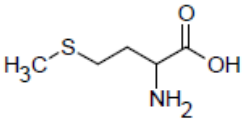
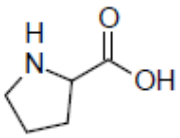
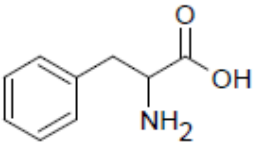
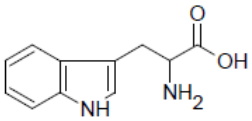
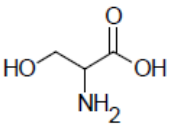
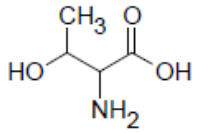
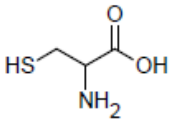
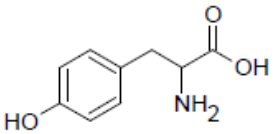
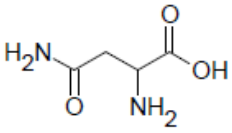
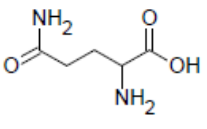
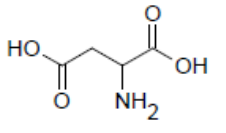
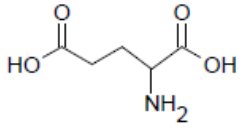
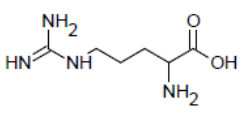
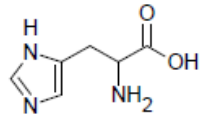
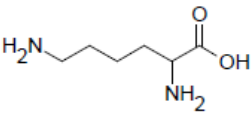
Appendices

Appendix A: The Chemistry of Amino Acids

Table A.1. α-Amino acids found in proteins.

			
Glycine (Gly)	Alanine (Ala)	Valine* (Val)	Leucine* (Leu)
			
Isoleucine* (Ile)	Methionine* (Met)	Proline (Pro)	Phenylalanine* (Phe)
			
Tryptophan* (Trp)	Serine (Ser)	Threonine* (Thr)	Cysteine (Cys)
			
Tyrosine (Tyr)	Asparagine (Asn)	Glutamine (Gln)	Aspartic Acid (Asp)
			
Glutamic Acid (Glu)	Arginine* (Arg)	Histidine* (His)	Lysine* (Lys)

*essencial amino acids

Appendix B: Literature Review

Table B.1. Binary systems (AA+H_2O).

Amino acids	Properties	Temperatures (K)	Reference
Glycine, DL-α-alanine, DL-α-amino-n-butyric acid, L-valine, L-leucine and diglycine	Density	298.15	Banipal and Singh, 2003
Glycine and β-alanine	Density and speed of sound	291.15 to 328.15	Chalikian et al., 1993
Glycine and L-alanine	Density	298 to 443	Cibulka et al., 2010
Glycine	Density	397.39 to 473.16	Hakin et al., 1998
L-alanine	Density	397 to 521	Hakin et al., 2000
Glycylglycine and L-serine	Density	298 to 423	Marriott et al., 2001
Proline-leucine	Density	283.15 to 318.15	Mendonça et al., 2004
Glycine, L-alanine, L-serine, L-glutamic acid, and L-aspartic acid	Density	308.15	Munde and Kishore, 2003
α- DL-aminobutyric acid, DL-norvaline and DL-norleucine	Density and viscosity	288.15, 293.15, 298.15 and 303.15	Romero and Negrete, 2004
L-asparagine, L-glutamine, L-histidine, L-aspartic, L-glutamic acid, L-lysine, L-arginine and L-histidine	Density and speed of sound	278.15, 288.15, 298.15, 308.15 and 318.15	Yasuda et al., 1998

Table B.2. Ternary systems (AA+salt+H_2O).

Amino acids	Salt	Properties	Temperatures (K)	Reference
L-proline and L-glutamine	$Cu(NO_3)_2$ and $NiCl_2$	Density and viscosity	308	Akhtar, 2007
L-alanine	NaBr, KCl, KBr and $MgCl_2$	Density and speed of sound	298.15	Badarayani and Kumar, 2002
Glycine	NaBr, KCl, KBr and $MgCl_2$	Density and speed of sound	298.15	Badarayani and Kumar, 2003a
L-alanine	KCl	Density and speed of sound	283.15 to 313.15	Badarayani and Kumar, 2003b
Glycine, L-alanine and glycylglycine	$(CH_3)_4NBr$, $(C_2H_5)_4NBr$ and $(C_4H_9)_4NBr$	Density	298.15	Badarayani and Kumar, 2004a
Glycylglycine	KCl, KBr and Na_2SO_4	Density, speed of sound and viscosity	298.15	Badarayani and Kumar, 2004b
Glycine, L-alanine and glycylglycine	$(CH_3)_4NBr$, $(C_2H_5)_4NBr$ and $(C_4H_9)_4NBr$	Viscosity	298.15	Badarayani and Kumar, 2004c
Glycine, L-alanine, DL-α-amino-n-butyric acid, L-valine and L-leucine	$C_8H_{20}BrN$	Density	298.15	Banerjee and Kishore, 2005
L-aspartic acid, L-glutamic acid, L-lysine and L-arginine	$NaCH_3COO$, NaC_2H_5COO and NaC_3H_7COO	Density	288.15, 298.15, 308.15 and 318.15	Banipal et al., 2007b
Glycine, alanine, DL-α-amino-n-butyric acid, valine, leucine, diglycine and glycylalanine	NaCl	Heat capacity and density	298.15	Bhat and Ahluwalia, 1985
Glycine, DL-alanine, DL-α-aminobutyric acid, DL-valine, β-alanine, ϒ-aminobutyric acid and δ-aminovaleric acid	Na_2SO_4	Density and viscosity	288.15, 298.15 and 308.15	Islam and Wadi, 2003
Glycine, L-alanine, and L-leucine	$MgCl_2$	Density	288.15, 298.15, and 308.15	Lark et al., 2004
Glycylglycine	NaF, NaCl and NaBr	Density	298.15 and 308.15	Lin et al., 2006

Continued

Glycine, alanine, α-amino-n-butyric acid, valine and leucine	$MgSO_4$	Density	298.15	Mallick and Kishore, 2006
Proline-leucine dipeptide	NaCl	Density	318.15	Mendonça et al., 2005
Glycine, DL-α-alanine, DL-α-aminobutyric acid, DL-norvaline, DL-norleucine, β-alanine, ϒ-aminobutyric acid, δ-aminovaleric acid, ε-aminocaproic acid, L-serine, L-threonine, DL-valine, L-glutamine, DL-methionine and DL-phenylalanine	NH_4Cl	Density and viscosity	298.15	Natarajan et al., 1990
Glycine, L-alanine, β-alanine, α-aminoisobutyric acid, L-serine and L-threonine	LiCl, NaCl and KCl	Density, speed of sound and viscosity	298.15	Ogawa et al., 1984
Glycine, L-alanine and L-valine	$MgCl_2$	Speed of sound and density	298.15	Pal and Kumar, 2005a
Glycine, L-alanine and L-valine	$MgCl_2$	Density	288.15 and 308.15	Pal and Kumar, 2005b
L-leucine, L-asparagine and glycylglycine	NaCl, $NaNO_3$ and KNO_3	Speed of sound and density	298.15 to 323.15	Riyazuddeen and Bansal, 2006
L-isoleucine, L-proline and L-glutamine	KCl, KNO_3 and K_2SO_4	Speed of sound and density	303.15, 308.15, 313.15, 318.15 and 323.15	Riyazuddeen and Basharat, 2006
L-alanine, L-proline, L-valine, and L-leucine	KCl and KNO_3	Density and speed of sound	298.15, 303.15, 308.15, 313.15, 318.15, and 323.15	Riyazuddeen and Khan, 2009
DL-alanine	NaCl	Density, speed of sound, refractive index and viscosity	298.15	Rodríguez et al., 2003
Glycine, DL-alanine, DL-serine, DL-threonine, L-histidine and glycylglycine	NaCl	Density	278.15, 288.15, 298.15 and 308.15	Shen et al., 2000

Continued

L-lysine monohydrochloride, L-histidine and L-arginine	$NaCH_3COO$, KCH_3COO and $Ca(CH_3COO)_2$	Density	303.15 to 323.15	Siddique and Naqvi, 2010
Glycine, L-alanine, L-leucine, L-valine, L-serine, D,L-glutamic acid/ sodium salt, glycyl-glycine and glycyl-glycylglycine	$NaCH_3COO$, NaSCN and Na_2SO_4	Density	298.15	Singh and Kishore, 2003
Glycine, alanine, valine, leucine and lysine	$NaCH_3(CH_2)_{11}OSO_3$ and $BrC_{16}H_{33}N(CH_3)_3$	Density	298.15	Singh et al., 2004
L-proline	HCl and NaOH	Density	278.15 to 368.15	Sorenson et al., 2003
Diglycine and triglycine	NaCl	Density and speed of sound	298.15	Soto et al., 2004
Glycine, DL-alanine, L-threonine, β-alanine, ϒ-aminobutyric acid and ε-aminocaproic acid	KSCN	Density and viscosity	288.15, 298.15 and 308.15	Wadi and Goyal, 1992
Glycine and DL-alanine	Na_2SO_4	Density and speed of sound	288.15, 298.15 and 308.15	Wadi and Ramasami, 1997
Glycine, DL-α-alanine, DL-α-amino-n-butyric acid, DL-valine and DL-leucine	$NaCH_3COO$	Density	308.15	Wang et al., 1999
Glycine, DL-alanine, DL-α-amino-n-butyric acid, DL-valine, DL-leucine, and L-serine	$CH_5N_3.HCl$	Density	278.15, 288.15, 298.15 and 308.15	Yan et al., 1998
Glycine, DL-α-alanine, DL-α-aminobutyric acid, DL-valine, DL-leucine and L--serine	$CaCl_2$	Density and viscosity	278.15, 288.15, 298.15 and 308.15	Yan et al., 2004
L-alanine, DL-serine, DL-threonine, L-histidine, glycine and glycylglycine	NaCl	Density	298.15	Yuan et al., 2006

Appendix C: Derivation of the Equation to Calculate the Partial Molar Volume

By the definition, the apparent molar volume of the AA ($V_\emptyset$) is:

$$V_\emptyset = \frac{V_T - V_B}{n_2} \quad \text{(C.1)}$$

where V_T is the volume of the ternary system, V_B is the solvent volume (water + salt) and n_2 is the number of moles of amino acid.

Considering w_i the mass of the specie i,

$$w_T = w_1 + w_2 + w_3 \quad \text{(C.2)}$$

1 ≡ water, 2 ≡ amino acid and 3 ≡ salt.

By the definition of molality,

$$m_i = \frac{n_i}{w_1} = \frac{w_i}{M_i w_1} \rightarrow w_i = m_i M_i w_1 \quad \text{(C.3)}$$

$$w_T = w_1 + m_2 M_2 w_1 + m_3 M_3 w_1 \quad \text{(C.4)}$$

By analogy,

$$w_B = w_1 + m_3 M_3 w_1 \quad \text{(C.5)}$$

Knowing that $V = \frac{w}{\rho}$

$$V_\emptyset = \frac{\left(w/\rho\right)_T - \left(w/\rho\right)_B}{n_2} = \frac{\frac{w_1(1+m_2M_2+m_3M_3)}{\rho_T} - \frac{w_1(1+m_3M_3)}{\rho_B}}{n_2} \quad \text{(C.6)}$$

Dividing by w_1,

$$V_\emptyset = \frac{\frac{1+m_2M_2+m_3M_3}{\rho_T} - \frac{1+m_3M_3}{\rho_B}}{m_2} = \frac{\frac{\rho_B+\rho_B m_2M_2+\rho_B m_3M_3-\rho_T-\rho_T m_3M_3}{\rho_T\rho_B}}{m_2} \quad \text{(C.7)}$$

$$V_\emptyset = \frac{\rho_B-\rho_T+m_3M_3(\rho_B-\rho_T)+\rho_B m_2M_2}{m_2\rho_T\rho_0} = \frac{-(\rho_T-\rho_B)(1+m_3M_3)+\rho_B m_2M_2}{m_2\rho_T\rho_B} \quad \text{(C.8)}$$

$$V_{\emptyset} = \frac{M_2}{\rho_T} - (1 + m_3 M_3)\frac{(\rho_T - \rho_B)}{m_2 \rho_T \rho_B} \quad \text{(C.9)}$$

At infinite dilution $m_2 \to 0$, $V_{\emptyset} \to V_{\emptyset}^0$ and $\rho_T \to \rho_B$.

$$\lim_{m_2 \to 0} \frac{\rho_T - \rho_B}{m_2} = \lim_{m_2 \to 0} a_v + b_v m_2 = a_v$$

Finally,

$$V_{\emptyset}^0 = \frac{M_2}{\rho_B} - (1 + m_3 M_3)\frac{a_v}{{\rho_B}^2} = \frac{1}{\rho_B}\left[M_2 - (1 + m_3 M_3)\frac{a_v}{\rho_B}\right] \quad \text{(C.10)}$$

If m_2^* is the number of moles of amino acid per kilogram of binary solvent and not per kilogram of water, the following formula can be used to calculate the apparent molar volume:

$$V_{\emptyset} = \frac{M_2}{\rho_T} - \frac{(\rho_T - \rho_B)}{m_2^* \rho_T \rho_B} \quad \text{(C.11)}$$

and, the partial molar volume is calculate through:

$$V_{\emptyset} = V_{\emptyset}^0 + S_v m_2^* \quad \text{(C.12)}$$

where S_v is an experimental slope.

It should be noted that in many publications studied the question of interpretation, concerning the formula used and if was properly used arose. In fact, many times it is not possible to understand if the concentration is expressed per kilogram of water or kilogram of binary solvent (water + salt).

Appendix D: Density Values

Table D.1. Density values measured for aqueous systems AA + H_2O.

m_2	ρ (g·cm^{-3})				
(mol·kg^{-1})	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K
			Water + AA		
Glycine					
0.0000	0.999967	0.999103	0.997048	0.994033	0.990213
0.0500	1.001662	1.000726	0.998634	0.995591	0.991760
0.0967	1.003226	1.002237	1.000108	0.997043	0.993196
0.1500	1.004999	1.003950	1.001778	0.998688	0.994824
0.2000	1.006647	1.005542	1.003334	1.000218	0.996338
0.2996	1.009890	1.008679	1.006402	1.003236	0.999326
0.3952	1.012951	1.011645	1.009298	1.006091	1.002154
0.4999	1.016258	1.014846	1.012427	1.009177	1.005208
Alanine					
0.0000	0.999967	0.999103	0.997048	0.994033	0.990213
0.0500	1.001505	1.000588	0.998498	0.995467	0.991639
0.1000	1.002998	1.002035	0.999916	0.996867	0.993028
0.1500	1.004476	1.003471	1.001323	0.998255	0.994406
0.2000	1.005941	1.004894	1.002717	0.999634	0.995771
0.3000	1.008835	1.007705	1.005474	1.002353	0.998469
0.4000	1.011683	1.010471	1.008184	1.005034	1.001124
0.5000	1.014473	1.013187	1.010849	1.007660	1.003732

Table D.2. Density values measured for ternary systems, AA + salt + H_2O.

m_2	ρ (g·cm^{-3})				
(mol·kg^{-1})	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K
0.1 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.008181	1.007059	1.004848	1.001746	0.997886
0.0500	1.009788	1.008614	1.006368	1.003241	0.999368
0.1000	1.011396	1.010170	1.007891	1.004740	1.000851
0.1502	1.012994	1.011720	1.009405	1.006229	1.002327
0.2000	1.014573	1.013247	1.010901	1.007703	1.003782
0.3001	1.017705	1.016284	1.013871	1.010632	1.006679
0.3999	1.020781	1.019269	1.016789	1.013506	1.009530
0.4995	1.023808	1.022201	1.019668	1.016343	1.012341
Alanine					
0.0000	1.008181	1.007059	1.004848	1.001746	0.997886
0.0500	1.009634	1.008474	1.006231	1.003114	0.999243
0.1000	1.011060	1.009862	1.007592	1.004459	1.000576
0.1500	1.012470	1.011234	1.008939	1.005792	1.001896
0.1996	1.013846	1.012575	1.010251	1.007086	1.003182
0.3000	1.016626	1.015283	1.012907	1.009710	1.005784
0.4000	1.019342	1.017925	1.015506	1.012278	1.008332
0.5000	1.022021	1.020533	1.018067	1.014810	1.010843
0.3 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.023476	1.021948	1.019470	1.016209	1.012266
0.0500	1.024988	1.023417	1.020916	1.017620	1.013685
0.1000	1.026489	1.024878	1.022341	1.019035	1.015083
0.1500	1.027968	1.026321	1.023751	1.020456	1.016455
0.2000	1.029443	1.027750	1.025154	1.021815	1.017827
0.3000	1.032376	1.030592	1.027945	1.024567	1.020559
0.3999	1.035256	1.033398	1.030694	1.027232	1.023234
0.5000	1.038089	1.036161	1.033403	1.029976	1.025897
Alanine					
0.0000	1.023476	1.021948	1.019470	1.016209	1.012266
0.0500	1.024777	1.023224	1.020724	1.017452	1.013493
0.0999	1.026083	1.024502	1.021980	1.018693	1.014728
0.1500	1.027393	1.025779	1.023236	1.019937	1.015959
0.1992	1.028653	1.027012	1.024448	1.021134	1.017155
0.2995	1.031230	1.029523	1.026919	1.023580	1.019580
0.4000	1.033772	1.032012	1.029363	1.025999	1.021976
0.5000	1.036244	1.034424	1.031741	1.028353	1.024313

Continued

0.7 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.051323	1.049183	1.046298	1.042798	1.038728
0.0500	1.052669	1.050496	1.047593	1.044070	1.040000
0.1000	1.054001	1.051797	1.048868	1.045339	1.041258
0.1499	1.055333	1.053091	1.050132	1.046595	1.042511
0.2000	1.056615	1.054344	1.051375	1.047816	1.043718
0.3000	1.059198	1.056868	1.053856	1.050275	1.046152
0.4000	1.061777	1.059390	1.056323	1.052720	1.048587
0.5000	1.064272	1.061840	1.058733	1.055096	1.050938
Alanine					
0.0000	1.051323	1.049183	1.046298	1.042798	1.038728
0.0500	1.052443	1.050283	1.047388	1.043879	1.039797
0.1000	1.053578	1.051402	1.048491	1.044967	1.040888
0.1500	1.054706	1.052507	1.049577	1.046079	1.041951
0.2000	1.055811	1.053591	1.050651	1.047114	1.043012
0.3000	1.058016	1.055747	1.052785	1.049229	1.045115
0.3957	1.060097	1.057791	1.054797	1.051228	1.047098
0.5000	1.062325	1.059985	1.056961	1.053380	1.049227
1.0 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.070293	1.067792	1.064697	1.061062	1.056925
0.0500	1.071508	1.068979	1.065865	1.062222	1.058078
0.1000	1.072734	1.070186	1.067046	1.063425	1.059217
0.1500	1.073949	1.071367	1.068210	1.064551	1.060370
0.2000	1.075138	1.072547	1.069374	1.065707	1.061523
0.3000	1.077519	1.074884	1.071669	1.067969	1.063764
0.4000	1.079887	1.077197	1.073949	1.070259	1.066025
0.5000	1.082224	1.079482	1.076203	1.072468	1.068221
Alanine					
0.0000	1.070293	1.067792	1.064697	1.061062	1.056925
0.0500	1.071283	1.068790	1.065682	1.062033	1.057893
0.1000	1.072367	1.069858	1.066738	1.063084	1.058936
0.1501	1.073388	1.070859	1.067729	1.064066	1.059917
0.2000	1.074343	1.071802	1.068661	1.064994	1.060839
0.3000	1.076330	1.073761	1.070601	1.066913	1.062753
0.4000	1.078274	1.075674	1.072495	1.068795	1.064624
0.5000	1.080193	1.077571	1.074367	1.070652	1.066473

Continued

1.3 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.087949	1.085179	1.081890	1.078139	1.073974
0.0500	1.089087	1.086320	1.082992	1.079244	1.075066
0.1000	1.090222	1.087423	1.084091	1.080329	1.076135
0.1500	1.091360	1.088523	1.085173	1.081409	1.077212
0.1999	1.092443	1.089583	1.086220	1.082443	1.078232
0.2987	1.094575	1.091690	1.088311	1.084516	1.080292
0.4000	1.096779	1.093862	1.090436	1.086617	1.082381
0.5000	1.098900	1.095948	1.092521	1.088684	1.084429
Alanine					
0.0000	1.087949	1.085179	1.081890	1.078139	1.073974
0.0500	1.088799	1.086034	1.082750	1.078992	1.074830
0.1000	1.089729	1.086949	1.083653	1.079892	1.075721
0.1500	1.090631	1.087846	1.084533	1.080774	1.076597
0.2000	1.091547	1.088748	1.085407	1.081641	1.077474
0.3000	1.093269	1.090459	1.087116	1.083350	1.079159
0.4000	1.095043	1.092205	1.088843	1.085073	1.080863
0.5000	1.096777	1.093916	1.090540	1.086745	1.082541
2.0 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1.124496	1.121319	1.117777	1.113895	1.109681
0.0500	1.125427	1.122233	1.118662	1.114782	1.110564
0.1000	1.126393	1.123172	1.119584	1.115702	1.111475
0.1500	1.127347	1.124120	1.120504	1.116607	1.112380
0.1998	1.128138	1.124903	1.121291	1.117394	1.113153
0.3000	1.129973	1.126723	1.123089	1.119178	1.114939
0.4000	1.131799	1.128496	1.124850	1.120921	1.116665
0.5049	1.133654	1.130338	1.126686	1.122763	1.118491
Alanine					
0.0000	1.124496	1.121319	1.117777	1.113895	1.109681
0.0500	1.125145	1.121960	1.118392	1.114502	1.110300
0.1000	1.125884	1.122681	1.119110	1.115231	1.111022
0.1500	1.126590	1.123382	1.119802	1.115927	1.111712
0.2000	1.127325	1.124100	1.120526	1.116640	1.112422
0.3000	1.128694	1.125476	1.121896	1.118011	1.113797
0.4000	1.130103	1.126857	1.123256	1.119359	1.115135
0.5000	1.131445	1.128189	1.124591	1.120694	1.116470

Appendix E: Speed of Sound Values

Table E.1. Speed of Sound values measured for aqueous systems, AA + H_2O.

m_2	u (m·s^{-1})				
(mol·kg^{-1})	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K
			Water + AA		
Glycine					
0.0000	1426.17	1465.93	1496.70	1519.85	1536.45
0.0500	1429.44	1469.05	1499.75	1522.83	1539.44
0.0967	1432.25	1471.66	1502.20	1525.14	1541.68
0.1500	1435.45	1474.62	1504.97	1527.77	1544.22
0.2000	1438.42	1477.38	1507.56	1530.22	1546.56
0.2996	1444.27	1482.81	1512.67	1535.04	1551.16
0.3952	1449.82	1487.95	1517.48	1539.62	1555.51
0.4999	1455.81	1493.50	1522.69	1544.56	1560.23
Alanine					
0.0000	1426.17	1465.93	1496.70	1519.85	1536.45
0.0500	1430.30	1469.95	1500.37	1523.38	1540.11
0.1000	1434.11	1473.45	1503.62	1526.42	1542.98
0.1500	1437.90	1476.92	1506.85	1529.44	1545.83
0.2000	1441.67	1480.36	1510.06	1532.43	1548.67
0.3000	1449.10	1487.20	1516.42	1538.39	1554.26
0.4000	1456.47	1493.95	1522.67	1544.26	1559.78
0.5000	1463.69	1500.61	1528.84	1550.01	1565.23

Table E.2. Speed of sound values measured for ternary systems, AA + salt + H_2O.

m_2	u (m·s^{-1})				
(mol·kg^{-1})	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K
0.1 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1440.17	1479.34	1509.55	1532.29	1548.79
0.0500	1443.03	1481.92	1512.06	1534.66	1550.94
0.1000	1445.89	1484.57	1514.55	1537.03	1553.22
0.1502	1448.75	1487.24	1517.03	1539.38	1555.44
0.2000	1451.58	1489.85	1519.48	1541.70	1557.71
0.3001	1457.19	1495.06	1524.36	1546.32	1562.15
0.3999	1462.77	1500.22	1529.18	1550.88	1566.43
0.4995	1468.27	1505.32	1533.97	1555.41	1570.82
Alanine					
0.0000	1440.17	1479.34	1509.55	1532.29	1548.79
0.0500	1443.88	1482.77	1512.69	1535.24	1551.65
0.1000	1447.56	1486.14	1515.83	1538.17	1554.39
0.1500	1451.20	1489.48	1518.93	1541.09	1557.13
0.1996	1454.77	1492.77	1521.97	1543.93	1559.81
0.3000	1462.00	1499.41	1528.11	1549.67	1565.19
0.4000	1469.11	1505.92	1534.16	1555.32	1570.51
0.5000	1476.15	1512.37	1540.13	1560.92	1575.77
0.3 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1465.65	1503.35	1532.29	1554.12	1569.73
0.0500	1468.48	1505.97	1534.74	1557.04	1572.03
0.1000	1471.19	1508.46	1537.08	1559.24	1574.17
0.1500	1473.86	1510.94	1539.40	1561.35	1576.25
0.2000	1476.54	1513.40	1541.71	1563.49	1578.35
0.3000	1481.91	1518.32	1546.33	1567.87	1582.56
0.3999	1487.19	1523.22	1550.93	1572.11	1586.68
0.5000	1492.43	1528.07	1555.40	1576.52	1590.80
Alanine					
0.0000	1465.65	1503.35	1532.29	1554.12	1569.73
0.0500	1469.10	1506.46	1535.20	1556.84	1572.19
0.0999	1472.52	1509.63	1538.12	1559.58	1574.77
0.1500	1475.99	1512.81	1541.08	1562.34	1577.37
0.1992	1479.38	1515.90	1543.95	1565.02	1579.90
0.2995	1486.27	1522.21	1549.80	1570.48	1585.05
0.4000	1493.12	1528.50	1555.62	1575.91	1590.15
0.5000	1499.81	1534.62	1561.29	1581.24	1595.14

Continued

0.7 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1513.84	1548.48	1575.03	1594.92	1609.29
0.0500	1516.37	1550.88	1577.28	1596.98	1611.24
0.1000	1518.89	1553.19	1579.42	1599.02	1613.17
0.1499	1521.43	1555.50	1581.56	1601.09	1615.07
0.2000	1523.87	1557.75	1583.67	1603.08	1616.97
0.3000	1528.81	1562.29	1587.90	1607.12	1620.79
0.4000	1533.76	1566.85	1592.13	1611.08	1624.64
0.5000	1538.60	1571.32	1596.27	1614.98	1628.38
Alanine					
0.0000	1513.84	1548.48	1575.03	1594.92	1609.29
0.0500	1517.02	1551.32	1577.70	1597.42	1611.67
0.1000	1520.25	1554.28	1580.43	1600.00	1614.13
0.1500	1523.44	1557.25	1583.15	1602.61	1616.53
0.2000	1526.59	1560.13	1585.84	1605.10	1618.93
0.3000	1532.86	1565.86	1591.17	1610.11	1623.66
0.3957	1538.87	1571.37	1596.23	1614.86	1628.14
0.5000	1545.28	1577.27	1601.68	1619.98	1632.95
1.0 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1548.24	1580.50	1605.20	1623.84	1636.93
0.0500	1550.59	1582.64	1607.22	1625.79	1639.10
0.1000	1553.01	1584.86	1609.27	1627.79	1640.94
0.1500	1555.40	1587.04	1611.30	1629.68	1642.80
0.2000	1557.74	1589.22	1613.33	1631.60	1644.63
0.3000	1562.45	1593.55	1617.35	1635.38	1648.25
0.4000	1567.16	1597.84	1621.36	1639.21	1651.91
0.5000	1571.82	1602.09	1625.34	1642.93	1655.51
Alanine					
0.0000	1548.24	1580.50	1605.20	1623.84	1636.93
0.0500	1551.22	1583.24	1607.70	1626.17	1638.85
0.1000	1554.35	1586.11	1610.37	1628.65	1641.22
0.1501	1557.35	1588.87	1612.94	1631.06	1643.47
0.2000	1560.24	1591.53	1615.43	1633.39	1645.66
0.3000	1566.16	1596.95	1620.46	1638.10	1650.09
0.4000	1571.99	1602.28	1625.42	1642.72	1654.47
0.5000	1577.74	1607.58	1630.33	1647.32	1658.81

Continued

1.3 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1581.52	1611.66	1634.34	1651.48	1663.55
0.0500	1583.95	1614.25	1636.51	1653.54	1665.45
0.1000	1586.24	1616.32	1638.46	1655.38	1667.20
0.1500	1588.54	1618.40	1640.39	1657.21	1669.03
0.1999	1590.73	1620.42	1642.27	1658.98	1670.71
0.2987	1595.05	1624.42	1646.03	1662.51	1674.11
0.4000	1599.54	1628.57	1649.85	1666.12	1677.57
0.5000	1603.88	1632.57	1653.62	1669.68	1680.92
Alanine					
0.0000	1581.52	1611.66	1634.34	1651.48	1663.55
0.0500	1584.09	1613.75	1636.49	1653.48	1665.51
0.1000	1586.93	1616.37	1638.91	1655.75	1667.65
0.1500	1589.70	1618.93	1641.29	1657.99	1669.77
0.2000	1592.53	1621.51	1643.66	1660.20	1671.89
0.3000	1597.91	1626.51	1648.31	1664.56	1675.99
0.4000	1603.41	1631.55	1652.98	1668.93	1680.10
0.5000	1608.82	1636.50	1657.58	1673.23	1684.15
2.0 mol·kg^{-1} ammonium sulfate					
Glycine					
0.0000	1653.70	1678.71	1697.45	1711.59	1721.48
0.0500	1655.77	1681.02	1699.39	1713.40	1723.19
0.1000	1657.81	1682.87	1701.10	1715.02	1724.76
0.1500	1659.83	1684.73	1702.81	1716.61	1726.32
0.1998	1661.50	1686.28	1704.28	1718.01	1727.65
0.3000	1665.39	1689.85	1707.63	1721.16	1730.70
0.4000	1669.23	1693.36	1710.91	1724.22	1733.65
0.5049	1673.18	1697.00	1714.33	1727.48	1736.77
Alanine					
0.0000	1653.70	1678.71	1697.45	1711.59	1721.48
0.0500	1655.78	1680.34	1699.17	1713.20	1722.99
0.1000	1658.20	1682.54	1701.24	1715.16	1724.91
0.1500	1660.53	1684.72	1703.25	1717.06	1726.71
0.2000	1662.92	1686.92	1705.30	1718.99	1728.55
0.3000	1667.46	1691.17	1709.26	1722.72	1732.10
0.4000	1672.09	1695.41	1713.20	1726.41	1735.57
0.5000	1676.55	1699.55	1717.07	1730.04	1739.01

Appendix F: Ammonium Sulfate Densities Values

Table F.1. Ammonium sulfate densities values for glycine and alanine.

Glycine								
T (K)	**$m_{(NH4)2SO4}$**	**ρ (g·m^{-3})**	**T (K)**	**$m_{(NH4)2SO4}$**	**ρ (g·m^{-3})**	**T (K)**	**$m_{(NH4)2SO4}$**	**ρ (g·m^{-3})**
278.15	0.1	1.008162	288.15	0.7	1.049193	308.15	2.0	1.113914
278.15	0.1	1.008170	288.15	0.7	1.049217	318.15	2.0	1.109673
278.15	0.1	1.008176	298.15	0.7	1.046302	318.15	2.0	1.109716
288.15	0.1	1.007043	298.15	0.7	1.046314	278.15	2.0	1.124454
288.15	0.1	1.007047	298.15	0.7	1.046319	278.15	2.0	1.124487
288.15	0.1	1.007051	308.15	0.7	1.042798	278.15	2.0	1.124472
298.15	0.1	1.004836	308.15	0.7	1.042808	278.15	2.0	1.124489
298.15	0.1	1.004838	308.15	0.7	1.042822	278.15	2.0	1.124484
298.15	0.1	1.004840	318.15	0.7	1.038737	278.15	2.0	1.124500
308.15	0.1	1.001734	318.15	0.7	1.038756	278.15	2.0	1.124550
308.15	0.1	1.001734	318.15	0.7	1.038749	288.15	2.0	1.121296
308.15	0.1	1.001736	278.15	1.0	1.070308	288.15	2.0	1.121327
318.15	0.1	0.997879	288.15	1.0	1.067817	288.15	2.0	1.121313
318.15	0.1	0.997876	298.15	1.0	1.064722	288.15	2.0	1.121329
318.15	0.1	0.997876	308.15	1.0	1.061094	288.15	2.0	1.121325
318.15	0.1	0.997879	318.15	1.0	1.056945	288.15	2.0	1.121340
318.15	0.1	0.997876	278.15	1.0	1.070308	288.15	2.0	1.121393
318.15	0.1	0.997876	288.15	1.0	1.067773	298.15	2.0	1.117764
278.15	0.3	1.023483	298.15	1.0	1.064679	298.15	2.0	1.117797
278.15	0.3	1.023487	308.15	1.0	1.061041	298.15	2.0	1.117781
278.15	0.3	1.023502	318.15	1.0	1.056914	298.15	2.0	1.117797
288.15	0.3	1.021951	278.15	1.3	1.087948	298.15	2.0	1.117794
288.15	0.3	1.021956	278.15	1.3	1.087998	298.15	2.0	1.117809
288.15	0.3	1.021969	288.15	1.3	1.085197	298.15	2.0	1.117861
298.15	0.3	1.019477	288.15	1.3	1.085235	308.15	2.0	1.113879
298.15	0.3	1.019479	298.15	1.3	1.081916	308.15	2.0	1.113913
298.15	0.3	1.019484	298.15	1.3	1.081931	308.15	2.0	1.113898
308.15	0.3	1.016218	308.15	1.3	1.078173	308.15	2.0	1.113915
308.15	0.3	1.016221	308.15	1.3	1.078190	308.15	2.0	1.113908
308.15	0.3	1.016216	318.15	1.3	1.073995	308.15	2.0	1.113924
318.15	0.3	1.012273	318.15	1.3	1.074018	308.15	2.0	1.113977
318.15	0.3	1.012279	278.15	2.0	1.124514	318.15	2.0	1.109663
318.15	0.3	1.012284	278.15	2.0	1.124544	318.15	2.0	1.109695
318.15	0.3	1.016202	288.15	2.0	1.121318	318.15	2.0	1.109680
278.15	0.7	1.051315	288.15	2.0	1.121354	318.15	2.0	1.109697
278.15	0.7	1.051332	298.15	2.0	1.117766	318.15	2.0	1.109692
278.15	0.7	1.051366	298.15	2.0	1.117794	318.15	2.0	1.109707
288.15	0.7	1.049188	308.15	2.0	1.113880	318.15	2.0	1.109759

Continued

Alanine								
T (K)	$m_{(NH4)2SO4}$	ρ (g·m^{-3})	T (K)	$m_{(NH4)2SO4}$	ρ (g·m^{-3})	T (K)	$m_{(NH4)2SO4}$	ρ (g·m^{-3})
278.15	0.1	1.008197	278.15	0.7	1.051310	288.15	1.3	1.085125
278.15	0.1	1.008201	288.15	0.7	1.049152	288.15	1.3	1.085157
288.15	0.1	1.007072	288.15	0.7	1.049166	298.15	1.3	1.081847
288.15	0.1	1.007083	298.15	0.7	1.046274	298.15	1.3	1.081867
298.15	0.1	1.004861	298.15	0.7	1.046280	308.15	1.3	1.078090
298.15	0.1	1.004864	308.15	0.7	1.042775	308.15	1.3	1.078101
308.15	0.1	1.001762	308.15	0.7	1.042787	318.15	1.3	1.073930
308.15	0.1	1.001763	318.15	0.7	1.038694	318.15	1.3	1.073951
318.15	0.1	0.997900	318.15	0.7	1.038703	278.15	2.0	1.124472
318.15	0.1	0.997901	278.15	0.7	1.051320	278.15	2.0	1.124492
278.15	0.3	1.023445	288.15	0.7	1.049187	288.15	2.0	1.121241
278.15	0.3	1.023462	298.15	0.7	1.046308	288.15	2.0	1.121271
288.15	0.3	1.021926	308.15	0.7	1.042803	298.15	2.0	1.117683
288.15	0.3	1.021940	318.15	0.7	1.038725	298.15	2.0	1.117698
298.15	0.3	1.019453	278.15	1.0	1.070262	308.15	2.0	1.113803
298.15	0.3	1.019458	288.15	1.0	1.067786	308.15	2.0	1.113831
308.15	0.3	1.016194	298.15	1.0	1.064689	318.15	2.0	1.109591
308.15	0.3	1.016196	308.15	1.0	1.061051	318.15	2.0	1.109623
318.15	0.3	1.012245	318.15	1.0	1.056917	298.15	2.0	1.117774
318.15	0.3	1.012251	278.15	1.3	1.087910	298.15	2.0	1.117779
278.15	0.7	1.051291	278.15	1.3	1.087939			

Appendix G: Example of Calculation of Adiabatic Compressibility through the Speed of Sound

In order to calculate the partial molar adiabatic compressibilities it is necessary to represent $\Delta[(\rho u)^2]/m_2$ in function of molality of the amino acid to obtain the parameter a_k, according with equation 2.6 to binary systems and 2.8 to ternary systems. With the parameter a_k, the speed of sound and density of the solvent, and using equation 2.5 and 2.7 for binary and ternary systems, respectively, it is possible to calculate the adiabatic compressibility. In the following figures and tables two examples of this procedure is shown.

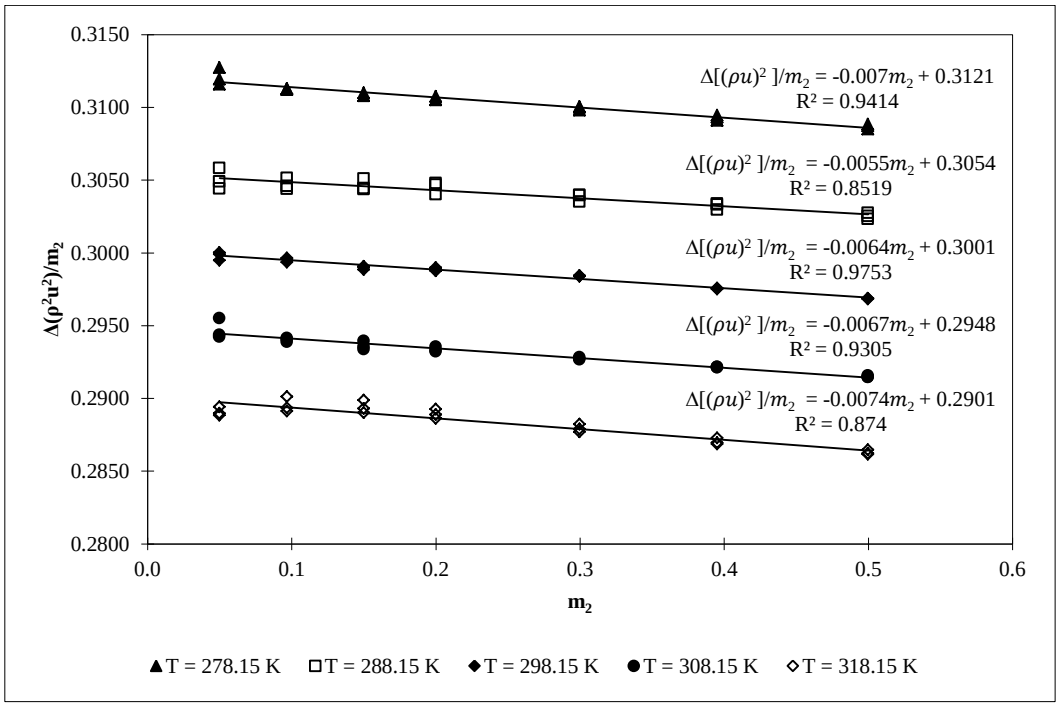


Figure G.1. $\Delta(\rho^2u^2)/m_2$ ($kg^3 \cdot m^{-3} \cdot s^{-2} \cdot mol^{-1}$) of glycine in water at different temperatures and molalities.

Table G.1. Values needed for the calculation of adiabatic compressibility of glycine in water.

T (K)	ρ ($kg \cdot m^{-3}$)	u ($m \cdot s^{-1}$)	a_k ($kg^3 \cdot m^{-4} \cdot s^{-2} \cdot mol^{-1}$)	K_s^0($cm^3 \cdot mol^{-1} \cdot GPa^{-1}$)
278.15	999.967	1426.17	0.3121	-38.5
288.15	999.103	1465.93	0.3054	-31.4
298.15	997.048	1496.70	0.3001	-26.8
308.15	994.033	1519.85	0.2948	-23.7
318.15	990.213	1536.45	0.2901	-21.7

Appendix G: Example of Calculation of Adiabatic Compressibility through the Speed of Sound

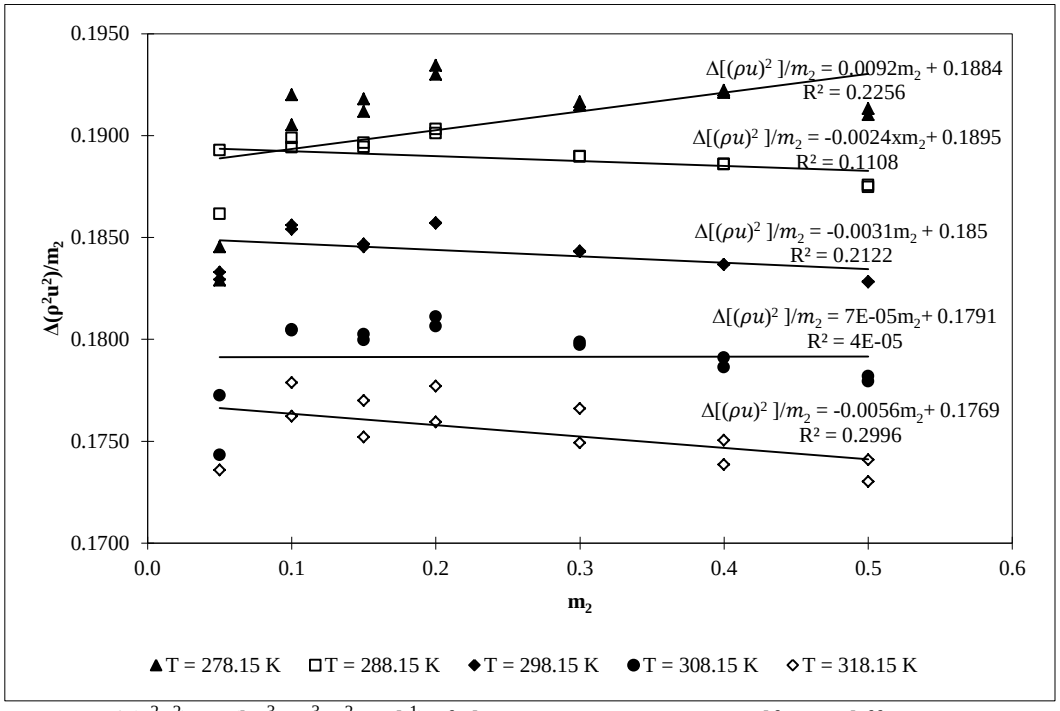


Figure G.2. $\Delta(\rho^2u^2)/m_2$ ($kg^3 \cdot m^{-3} \cdot s^{-2} \cdot mol^{-1}$) of alanine in 2.0 ammonium sulfate at different temperatures and molalities.

Table G.2. Values needed for the calculation of adiabatic compressibility of alanine in 2.0 ammonium sulfate.

T (K)	ρ ($kg \cdot m^{-3}$)	u ($m \cdot s^{-1}$)	a_k ($kg^3 \cdot m^{-4} \cdot s^{-2} \cdot mol^{-1}$)	K_s^0($cm^3 \cdot mol^{-1} \cdot GPa^{-1}$)
278.15	1124.482	1653.510	0.1884	10.0
288.15	1121.256	1678.170	0.1895	10.0
298.15	1117.691	1697.150	0.1850	10.5
308.15	1113.817	1711.325	0.1791	11.0
318.15	1109.607	1721.240	0.1749	11.3

As can be seen from the examples presented in the case of glycine in water the obtained values can be considered as acceptable. However, in the case of alanine in 2.0 ammonium sulfate a valid relation of $\Delta[(\rho u)^2]/m_2$ as a function of the molality of the amino acid was not found. Then, the adiabatic compressibility values calculated are not reliable. The same happened to all other solutions with sulfate concentrations between 0.1 and 2.0, and for both amino acids.