

**Partial Molar Volumes of Amino Acids in Aqueous MgSO<sub>4</sub> Solutions  
between 278.15 and 308.15 K**

**Ana Carolina Costa Mota**

Dissertation presented to the  
**Escola Superior de Tecnologia e Gestão**  
**Instituto Politécnico de Bragança**

To obtain the degree of Master in

**Biomedical Technology**

**September 2014**

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This Dissertation include the critiques and suggestions made by the Jury

September 2014

*Adoramos a perfeição porque não a podemos ter;  
repugná-la-íamos se a tivéssemos. O perfeito é  
desumano, porque o humano é imperfeito.*

*Fernando Pessoa*



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## Abstract

The main objective of this work is to contribute to the understanding of the molecular interactions between ions and protein groups in aqueous solutions, using amino acids as model compounds, by determining the partial molar volumes of glycine, L-alanine, DL-2-aminobutyric acid and L-valine in aqueous magnesium sulphate solutions.

The densities of aqueous solutions of magnesium sulphate (0.1, 0.3, 0.7 and 1.0) mol·kg<sup>-1</sup> containing the selected amino acids were measured at (278.15, 288.15, 298.15 and 308.15) K, using a digital density meter. After, the partial molar volumes at infinite dilution of the amino acids were calculated, and then used to obtain the corresponding transfer volumes and hydration numbers.

The dehydration effect on the studied amino acids was observed, increasing temperature or salt molality. The positive values of  $\Delta_{tr}V_{m,A}^0$  suggest that the interactions ion/hydrophilic group (zwitterionic centers) are predominant, and applying the methodology proposed by Friedman and Krishnan (1973), it was concluded that they are mainly pairwise. Regarding the alkyl chain effect, the increase of the hydrophobic part of amino acids (glycine > L-alanine > DL-2-aminobutyric > L-valine) does not result in a decreasing trend of the partial molar volumes of transfer.

To further analyse the alkyl chain effect of the amino acids, a group contribution method was successfully applied to model the partial molar volumes data. The contribution of the zwitterionic (NH<sub>3</sub><sup>+</sup>, COO<sup>-</sup>) groups to the value of the standard partial molar volume predominates and increases with increasing magnesium sulphate concentration; in general, the contribution of the alkyl groups is much smaller, having a very weak decreasing trend with increasing salt molality.

**Keywords:** Partial Molar Volumes, Amino Acids, Hydration Numbers, Electrolyte.





## Resumo

O principal objetivo deste trabalho é contribuir para a compreensão das interações moleculares entre íons e grupos de proteínas em água, utilizando aminoácidos como compostos modelo, através da determinação dos volumes molares parciais da glicina, L-alanina, DL-2-ácido aminobutírico e L-valina, em soluções aquosas de sulfato de magnésio.

As densidades das soluções aquosas de sulfato de magnésio (0,1, 0,3, 0,7 e 1,0) mol·kg<sup>-1</sup> contendo os aminoácidos selecionados foram medidas a (278,15; 288,15; 298,15 e 308,15) K, usando um densímetro digital. Depois, os volumes molares parciais a diluição infinita dos aminoácidos foram calculados, e, em seguida, utilizados para a obtenção dos correspondentes volumes de transferência e números de hidratação.

Observou-se um efeito de desidratação nos aminoácidos estudados, aumentando a temperatura ou a molalidade de sal. Os valores positivos de  $\Delta_{tr}V_{m,A}^0$  sugerem que as interações íão/grupo hidrofílico (centros zeuteriônicos) são predominantes, e aplicando a metodologia proposta por Friedman e Krishnan (1973), concluiu-se que ocorrem principalmente entre pares. Relativamente ao efeito da cadeia alquílica, o aumento da parte hidrofóbica dos aminoácidos (glicina > L-alanina > DL-2-ácido aminobutírico > L-valina) não resulta numa diminuição dos volumes molares parciais de transferência.

Para analisar melhor o efeito da cadeia alquílica dos aminoácidos, aplicou-se um método de contribuição de grupos para modelar os dados dos volumes molares parciais. A contribuição dos grupos zeuteriônicos (NH<sub>3</sub><sup>+</sup>, COO<sup>-</sup>) para o valor do volume molar parcial padrão predomina e aumenta com o aumento da concentração de sulfato de magnésio; em geral, a contribuição dos grupos alquilo é muito menor, tendo uma tendência muito fraca de diminuição com o aumento da molalidade de sal.

**Palavras-chave:** Volumes Molares Parciais, Aminoácidos, Números de Hidratação, Eletrólito.



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## Notation

$a, b, c$  Empirical constants

$a_v$  Parameter (the intercept)

$b_v$  Linear equation slope

$m_A$  Amino acid molality ( $\text{mol}\cdot\text{kg}^{-1}$ )

$m_s$  Salt molality ( $\text{mol}\cdot\text{kg}^{-1}$ )

$M$  Mass (g)

$MM$  Molar mass ( $\text{g}\cdot\text{mol}^{-1}$ )

$n_C$  Number of carbon atoms in the alkyl chain of the amino acids

$n_H$  Hydration number

$T$  Absolute temperature (K)

$V_{A,S}$  Pair interaction coefficient

$V_{A,SS}$  Triplet interaction coefficient

$V_{2,\phi}$  Apparent molar volume

$V_{m,A}^0$  Partial molar volume

$w$  Mass fraction

$\Delta_{\text{tr}}V_{m,A}^0$  Partial molar volume of transfer

## Greek symbols

$\rho_0$  Density of pure water for binary systems or density of binary (salt + water) for ternary systems

$\rho_B$  Density of binary solutions

$\rho_T$  Density of ternary solutions

$\Delta\rho$   $\rho - \rho_0$

### **Abbreviations**

A Amino acid

Aaba DL-2-aminobutyric acid

Ala Alanine

Gly Glycine

PMV Partial molar volume(s)

PME Partial molar expansion

Val Valine

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# Chapter 1

## Introduction

### 1.1. Motivation and Objectives

Amino acids are the building blocks of proteins, with significant biological and industrial importance. These biomolecules are used in many applications, mainly, in the pharmaceutical, food and chemical industries. Due to their importance in biochemistry, several studies with amino acids aim to understand and describe their physicochemical properties.

The main objective of this work is to contribute to the understanding of the molecular interpretation between ions and protein groups in aqueous solutions, by performing the experimental measurement of partial molar volumes (PMV) of selected model compounds in aqueous saline solutions.

In this work, four amino acids (glycine, L-alanine, DL-2-aminobutyric acid and L-valine) were selected as model compounds, as they are the fundamental structural units of proteins, and magnesium sulphate was chosen as the electrolyte since it provokes dramatic and, sometimes, opposite effects on amino acid solubility's. The volumetric properties of these solutions will provide important information about solute-solute and solute-solvent interactions that govern protein hydration, denaturation, and aggregation.

The results, combined with information gathered from the literature review, aim to contribute to a better understanding of the forces that manage important biological structures, in other words, for a better molecular interpretation of the interactions between the ions ( $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ ), and the groups of proteins ( $\text{NH}_3^+$ ,  $\text{COO}^-$  and alkyl chain).

## **1.2. Structure of the Work**

At the present chapter, some introductory concepts will be presented such as the definition of molecular interactions present in aqueous saline solutions containing amino acids, and the definition of partial molar volumes. In Chapter 2, a review of the studies published in the literature about partial molar volumes, and related properties, in aqueous saline solutions containing amino acids is presented.

The experimental methods and materials are presented in Chapter 3, and the discussion of the results obtained is made in Chapter 4. Finally, in Chapter 5, the conclusions and some suggestions for future work are given.

## **1.3. Introductory Concepts**

For a better understanding of this work, in this section, important theoretical concepts related to the chemistry of aqueous saline solutions can be found.

Proteins are large complex molecules. Therefore, direct study of electrolyte-protein interactions is difficult. The wide range of interactions present, are affected with the change in the concentration of the electrolyte studied. Salt induced electrostatic forces are known to play an important role in modifying the protein structure by affecting properties like solubility, denaturation and the activity of enzymes. These interactions are also affected by the surrounding solutes and solvent, for this reason, the physicochemical behaviours of proteins are strongly influenced by the presence of solutes. Because of direct solute-solvent interactions and/or alteration of the water structure, these solutes can change many properties of globular proteins such as their hydration, solubility and stability.

Despite the ample use and importance of amino acids in many industries, their interactions with electrolytes, and their physicochemical properties in electrolyte solutions, have been subject of a few investigations.

The detailed three-dimensional structure of proteins and nucleic acids provides critical information about the molecules, but they provide no information about the stability of a molecule or the energetic of its interactions. The interactions of water with the various

functional groups of proteins play crucial role in determining the conformational stability of proteins (Dhir 2012).

### 1.3.1. Molecular Interactions in Aqueous Saline Solutions of Biomolecules

It is widely appreciated that water molecules play an invaluable role in governing the structure, stability and function of biomolecules (Voet *et al.* 1999; Dhir 2012).

Water molecules interact strongly with ions. A particularly strong interaction occurs when an ionic substance such as sodium chloride (or magnesium sulphate) dissolves in water. Owing to its high polarity, the H<sub>2</sub>O molecules closest to the dissolved ion are strongly attached to it, forming what is known as the inner or primary hydration shell (Dhir 2012).

The water molecule is polar, with charges of contrary signs. In the liquid state, and further in the solid state, molecules interact by hydrogen bonding. The properties of the water molecule have direct consequences on the behaviour of biomolecules.

In this section, the main types of non-covalent reversible bonding involved in molecular interactions in most biological systems will be briefly described, as presented in three reference books of Chemistry (Chang 2000; Atkins and Paula 2005; Campos 2005).

#### 1.3.1.1. Ion-Ion Interactions

A charged group of a substrate attracts a group of opposite charge sign of a protein. The electrostatic attraction force is given by Coulomb's Law, here exemplified for the sodium chloride salt (Equation 1.1):

$$V = - \frac{q_{Na^+} \times q_{Cl^-}}{4\pi\epsilon_0 r} \quad (1.1)$$

where  $q_{Na^+}$  and  $q_{Cl^-}$  are the charges of the two ions,  $r$  is the distance between ions and  $\epsilon_0$  is the permittivity of the vacuum ( $8.854 \times 10^{-12} \text{ C}^2 \cdot \text{N}^{-1} \cdot \text{m}^{-2}$ ). This type of interaction is also called ionic bond (Campos 2005). The negative sign shows that the interaction is attractive (Chang 2000; Atkins and Paula 2005).

### 1.3.1.2. Dipole-Dipole Interactions

An intermolecular interaction of the dipole-dipole type occurs between polar molecules, which possess permanent dipole moments. Consider the electrostatic interaction between the two dipoles  $\mu_A$  and  $\mu_B$  separated by distance  $r$ , as shown in Figure 1.1 (Chang 2000).

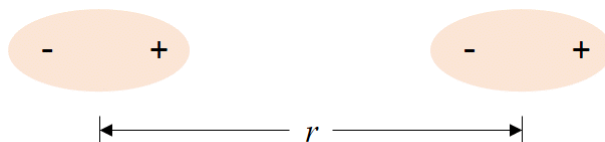


Figure 1.1. Schematic drawing showing two permanent dipoles for attractive interaction (adapted from Chang (2000)).

In this example, the potential energy of interaction is given by Equation 1.2:

$$V = -\frac{2\mu_A\mu_B}{4\pi\epsilon_0r^3} \quad (1.2)$$

where the negative sign indicates that the interaction is attractive; that is, energy is released when these two molecules interact. Reversing the charge signs of one of the dipoles makes  $V$  a positive quantity. Then the interaction between the two molecules is repulsive (Chang 2000).

### 1.3.1.3. Ion-Dipole Interactions

The existence of permanent dipoles will also allow the binding between the polar molecules and ions. Thus, the dissolution of a salt in water results from ion-dipole attractions that develop between the poles of the water molecule (O,  $\delta^-$ ) (H,  $\delta^+$ ) and ionic poles (e.g.  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ ) (Campos 2005).

The potential energy of a dipole  $\mu$  in the presence of a charge  $q$  is calculated by taking into account the interaction of the charge with the two partial charges of the dipole, one resulting in repulsion and the other in attraction (Equation 1.3).

$$V = -\frac{q\mu}{4\pi\epsilon_0r^2} \quad (1.3)$$



Equation 1.3 holds only when the ion and the dipole lie along the same axis (Chang 2000; Atkins and Paula 2005).

#### 1.3.1.4. Ion-Induced Dipole and Dipole-Induced Dipole Interactions

A non-polar molecule may acquire a temporary induced dipole moment,  $\mu_{\text{ind}}$ , as a result of the influence of an electric field generated by a nearby ion or polar molecule. The field distorts the electron distribution of the molecule, and gives rise to an electric dipole. The molecule is said to be *polarizable*. The magnitude of the induced dipole moment is proportional to the strength of the electric field,  $\mathcal{E}$ :

$$\mu_{\text{ind}} = \alpha' \mathcal{E} \quad (1.4)$$

where  $\alpha'$ , the proportionality constant, is the polarizability of the molecule (Atkins and Paula 2005). The potential energy of interaction between ion-induced dipole is given by Equation 1.5:

$$V = -\frac{1}{2} \times \frac{\alpha q^2}{4\pi\epsilon_0 r^4} \quad (1.5)$$

where  $\alpha = \alpha'/4\pi\epsilon_0$  (Chang 2000).

A polar molecule with dipole moment  $\mu$  can induce a dipole moment in a polarizable molecule because the partial charges of the polar molecule give rise to an electric field that distorts the second molecule. That induced dipole interacts with the permanent dipole of the first molecule, and the two are attracted together. The formula for the dipole-induced dipole interaction energy is:

$$V = -\frac{\alpha\mu^2}{4\pi\epsilon_0 r^6} \quad (1.6)$$

where  $\alpha$  is the polarizability of the non-polar molecule (Chang 2000; Atkins and Paula 2005).

#### 1.3.1.5. Dispersion Interactions

The dispersion interaction, or London forces, between non-polar species arises from the transient dipoles that they possess as a result of fluctuations in the instantaneous positions of their electrons. Suppose, for instance, that the electrons in one molecule flicker

into an arrangement that results in partial positive and negative charges and thus gives it an instantaneous dipole moment  $\mu_1$ . While it exists, this dipole can polarize the other molecule and induce in it an instantaneous dipole moment  $\mu_2$ . The two dipoles attract each other and the potential energy of the pair is lowered. The London formula is given by:

$$V = -\frac{3}{2} \times \frac{\alpha_A \alpha_B}{r^6} \times \frac{I_A I_B}{I_A + I_B} \quad (1.7)$$

where  $I_A$  and  $I_B$  are the ionization energies of the two molecules (Atkins and Paula 2005).

#### **1.3.1.6. Hydrogen Bonding**

It is the strongest bond of designated "weak bonds". This is a type of bond with very specific characteristics but abundantly represented in biological systems (from the outset, in water). In this type of bonding, a hydrogen atom is shared by two other atoms. That which the H is more strongly attached is designated hydrogen donor, while the other is a hydrogen acceptor.

The donor in biological systems is an oxygen or a nitrogen atom covalently bonded to a hydrogen. The acceptor has a partial negative charge (represented by a pair of unshared electrons) which attracts hydrogen (Campos 2005).

#### **1.3.2. Salt Effects on Protein Aqueous Solutions**

A protein contains multiple charged groups so its solubility depends on the concentrations of dissolved salts, the polarity of the solvent, the pH and the temperature. In the case of ion specific effects in several biological systems, some of the earliest and reference works in this area were published by Franz Hofmeister in the 1880s and Kunz *et al.* (2004), respectively.

In Figure 1.2 a typical Hofmeister series is presented in which the ions effect on important aspects of the behaviour of proteins in aqueous solutions is established.

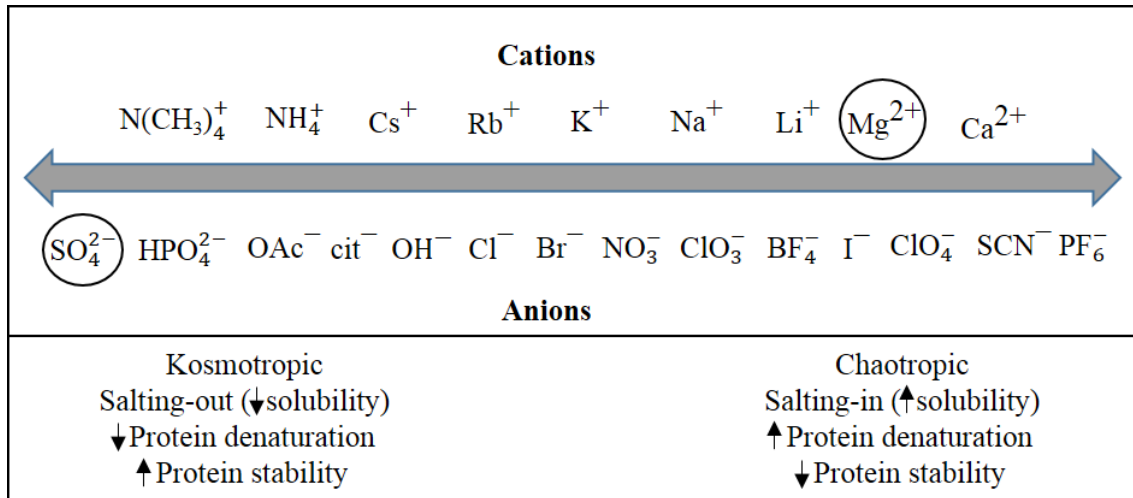


Figure 1.2. Classification of cations and anions in a typical Hofmeister series (adapted from Kunz (2010)).

The salting-in phenomenon can be described as the increase of the solubility of a protein as salt is added. The opposite effect, salting-out, is the basis of one of the most commonly used protein purification procedures (Voet *et al.* 1999).

According to Kunz (2010), in the case of cations the series goes from soft weakly hydrated ions on the left, to hard strongly hydrated ions on the right, being the opposite in the case of the anions. To support this classification, Table 1.1 shows the hydration energies of important ions.

Table 1.1. Molar entropy of hydration,  $\Delta_{hyd}S$ , and Gibbs free energy of hydration,  $\Delta_{hyd}G$ , at 298.15 K (Marcus 1991; Marcus 1997).

Ion	$\Delta_{hyd}G$ (kJ mol <sup>-1</sup> )
SCN <sup>-</sup>	-280
NO <sub>3</sub> <sup>-</sup>	-300
Cl <sup>-</sup>	-340
CH <sub>3</sub> COO <sup>-</sup>	-365
SO <sub>4</sub> <sup>2-</sup>	-1080
NH <sub>4</sub> <sup>+</sup>	-285
K <sup>+</sup>	-304
Na <sup>+</sup>	-365
Li <sup>+</sup>	-475
Ca <sup>2+</sup>	-1515
Cd <sup>2+</sup>	-1755

continued	
Ion	$\Delta_{\text{hyd}}G$ (kJ mol <sup>-1</sup> )
Mn <sup>2+</sup>	-1760
Mg <sup>2+</sup>	-1830
Zn <sup>2+</sup>	-1955

The cation (Mg<sup>2+</sup>) and anion (SO<sub>4</sub><sup>2-</sup>) studied in this work are highlighted in Figure 1.2 and Table 1.1. As can be seen both Mg<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup> are strongly hydrated ions.

In general, specific cation effects are less pronounced than specific anion effects, because anions have stronger interactions with water than cations of the same size and absolute charge density. However, this is only true when ion–water interactions are dominant for the specific ion effects. When direct ion/ion or ion/charged head-group interactions are dominant, specific cation effects can be of the same order of magnitude as specific anion effects (Kunz 2010).

In terms of salts, for example, high salt concentration of Na<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COONa and MgSO<sub>4</sub> stabilize proteins whereas salts such as MgCl<sub>2</sub>, CaCl<sub>2</sub> and KSCN denature proteins (Mallick and Kishore 2006).

### 1.3.3. Partial Molar Volumes

The partial molar volume (PMV) of a solute may be visualized by considering a large reservoir of its solution, so that the addition of one mole of the solute does not alter the concentration of the solution. The change in the volume of the solution upon addition of one mole of solute to this large reservoir is the partial molar volume when the temperature,  $T$ , pressure,  $P$ , and the number of moles of the other components if present remains unchanged (Dhir 2012).

Partial molar volume is a thermodynamic property which can be obtained from the density, being very useful in the study of solute-solvent interactions in solutions. With the addition of salts is intended to observe the effect these have on the amino acids, that is, to verify that the amino acids expand or contract in the presence of salts.

To derive the expressions for the PMV at infinite dilution, the following nomenclature for the chemical species was adopted: 1  $\equiv$  water, A  $\equiv$  amino acid and 3  $\equiv$  salt.

First, the concept of apparent molar volume of the amino acid ( $V_{A,\phi}$ ) in aqueous saline solutions is defined as:

$$V_{A,\phi} = \frac{V_T - V_B}{n_A} \quad (1.8)$$

where  $V_T$  is the volume of the ternary system,  $V_B$  is the volume of the binary system and  $n_A$  is the number of moles of amino acid.

$$M_T = M_1 + M_A + M_3 \quad (1.9)$$

where  $M_T$  is the total mass,  $M_1$  is the mass of water,  $M_A$  is the mass of amino acid and  $M_3$  is the mass of salt.

Considering the definition of molality, we have:

$$m = \frac{n_i}{M_1} = \frac{M_i / MM_i}{M_1} (=) M_i = m \times MM_i \times M_1 \quad (1.10)$$

where  $m$  is the molality and  $MM_i$  is the molar mass of the species  $i$ .

Substituting Equation 1.10 into 1.9, the following result is obtained:

$$M_T = M_1 + m_A \times MM_A \times M_1 + m_3 \times MM_3 \times M_1 \quad (1.11)$$

By analogy:

$$M_B = M_1 + m_3 \times MM_3 \times M_1 \quad (1.12)$$

Considering that  $V = \frac{M}{\rho}$  then:

$$V_T = \frac{M_T}{\rho_T} = \frac{M_1 \times (1 + m_A \times MM_A + m_3 \times MM_3)}{\rho_T} \quad (1.13)$$

$$V_B = \frac{M_B}{\rho_B} = \frac{M_1 \times (1 + m_3 \times MM_3)}{\rho_B} \quad (1.14)$$

By replacing  $V_T$  and  $V_B$  in Equation 1.8:

$$V_{A,\phi} = \frac{\frac{M_1 \times (1 + m_A \times MM_A + m_3 \times MM_3)}{\rho_T} - \frac{M_1 \times (1 + m_3 \times MM_3)}{\rho_B}}{n_A} \quad (1.15)$$

Dividing Equation 1.15 by  $M_1$ :

$$V_{A,\phi} = \frac{MM_A}{\rho_T} - (1 + m_3 \times MM_3) \times \frac{(\rho_T - \rho_B)}{m_A \times \rho_T \times \rho_B} \quad (1.16)$$

At infinite dilution,  $m_A \rightarrow 0$ ,  $\rho_T \rightarrow \rho_B$ , and the apparent molar volume and the partial molar volume become equal ( $V_{A,\phi} = V_{m,A}^o$ ). To overcome the evident mathematical difficulty present in Equation 1.16, the difference between the density of the ternary and binary solutions, divided by the amino acid molality, is represented by a linear relation using the experimental measured density data:

$$\lim_{m_A \rightarrow 0} \frac{\rho_T - \rho_B}{m_A} = \lim_{m_A \rightarrow 0} a_v + b_v \times m_A = a_v$$

Applying the limit to Equation 1.18, the following result is obtained:

$$V_{m,A}^o = \frac{MM_A}{\rho_B} - (1 + m_3 \times MM_3) \times \frac{a_v}{\rho_B^2} = \frac{1}{\rho_B} \times \left[ MM_A - (1 + m_3 \times MM_3) \times \frac{a_v}{\rho_B} \right] \quad (1.17)$$

Similarly, the equation to calculate  $V_{A,\phi}$  in binary systems can be obtained:

$$V_{m,A}^o = \frac{MM_A}{\rho_0} - \frac{a_v}{\rho_0^2} \quad (1.18)$$

### 1.3.4. Interpretation of Intermolecular Interactions

The stabilization of biological macromolecules is commonly linked to several non-covalent interactions including hydrogen bonding, electrostatic and hydrophobic interactions, that are affected by the solvent of macromolecules and surrounding solutes (Zhao 2006). Volumetric properties (e.g. standard partial molar volumes) as well as changes in enthalpy and free energy in water and solutions of organic solvents or salts, can provide valuable clues to understand 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 understanding 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 hydrophobicity criteria proposed by Hepler (1969) uses the partial molar volume derivatives with temperature to reflect about the hydrophobicity of the solute:

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

- If  $(\partial V_{m,A}^0/\partial T)_P < 0$  and  $(\partial^2 V_{m,A}^0/\partial T^2)_P > 0$ , the solute is hydrophobic;

where  $(\partial V_{m,A}^0/\partial T)_P$  is the partial molar expansion.

Given these criteria, the studies presented by Romero and Negrete (2004) have suggested that the hydrophilic interactions between water and amino acids are stronger than the hydrophobic interactions, but they decrease with increasing length of the hydrophobic chains.

The zwitterionic parts of amino acids are hydrated by hydrophilic hydration whereas its apolar part is hydrated by hydrophobic hydration. The overlap of different hydrated spheres of amino acids and co-solutes occurs. Thus, a water molecule mainly from the hydrophobic hydration sphere gets out. A good indicator of these released water molecules due to different types of overlap among hydration spheres, is the volume change during transfer (Das *et al.* 2004). Hence, the co-sphere overlap model can be used to rationalize the values in terms of solute-co-solute interactions (Siddique and Naqvi 2010).

This model states that when two solute particles come sufficiently close together so that their co-spheres overlap, some co-sphere material is displaced and this is accompanied by the change in thermodynamic parameters (Banipal *et al.* 2007).

For the ternary systems (salt + amino acids + water), the overlap of co-solute ions and amino acids comes into play because of interactions between: (i) the  $(-\text{NH}_3^+, -\text{COO}^-)$  charged ends of amino acids and ions of the co-solute (called ion-charged/hydrophilic group or ion-ion interactions); (ii) the hydrophobic parts of the amino acids and co-solute ions or the charged ends/hydrophilic parts of amino acids and the hydrophobic parts of the co-solutes (called ion-hydrophobic group interactions); and (iii) the hydrophobic parts of the amino acids and hydrophobic parts of ions of co-solutes (called hydrophobic-hydrophobic group interactions) (Siddique and Naqvi 2010).

Akhtar (2007) investigated the thermodynamic of amino acids in aqueous salt solutions and identify the significant hydration characteristics of the solutes: (i)  $\text{NH}_3^+$  and  $\text{COO}^-$  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  $\text{NH}_3^+$  group is greater than that of the  $\text{COO}^-$  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 owing to the different types of interactions above described are shown in Figure 1.3. Observing Figure 1.3, it is verified that the overlap of two hydrophobic hydration co-spheres relaxes some water molecules from the solvation sphere to the bulk giving rise to a negative change in volume. For hydrophilic ionic species the volume of water molecules is smaller in the solvation shell due to (i) the effect of electrostriction (electrostriction is a property of all electrical non-conductors, or dielectrics that causes them to change their shape under the application of an electric field) and (ii) a decrease in the hydrogen-bonded network of water molecules in the solvation sphere than in the bulk (the so-called structure- breaking effect).

The structure-breaking influence of ionic species on the hydrophobic hydration sphere of apolar groups gives a negative volume effect. The overlap of co-spheres of two ionic species relaxes some solvation water to bulk so that overall structure is increased, giving rise to positive volume change (Mishra and Ahluwalia 1984).



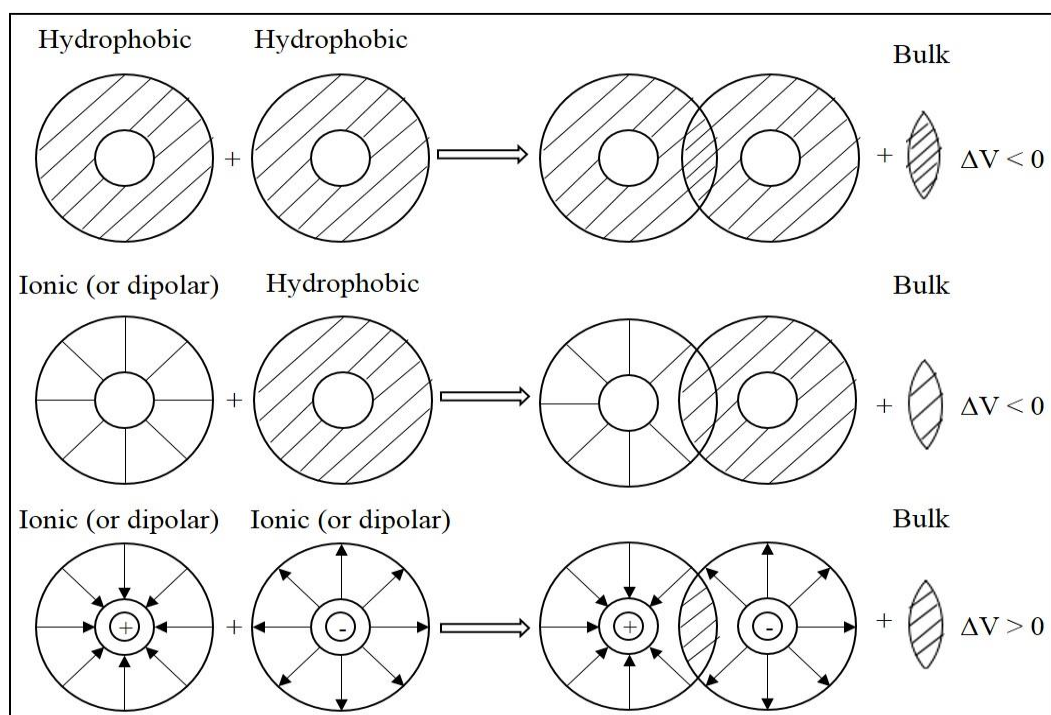


Figure 1.3. Solute-solute interactions through the overlap of hydration co-spheres and the resulting volume changes in aqueous solutions (adapted from Mishra and Ahluwalia (1984)).



## Chapter 2

### State of the Art

A very complete review on the topic partial molar volumes of amino acids in aqueous solutions was published in the last decade by Zhao (2006). More recently, an update was made by Martins (2012) for the amino acids glycine and alanine. The above information was considered and completed with experimental data published until May 2014 for the four amino acids studied in this work.

This information will be useful, in Chapter 3, to assess the data quality of salt/amino acid binary and ternary aqueous systems obtained in this work. Additionally, the effect of different salts will be compared in Chapter 4.

First, the results from a literature review related to the partial molar volumes of glycine, alanine (L or DL isomers), DL-2-aminobutyric acid and valine (L or DL isomers) in water is presented in Table 2.1. Due to the large number of references available for glycine and alanine, it was decided to report only the more recent, and/or the references that also included data for the other amino acids (the complete set can be found in Martins (2012), Zhao (2006) and in Appendix B).

Similar information is available in Tables 2.2, 2.3, 2.4 and 2.5, for the partial molar volumes of glycine, alanine, DL-2-aminobutyric acid and valine, respectively, in aqueous saline solutions.

Table 2.1. References containing partial molar volumes and other properties for the binary systems (amino acid + water) published in the literature.

Amino acids	Temperature (K)	Reference
glycine	298.15	Banipal and Singh (2003), Pal and Kumar (2004), Banipal <i>et al.</i> (2004), Zhao (2006), Mallick and Kishore (2006), Talele and Kishore (2013)
	288.15 to 318.15	Rima <i>et al.</i> (2013), Dhir (2012) and Banipal <i>et al.</i> (2008)
	298 to 443	Cibulka <i>et al.</i> (2010)
	308.15	Munde and Kishore (2003)
	278.15 to 318.15	Martins <i>et al.</i> (2014)
alanine	397 to 521	Hakin <i>et al.</i> (2000)
	298 to 443	Cibulka <i>et al.</i> (2010)
	308.15	Munde and Kishore (2003)
	298.15	Banipal and Singh (2003), Banipal <i>et al.</i> (2004), Zhao (2006), Talele and Kishore (2013), Mallick and Kishore (2006)
	288.15 to 318.15	Rima <i>et al.</i> (2013), Dhir (2012) and Banipal <i>et al.</i> (2008)
	278.15 to 318.15	Martins <i>et al.</i> (2014)
DL-2-aminobutyric acid	298.15	Banipal and Singh (2003), Banipal <i>et al.</i> (2004), Zhao (2006), Talele and Kishore (2013), Mallick and Kishore (2006)
	288.15 to 303.15	Romero and Negrete (2004)
	288.15 to 318.15	Rima <i>et al.</i> (2013), Dhir (2012) and Banipal <i>et al.</i> (2008)
valine	298.15	Banipal and Singh (2003), Zhao (2006), Mallick and Kishore (2006), and Singh and Kishore (2003)
	288.15 to 318.15	Banipal <i>et al.</i> (2008) and Dhir (2012)
	288.15, 298.15 and 308.15	Islam and Wadi (2003)
	297.15	Dipaola and Belleau (1978)

Table 2.2. References for the partial molar volumes and other properties for the ternary system glycine + salt + water published in the literature.

Salt	Temperature (K)	Reference
NaCl	298.15	Bhat and Ahluwalia (1985)
	298.15	Yuan <i>et al.</i> (2006)
	298.15	Ogawa <i>et al.</i> (1984)
	278.15, 288.15, 298.15 and 308.15	Shen <i>et al.</i> (2000)
Na <sub>2</sub> SO <sub>4</sub>	288.15, 298.15 and 308.15	Islam and Wadi (2003)
	288.15, 298.15 and 308.15	Wadi and Ramasami (1997)
	298.15	Singh and Kishore (2003)
MgCl <sub>2</sub>	288.15, 298.15, and 308.15	Lark <i>et al.</i> (2004)
	288.15 and 308.15	Pal and Kumar (2005b)
	298.15	Pal and Kumar (2005a)
	298.15	Badarayani and Kumar (2003a)
NaBr and KBr		Badarayani and Kumar (2003a)
(CH <sub>3</sub> ) <sub>4</sub> NBr, (C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> NBr and (C <sub>4</sub> H <sub>9</sub> ) <sub>4</sub> NBr		Badarayani and Kumar (2004a)
C <sub>8</sub> H <sub>20</sub> BrN		Banerjee and Kishore (2005)
MgSO <sub>4</sub>		Mallick and Kishore (2006)
NH <sub>4</sub> Cl		Natarajan <i>et al.</i> (1990)
LiCl	298.15	Ogawa <i>et al.</i> (1984)
NaSCN		Singh and Kishore (2003)
NaCH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> OSO <sub>3</sub> and BrC <sub>16</sub> H <sub>33</sub> N(CH <sub>3</sub> ) <sub>3</sub>		Singh <i>et al.</i> (2004)
KCl		Badarayani and Kumar (2003a)
		Ogawa <i>et al.</i> (1984)
Mg(CH <sub>3</sub> COO) <sub>2</sub>		Banipal <i>et al.</i> (2006)
C <sub>15</sub> H <sub>34</sub> BrN and C <sub>17</sub> H <sub>38</sub> BrN		Talele and Kishore (2013)
NaCH <sub>3</sub> COO	298.15	Banipal <i>et al.</i> (2004)
	298.15	Singh and Kishore (2003)
	308.15	Wang <i>et al.</i> (1999)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Martins <i>et al.</i> (2014)
CH <sub>5</sub> N <sub>3</sub> .HCl	278.15, 288.15, 298.15 and 308.15	Yan <i>et al.</i> (1998)
CaCl <sub>2</sub>		Yan <i>et al.</i> (2004)
ZnCl <sub>2</sub>	288.15, 298.15, 308.15 and 318.15	Banipal <i>et al.</i> (2008)
KSCN	288.15, 298.15 and 308.15	Wadi and Goyal (1992)

Table 2.3. References for the partial molar volumes and other properties for the ternary system alanine + salt + water published in the literature.

Salt	Temperature (K)	Reference
KCl	283.15 to 313.15 298.15	Badarayani and Kumar (2003b) Ogawa <i>et al.</i> (1984)
Mg(CH <sub>3</sub> COO) <sub>2</sub>		Banipal <i>et al.</i> (2006)
C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub>		Banipal <i>et al.</i> (2004)
(CH <sub>3</sub> ) <sub>4</sub> NBr, (C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> NBr and (C <sub>4</sub> H <sub>9</sub> ) <sub>4</sub> NBr		Badarayani and Kumar (2004a)
C <sub>8</sub> H <sub>20</sub> BrN		Banerjee and Kishore (2005)
MgSO <sub>4</sub>	298.15	Mallick and Kishore (2006)
NH <sub>4</sub> Cl		Natarajan <i>et al.</i> (1990)
LiCl		Ogawa <i>et al.</i> (1984)
NaSCN		Singh and Kishore (2003)
NaCH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> OSO <sub>3</sub> and BrC <sub>16</sub> H <sub>33</sub> N(CH <sub>3</sub> ) <sub>3</sub>		Singh <i>et al.</i> (2004)
NaCl	298.15	Ogawa <i>et al.</i> (1984)
	298.15	Bhat and Ahluwalia (1985)
	298.15	Rodriguez <i>et al.</i> (2003)
	298.15	Yuan <i>et al.</i> (2006)
	278.15, 288.15, 298.15 and 308.15	Shen <i>et al.</i> (2000)
Na <sub>2</sub> SO <sub>4</sub>	288.15, 298.15 and 308.15	Islam and Wadi (2003)
	288.15, 298.15 and 308.15	Wadi and Ramasami (1997)
	298.15	Singh and Kishore (2003)
MgCl <sub>2</sub>	288.15, 298.15, and 308.15	Lark <i>et al.</i> (2004)
	288.15 and 308.15	Pal and Kumar (2005b)
	298.15	Pal and Kumar (2005a)
KSCN	288.15, 298.15 and 308.15	Wadi and Goyal (1992)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Martins <i>et al.</i> (2014)
CH <sub>5</sub> N <sub>3</sub> .HCl	278.15, 288.15, 298.15 and 308.15	Yan <i>et al.</i> (1998)
CaCl <sub>2</sub>		Yan <i>et al.</i> (2004)
NaCH <sub>3</sub> COO	298.15	Singh and Kishore (2003)
	308.15	Wang <i>et al.</i> (1999)

Table 2.4. References for the partial molar volumes and other properties for the ternary system DL-2-aminobutyric acid + salt + water published in the literature.

Salt	Temperature (K)	Reference
C <sub>8</sub> H <sub>20</sub> BrN		Banerjee and Kishore (2005)
NaCl		Bhat and Ahluwalia (1985)
NH <sub>4</sub> Cl		Natarajan <i>et al.</i> (1990)
C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub>	298.15	Banipal <i>et al.</i> (2004)
Mg(CH <sub>3</sub> COO) <sub>2</sub>		Banipal <i>et al.</i> (2006)
MgSO <sub>4</sub>		Mallick and Kishore (2006)
C <sub>15</sub> H <sub>34</sub> BrN and C <sub>17</sub> H <sub>38</sub> BrN		Talele and Kishore (2013)
Na <sub>2</sub> SO <sub>4</sub>	288.15, 298.15 and 308.15	Islam and Wadi (2003)
NaCH <sub>3</sub> COO	308.15	Wang <i>et al.</i> (1999)
CH <sub>5</sub> N <sub>3</sub> .HCl	278.15, 288.15, 298.15 and 308.15	Yan <i>et al.</i> (1998)
CaCl <sub>2</sub>		Yan <i>et al.</i> (2004)
ZnCl <sub>2</sub>	288.15, 298.15, 308.15 and 318.15	Banipal <i>et al.</i> (2008)

Table 2.5. References for the partial molar volumes and other properties for the ternary systems valine + salt + water published in the literature.

Salt	Temperature (K)	Reference
BaCl <sub>2</sub>		Roy <i>et al.</i> (2013)
C <sub>8</sub> H <sub>20</sub> BrN		Banerjee and Kishore (2005)
NaCl		Bhat and Ahluwalia (1985)
MgSO <sub>4</sub>	298.15	Mallick and Kishore (2006)
NaSCN		Singh and Kishore (2003)
NH <sub>4</sub> Cl		Natarajan <i>et al.</i> (1990)
NaCH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> OSO <sub>3</sub> and BrC <sub>16</sub> H <sub>33</sub> N(CH <sub>3</sub> ) <sub>3</sub>		Singh <i>et al.</i> (2004)
MgCl <sub>2</sub>	298.15 288.15 and 308.15	Pal and Kumar (2005a) Pal and Kumar (2005b)
KCl and KNO <sub>3</sub>	298.15, 303.15, 308.15, 313.15, 318.15, and 323.15	Riyazuddeen and Khan (2009)
NaCH <sub>3</sub> COO	298.15 308.15	Singh and Kishore (2003) Wang <i>et al.</i> (1999)
CH <sub>5</sub> N <sub>3</sub> .HCl	278.15, 288.15, 298.15 and 308.15	Yan <i>et al.</i> (1998)
CaCl <sub>2</sub>		Yan <i>et al.</i> (2004)
Na <sub>2</sub> SO <sub>4</sub>	298.15 288.15, 298.15 and 308.15	Singh and Kishore (2003) Islam and Wadi (2003)
MnCl <sub>2</sub> ·4H <sub>2</sub> O	288.15, 298.15, 308.15 and 318.15	Banipal <i>et al.</i> (2012)





# Chapter 3

## Experimental Part

In this chapter, the experimental work will be presented. First, the materials and methods are described. Then, the experimental data obtained regarding the densities for the binary system (water + amino acid and water + salt) and ternary systems (salt + water + amino acid) are presented. Finally, the partial molar volumes, calculated from the density data, are given.

### 3.1. Materials and Methods

#### 3.1.1. Chemicals

Table 3.1 shows the chemical compounds used as well as their source, purity and molecular mass. The molecular mass was calculated using the atomic weight of the elements published by Wieser and Coplen (2011). Moreover, all the solutions were prepared using deionized water.

Table 3.1. Specifications of the chemicals used.

Chemical name	Supplier	Mass fraction purity (%) <sup>a</sup>	Molecular mass (g/mol)
magnesium chloride hexahydrate	Panreac	≥ 99.0	203.29910
magnesium sulphate heptahydrate	Merck	≥ 99.0	246.48000
L-valine	Merck	≥ 99.0	117.14638
DL-2-aminobutyric acid	Merck	≥ 99.0	103.11983
L-alanine	Merck	≥ 99.0	89.09328
glycine	Merck	≥ 99.7	75.06673

<sup>a</sup> Declared by the supplier

The chemical structures of the four amino acids used in this study are presented in Figure 3.1.

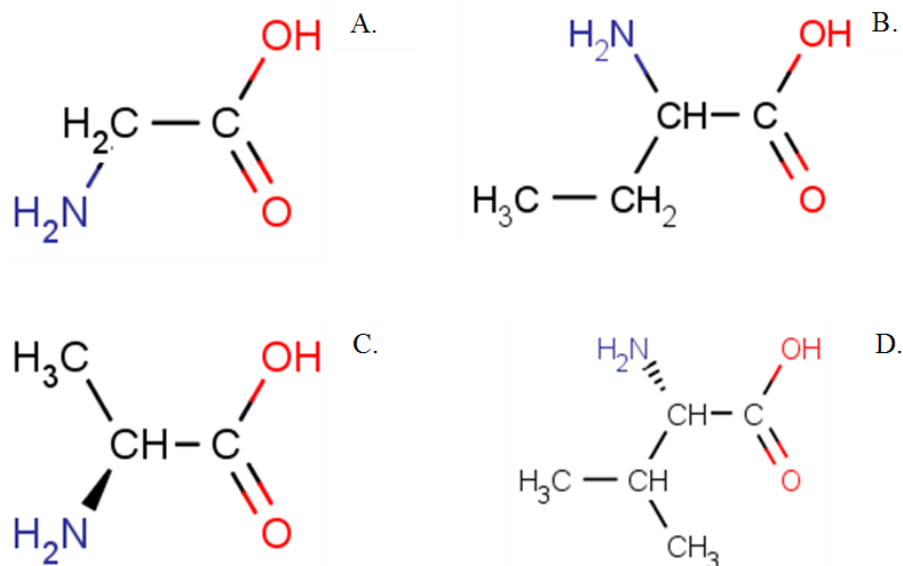


Figure 3.1. Chemical structures of the amino acids used in this work: A. glycine; B. DL-2-aminobutyric acid; C. L-alanine and D. L-valine.

### 3.1.2. Experimental Procedure

#### 3.1.2.1. Preparation of Solutions

The aqueous solutions were prepared using standard volumetric flasks (200 mL or 250 mL). All the glass material was cleaned with distilled water and, then, dried in an oven at 353.15 K. Flasks were also cleaned with acetone. All the solutions were prepared by weight using an analytical balance, *Denver Instrument* (Figure 3.2). The maximum weight is 220 g and the minimum weight is 0.01 g (uncertainty  $\pm 0.1$  mg). The uncertainty in the solute molality was estimated to be  $\pm 2 \times 10^{-5}$  mol $\cdot$ kg $^{-1}$ .

Initially, two flasks are prepared with approximately 90 grams of binary solution (water + salt) each. The weight of a cleaned and dried flask is registered and the balance tare; the required amount of the salt is added into it and the weight of the solute noted. Balance is tare again and the readjusted amount of deionized water is then added and its weight registered. The adopted procedure aimed to obtain the most similar molalities. For this purpose, a syringe BD Plastipak<sup>TM</sup> is used to accurately add the correct amount

of water to the salt. Binary solutions (water + salt) were prepared at the molalities of 0.1, 0.3, 0.7 and 1.0 molal.

After the binary solutions, the ternary solution with an amino acid molality of 0.4 molal is prepared. The cleaned and dried flask was placed in the *Denver Instrument* balance. The weight is registered and the balance tare. After, a certain mass of amino acid (calculated estimate) in a flask is weighed and, then, one of binary solutions previously prepared is added. The molality of the resulting solution is calculated.

The last step is the dilution of the ternary solution in order to obtain solutions with amino acid molalities of 0.3, 0.25, 0.20, 0.15 and 0.10 molal. The dilutions were made using the second binary solution. Both salt molalities and amino acid molalities are expressed as moles of solute per kilogram of water.



Figure 3.2. Analytical balance used throughout this work.

### 3.1.2.2. Density Measurements

The density measurements of aqueous magnesium sulphate solutions containing amino acids were performed using a vibrating-tube density meter with glass U-shaped tube *Anton Paar* DMA 5000 M (Figure 3.3). This device allows the measurement of density between 0 and 3 g·cm<sup>-3</sup> (error  $\pm$  0.000005 g·cm<sup>-3</sup>), with temperature varying between 273.15 and 368.15 K (error  $\pm$  0.01 K) and pressure range from 0 to 10 bar (Paar 2011). The measurement repeatability is  $\pm$  1 $\times$ 10<sup>-5</sup> g·cm<sup>-3</sup>.



Figure 3.3. Density meter *Anton Paar* DMA 5000 M.

At the beginning of each working day, an air check is performed. At each temperature, a water check is performed and compared to literature values (Spieweck and Bettin 1992). Approximately 20 mL of each solution, is placed in a flask under the density meter tube and, then, “Start” is pressed; the density meter collects the sample automatically and proceeds to temperature stabilization. The value of density is shown in the screen and the density meter returns the sample to the original flask. Then, the sample flask is replaced by the waste vessel and the equipment starts the cleaning process, first with de-ionized water and, after, with acetone. The measurement of each solution takes around ten minutes.

## 3.2. Experimental Data

In this section, the results of the binary systems (water + amino acid and water + salt) and of the ternary systems (salt + amino acid + water) are presented. The complete numerical density data sets are reported in Appendix A.

### 3.2.1. Binary Systems Water + Amino Acid

Four binary water + amino acid (glycine, L-alanine, DL-2-aminobutyric acid or L-valine) systems were studied.

### 3.2.1.1. Density Data

For the systems L-valine + water and DL-2-aminobutyric acid + water, two independent measurements were performed. Consistent results were obtained. Thus, the presented data are an average of both measurements.

To check the quality of the data, the density data measured in this work were compared to the results obtained by other authors, from 278.15 K to 308.15 K. As can be seen in Figure 3.4, the results of this study are in good agreement with the results of Yan *et al.* (1999) and Rima *et al.* (2013). These references were chosen as an example: the work of Rima *et al.* (2013) is recent and the work of Yan *et al.* (1999) presents experimental results for all amino acids.

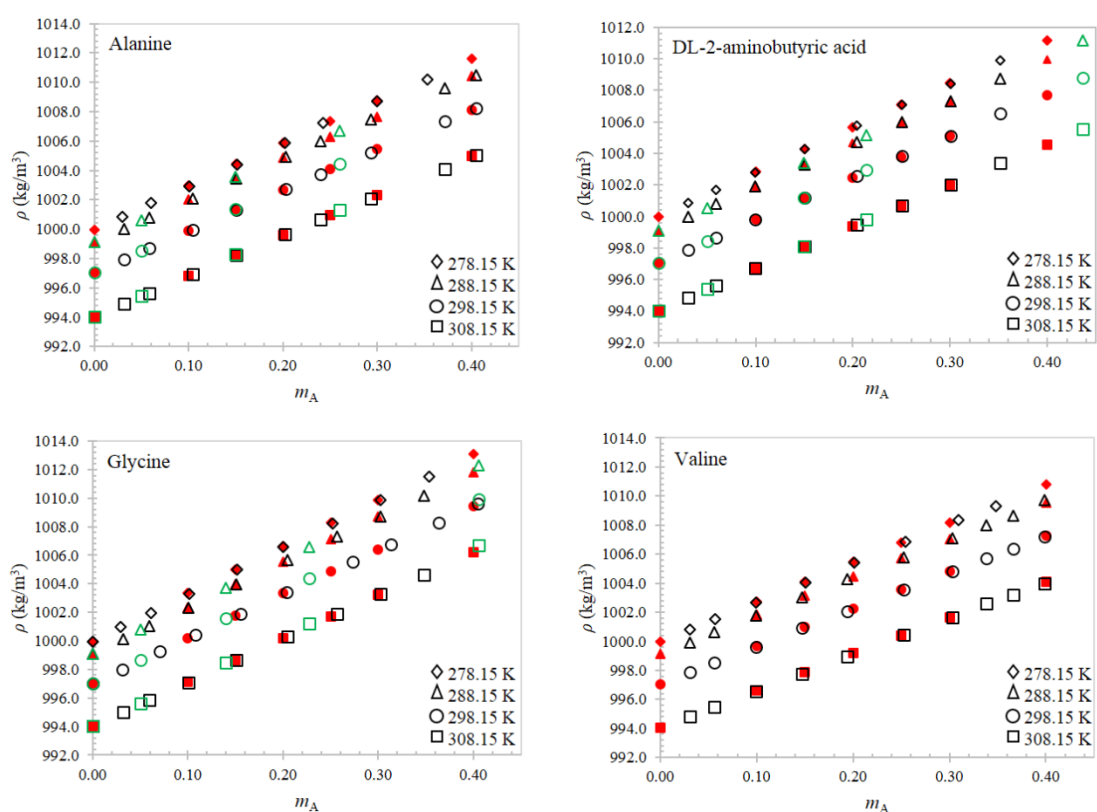


Figure 3.4. Density versus amino acid molality: red symbols (this work); black symbols (Yan *et al.* 1999) and green symbols (Rima *et al.* 2013).

Using the density data a plot is made, representing  $\Delta\rho/m_A$  as a function of the amino acid molality.

Considering Equation 3.1, a linear regression is performed, to calculate the parameter  $a_v$ :

$$\frac{\rho - \rho_0}{m_A} = a_v + b_v m_A \quad (3.1)$$

where  $\rho$  is the solution density,  $m_A$  is the amino acid molality and  $b_v$  is the linear equation slope. Figure 3.5 presents  $\Delta\rho/m_A$  as a function of the molality of the amino acid.

As can be observed in Figure 3.5 a good linear behaviour was obtained for all temperatures and for each amino acid ( $R^2 \geq 0.9955$ ). It is also possible to verify that the distance between straight lines becomes larger as temperature decreases, giving an insight on the temperature dependence of the partial molar volumes, as can be seen in the next section.

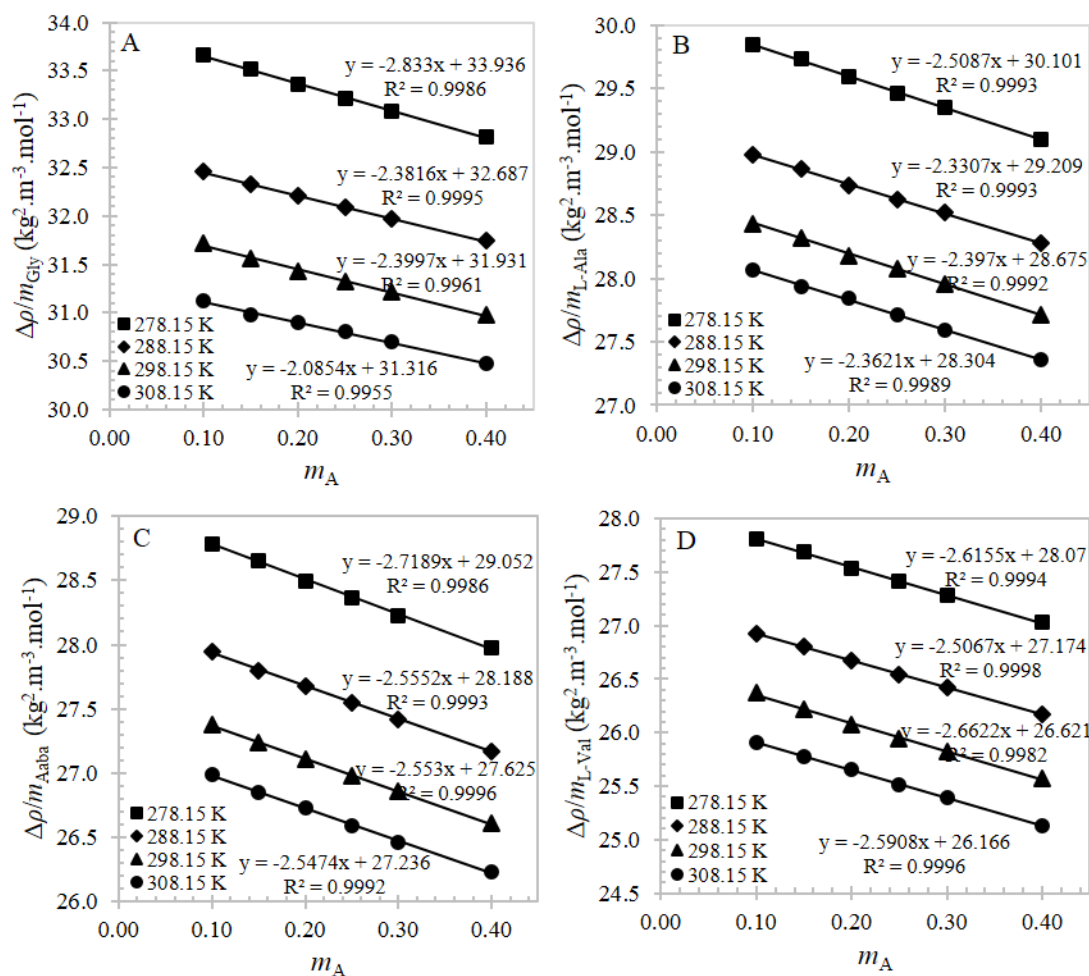


Figure 3.5.  $\Delta\rho/m_A$  versus amino acid molality: A. Gly; B. L-Ala; C. Aaba and D. L-Val.

### 3.2.1.2. Partial Molar Volumes

The next step is to calculate the partial molar volumes using Equation 1.18. Table 3.2 present a comparison between the results obtained in this work (the uncertainties are given in parenthesis) and by other studies for the PMV of glycine, L-alanine, DL-2-aminobutyric acid and L-valine, respectively, and for all studied temperatures. Again, due to the large number of references available for glycine and alanine, only the more recent ones and the values from Zhao (2006) were included for comparison purposes. In the supporting information, Appendix B, the complete PMV data tables are available.

Table 3.2. PMV ( $\text{cm}^3\text{mol}^{-1}$ ) of amino acids in aqueous solutions obtained in this work and found in the literature.

	278.15 K	288.15 K	298.15 K	308.15 K
<b>Reference</b>	<b>glycine</b>			
This work	41.13 (0.05)	42.39 (0.03)	43.17 (0.07)	43.82 (0.07)
Zhao (2006)	41.33	42.42	43.18	43.86
Martins <i>et al.</i> (2014)	41.17 (0.02)	42.39 (0.01)	43.20 (0.01)	43.76 (0.02)
Dhir (2012)	--	42.81 (0.05)	43.17 (0.02)	44.34 (0.05)
Talele and Kishore (2013)	--	--	43.14	--
Rima <i>et al.</i> (2013)	--	41.69	42.43	43.00
	<b>alanine</b>			
This work	58.99 (0.03)	59.91 (0.03)	60.51 (0.03)	60.98 (0.04)
Zhao (2006)	58.95	59.84	60.48	61.09
Martins <i>et al.</i> (2014)	58.91 (0.01)	59.83 (0.01)	60.46 (0.02)	60.92 (0.02)
Dhir (2012)	--	59.89 (0.04)	60.40 (0.03)	61.31 (0.12)
Talele and Kishore (2013)	--	--	60.43	--
Rima <i>et al.</i> (2013)	--	59.28	60.07	60.70
	<b>DL-2-aminobutyric acid</b>			
This work	74.07 (0.05)	74.97 (0.03)	75.64 (0.02)	76.18 (0.04)
Zhao (2006)	74.40	74.75	75.60	76.44
Dhir (2012)	--	75.62 (0.09)	75.97 (0.03)	76.47 (0.12)
Mallick and Kishore (2006) Talele and Kishore (2013)	--	--	75.51	--
Rima <i>et al.</i> (2013)	--	74.35	75.18	75.84
	<b>valine</b>			
This work	89.08 (0.03)	90.03 (0.02)	90.71 (0.05)	91.37 (0.02)
Zhao (2006)	89.42	89.92	90.87	91.56
Dhir (2012)	--	90.02 (0.05)	90.76 (0.02)	91.22 (0.07)
Talele and Kishore (2013)	--	--	90.39	--
Banipal <i>et al.</i> (2008)	--	90.05	90.74	91.52
Mallick and Kishore (2006)	--	--	90.39	--

In general, the values of partial molar volumes of this work are in good agreement with the results obtained by other authors, as reported by Zhao (2006). In this reference, an average obtained from the literature values was made, so it is also a good reference to compare our results. The maximum difference between the PMV of this work and Zhao (2006) is  $0.33 \text{ cm}^3\cdot\text{mol}^{-1}$  (for DL-2-aminobutyric acid at 278.15 K) and the minimum is  $0.01 \text{ cm}^3\cdot\text{mol}^{-1}$  (for valine at 288.15 K).

### 3.2.1.3. Partial Molar Expansion

Another consistency test that can be applied to the data is a comparison between the values of the partial molar expansion,  $E_{m,A}^0 = (\partial V_{m,A}^0 / \partial T)_P$ . For that, the partial molar volumes at infinite dilution in water were fitted to:

$$V_{m,A}^0 = a + bT + cT^2 \quad (3.2)$$

where  $T$  is the absolute temperature, and  $a$ ,  $b$  and  $c$  empirical constants.

After obtaining constants  $a$ ,  $b$  and  $c$ , the partial molar expansion was calculated for each temperature (278.15, 288.15, 298.15 and 308.15 K). Figure 3.6 presents four plots (one for each amino acid) representing the partial molar volume as a function of temperature.

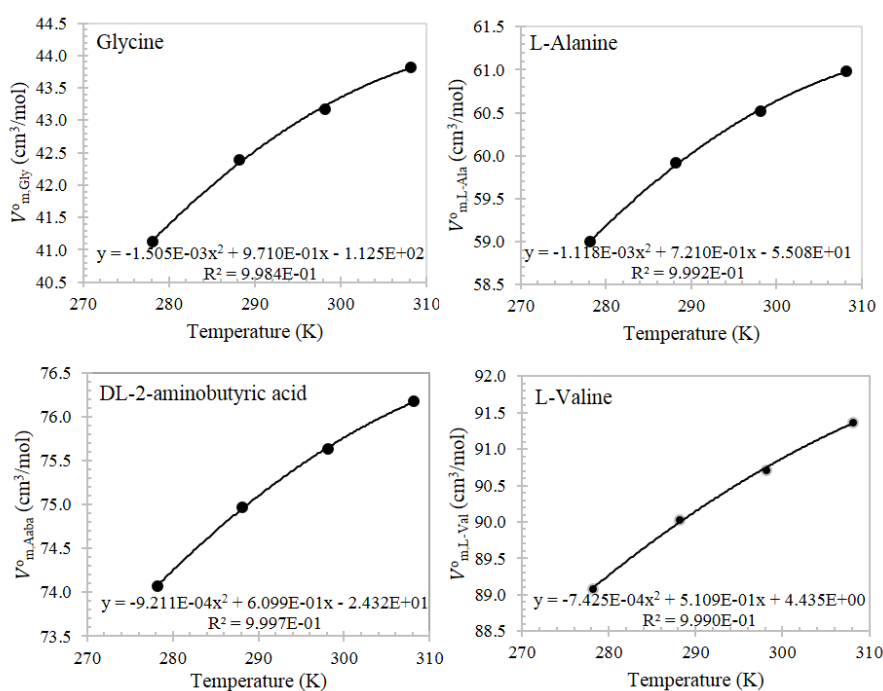


Figure 3.6. Partial molar volumes of all studied amino acids at all temperatures.



As can be seen, all plots of partial molar volumes have a parabolic behaviour and good coefficients of determination ( $R^2 \geq 0.9984$ ).

In Figure 3.7, the calculated partial molar expansions are presented for the four studied amino acids, as well as some values from the literature.

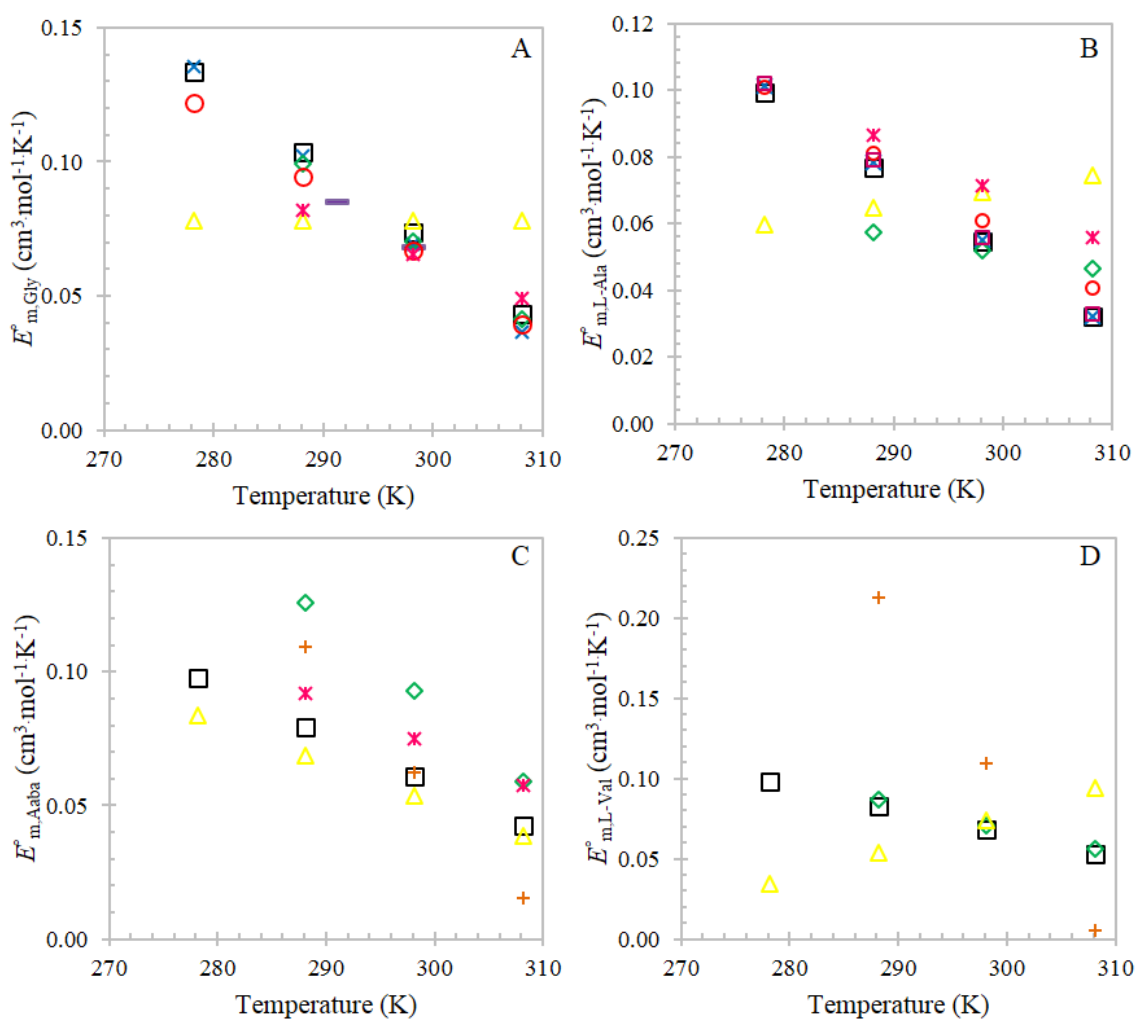


Figure 3.7. PME versus temperature:  $\square$  This work;  $\times$  (Martins *et al.* 2014);  $\diamond$  (Banipal *et al.* 2008);  $\circ$  (Shen *et al.* 2000);  $\triangle$  (Yan *et al.* 1999);  $+$  (Wadi *et al.* 1990);  $-$  (Chalikian *et al.* 1993);  $*$  (Rima *et al.* 2013);  $\square$  (Javornik *et al.* 2013) for A. Gly, B. Ala, C. Aaba and D. Val.

Relatively to the plot of Figure 3.7. A (glycine) it is verified that the values found in the literature are in good agreement with the results of this work except the results of Yan *et al.* (1999).

For L-alanine (Figure 3.7. B) we can see that the results of this work are in agreement with results of Martins *et al.* (2014) and Shen *et al.* (2000) whereas the results of Yan *et al.* (1999), Banipal *et al.* (2008) and Rima *et al.* (2013) are not in agreement. However, for the temperature of 288.15 K and 298.15 K the values of partial molar expansion of Rima *et al.* (2013) and Banipal *et al.* (2008), respectively, are close to the result of this work. The difference of partial molar expansion between this work and the work of Javornik *et al.* (2013) is almost imperceptible.

Regarding DL-2-aminobutyric acid (Figure 3.7. C) the results of this work are in close agreement with the results from Yan *et al.* (1999) and Rima *et al.* (2013) but not with the results of Banipal *et al.* (2008) and Wadi *et al.* (1990). However, at 298.15 K the partial molar expansion of Wadi *et al.* (1990) is similar to the result of this work.

Finally, in the case of L-valine (Figure 3.7. D) the values obtained by Banipal *et al.* (2008), are in good agreement with the results of this work which does not occur for the results published by Wadi *et al.* (1990) and Yan *et al.* (1999). But, again, at 298.15 K the PME of Yan *et al.* (1999) is similar to this work.

Additionally, the hydrophobicity criteria proposed by Hepler (1969) can be applied, considering the first and second molar volume derivatives with temperature (Table 3.3), to conclude that the four amino acids can be considered hydrophilic solutes.

Table 3.3. Results of the first and second derivatives of all amino acids at all studied temperatures.

T (K)	Gly	L-Ala	Aaba	L-Val
		$(\partial V_{m,A}^0/\partial T)_P$		
278.15	0.13373	0.09923	0.09748	0.09782
288.15	0.10364	0.07688	0.07909	0.08297
298.15	0.07354	0.05452	0.06067	0.06812
308.15	0.04344	0.03217	0.04224	0.05327
		$(\partial^2 V_{m,A}^0/\partial T^2)_P$		
--	-0.00301	-0.00224	-0.00184	-0.00149

For the second derivative, an order can be established between the four AA (Gly > L-Ala > Aaba > L-Val). That is not the case of the first derivative data for which the order varies with temperature.

### 3.2.2. Preliminary Tests

As a preliminary test, for validation purposes, density data were measured for the ternary mixture  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  + L-alanine + water, for a salt molality of 0.7 molal. This system was selected because it had been studied by Javornik *et al.* (2013) using the same equipment.

Figure 3.8 presents a graph of the partial molar volume of L-alanine where the results of this work and the results of Javornik *et al.* (2013) are presented as well as the error bars.

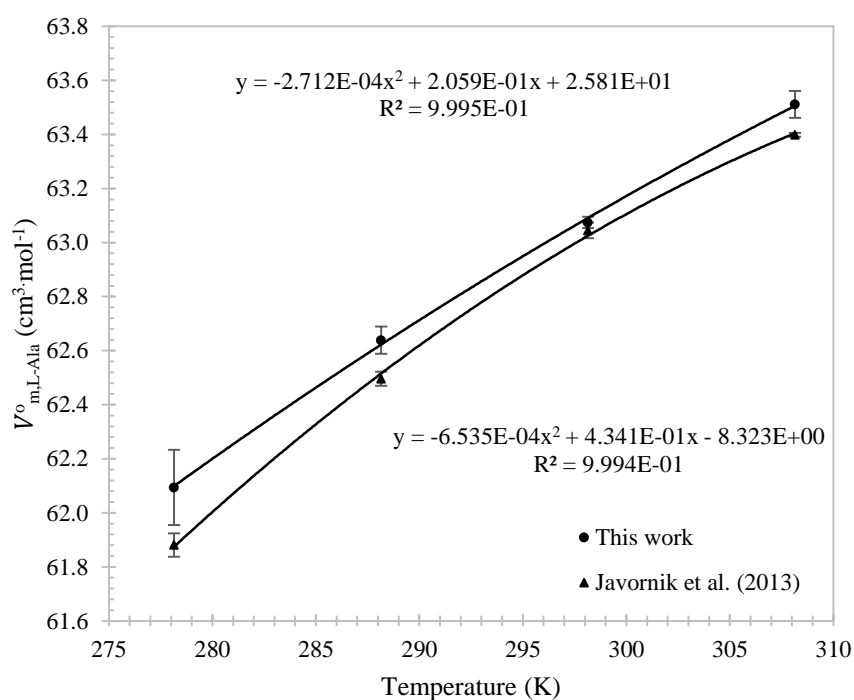


Figure 3.8. Partial molar volume of L-alanine of this work and Javornik *et al.* (2013).

As can be seen, a satisfactory agreement was obtained between both data sets. The largest difference occurs at 278.15 K but, at this temperature, the error bar of the experimental point of this work is the largest.

### 3.2.3. Ternary Systems $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ + Amino Acid + Water

First, the binary system water + salt was studied to obtain the reference values  $\rho_0$ . Thus, an average of all the independent values measured throughout this work (at least 8) is given in Appendix C.

In the present section, the results for the ternary systems obtained in this work, with magnesium sulphate heptahydrate, are presented. The complete numerical density data sets are reported in Appendix A. Table 3.4 shows the partial molar volumes of the amino acids in aqueous magnesium sulphate solutions at four different temperatures (278.15 K, 288.15 K, 298.15 K and 308.15 K) as well as different salt molalities.

Table 3.4. Partial molar volumes at infinite dilution of Gly, L-Ala, Aaba and L-Val at different temperatures and magnesium sulphate molalities (values in parentheses are estimated uncertainties).

$m_s$ (mol·kg <sup>-1</sup> )	$V_{m,A}^o$ (cm <sup>3</sup> ·mol <sup>-1</sup> )			
	278.15 K	288.15 K	298.15 K	308.15 K
glycine				
0.0000	41.13 (0.05)	42.39 (0.03)	43.17 (0.07)	43.82 (0.07)
0.1000	42.15 (0.05)	43.22 (0.06)	43.95 (0.02)	44.42 (0.12)
0.3000	43.43 (0.06)	44.29 (0.03)	44.90 (0.05)	45.41 (0.05)
0.7000	45.25 (0.05)	46.08 (0.06)	46.53 (0.02)	46.94 (0.10)
1.0000	46.51 (0.05)	46.99 (0.04)	47.40 (0.08)	47.74 (0.08)
L-alanine				
0.0000	58.99 (0.03)	59.91 (0.03)	60.51 (0.03)	60.98 (0.04)
0.1000	59.71 (0.04)	60.50 (0.04)	61.09 (0.03)	61.52 (0.06)
0.3000	60.73 (0.03)	61.54 (0.03)	62.04 (0.02)	62.34 (0.03)
0.7000	62.61 (0.04)	63.07 (0.09)	63.44 (0.07)	63.81 (0.05)
1.0000	63.27 (0.02)	63.68 (0.06)	64.11 (0.06)	64.43 (0.05)
DL-2-aminobutyric acid				
0.0000	74.07 (0.05)	74.97 (0.03)	75.64 (0.02)	76.18 (0.04)
0.1000	74.69 (0.04)	75.45 (0.04)	76.12 (0.02)	76.62 (0.02)
0.3000	76.00 (0.09)	76.67 (0.05)	77.20 (0.01)	77.70 (0.04)
0.7000	77.71 (0.06)	78.13 (0.05)	78.47 (0.04)	78.97 (0.03)
1.0000	78.57 (0.03)	78.91 (0.03)	79.32 (0.02)	79.76 (0.06)
L-valine				
0.0000	89.08 (0.03)	90.03 (0.02)	90.71 (0.05)	91.37 (0.02)
0.1000	89.87 (0.03)	90.69 (0.03)	91.36 (0.04)	91.97 (0.02)
0.3000	91.02 (0.03)	91.73 (0.04)	92.25 (0.05)	92.87 (0.02)
0.7000	92.94 (0.07)	93.42 (0.07)	93.85 (0.04)	94.35 (0.06)
1.0000	93.62 (0.03)	93.99 (0.05)	94.45 (0.10)	94.97 (0.13)

For all the systems, partial molar volumes increase when increasing salt molality or temperature. The estimated uncertainties are relatively low, being  $0.13 \text{ cm}^3 \text{ mol}^{-1}$  the maximum value, obtained for L-valine at 308.15 K, with molality of salt 1.0 molal.

In Figure 3.9, the graphs representing  $\Delta\rho/m_A$  versus amino acid molality for the four amino acids studied in this work at four temperatures and at 1.0 salt molality are presented. Also, as can be seen in Figure 3.9, the coefficient of determination is relatively good ( $R^2 \geq 0.8884$ ), taking into account that the higher the salt concentration, the more difficult it is to obtain a good coefficient of determination.

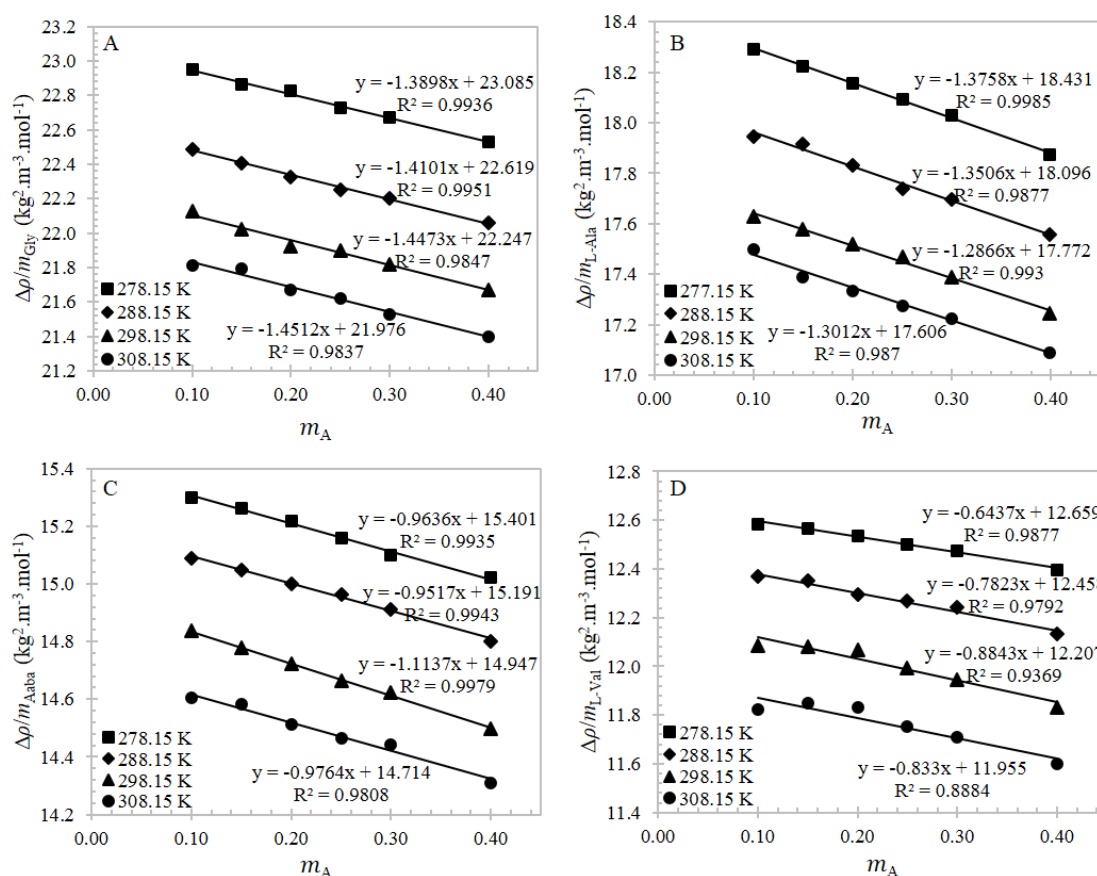


Figure 3.9.  $\Delta\rho/m_A$  versus amino acid molality: A. glycine; B. L-alanine; C. DL-2-aminobutyric acid and D. L-valine for 1.0 molal of salt molality.

To our knowledge, only one reference (Mallick and Kishore, 2006) is available, containing PMV data of amino acids in aqueous solutions of magnesium sulphate, only at 298.15 K. Figure 3.10 compares our results.

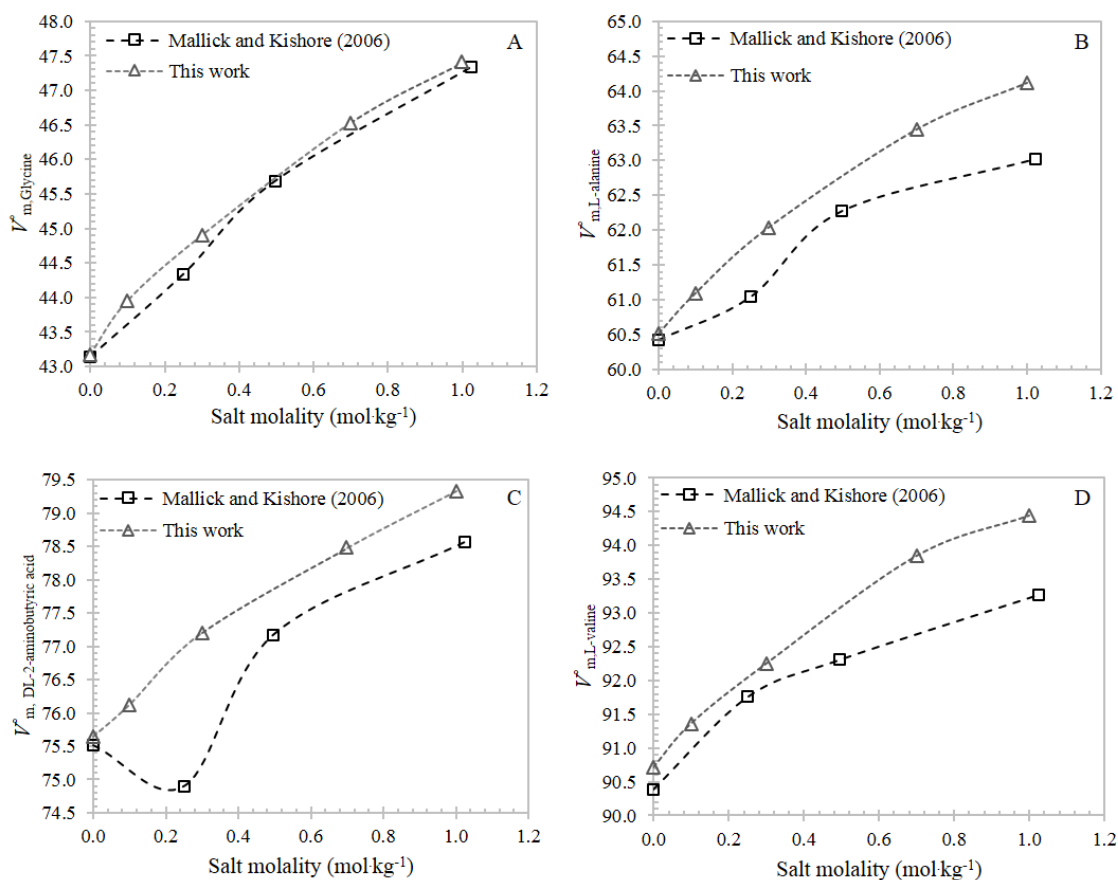


Figure 3.10. Comparison between PMV of this work and Mallick and Kishore (2006) at 298.15 K (lines are guide to the eyes).

As can be seen in Figure 3.10, our results are in agreement only in the case of glycine. The good quality and consistency of the experimental data obtained in this work was shown. Therefore, this information will be used in the next chapter to calculate the hydration numbers and partial molar volumes of transfer of the four amino acids in aqueous solutions of magnesium sulphate. A discussion on the salt effect in aqueous solutions containing amino acids will be done, comparing the results obtained here with the results obtained by other authors for the same amino acids in the presence of different salts.

## Chapter 4

### Discussion of results

In this chapter, the discussion of the results is performed. For this, hydration numbers and partial molar volumes of transfer were calculated and group contribution methods were applied to model, both the partial molar volumes and the partial molar volumes of transfer.

#### 4.1. Hydration Number

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

To perform the calculations of the hydration numbers, the Friedman and Krishnan (1973) method, specifically developed to amino acid solutions, using density data was applied. This has been the most used approach to interpret the dehydration of proteins and amino acids by electrolytes (Yan *et al.* 2004; Banipal *et al.* 2008). Accordingly, the hydration number is given by:

$$n_H = (V_{m,A}^o - V_{m,A,int}^o)/(V_e^o - V_b^o) \quad (4.1)$$

where  $V_e^o$  is the molar volume of electrostricted water,  $V_b^o$  is the molar volume of bulk water and  $V_{m,A,int}^o$  is the intrinsic volume of the amino acid.

Yan *et al.* (2004) published the  $(V_e^o - V_b^o)$  values: -2.6, -2.9, -3.3, -4.0  $\text{cm}^3\cdot\text{mol}^{-1}$  at 278.15, 288.15, 298.15 and 308.15 K, respectively. To calculate the intrinsic volume of the amino acid ( $V_{m,A,int}^o$ ), the following relationship was used (Millero *et al.* 1978):

$$V_{m,A,int}^{\circ} = (0.7/0.634)V_{m,A,cryst}^{\circ} \quad (4.2)$$

where  $V_{m,A,cryst}^{\circ}$  is the crystal volume determined from the work of Berlin and Pallansch (1968). Table 4.1 presents the hydration numbers of glycine, L-alanine and L-valine at all experimental conditions. No calculations were performed for DL-2-aminobutyric acid, since to the best of our knowledge no value is available for  $V_{m,Aaba,cryst}^{\circ}$ .

Table 4.1. Hydration numbers of Gly, L-Ala and L-Val at different temperatures and salt molalities.

$m_s/\text{mol}\cdot\text{kg}^{-1}$	$n_H$			
	278.15 K	288.15 K	298.15 K	308.15 K
	glycine			
0.0	4.13	3.27	2.64	2.01
0.1	3.76	2.98	2.40	1.86
0.3	3.25	2.61	2.11	1.61
0.7	2.55	2.00	1.62	1.23
1.0	2.06	1.68	1.35	1.03
	L-alanine			
0.0	4.91	4.08	3.41	2.69
0.1	4.63	3.88	3.23	2.56
0.3	4.24	3.52	2.94	2.35
0.7	3.52	2.99	2.52	1.98
1.0	3.26	2.78	2.31	1.83
	L-valine			
0.0	5.00	4.16	3.45	2.68
0.1	4.70	3.93	3.25	2.53
0.3	4.25	3.57	2.98	2.30
0.7	3.52	2.99	2.50	1.93
1.0	3.26	2.79	2.31	1.78

\*The highlighted values correspond to  $n_H(\text{L-Ala}) \geq n_H(\text{L-Val})$ .

The dehydration of the three amino acids above mentioned increases with salt molality and temperature. For L-alanine and L-valine the hydration number is very similar and higher than glycine but, for glycine, the decrease in the hydration number with the electrolyte concentration is larger than for the others amino acids. So, the absolute magnitude of dehydration follows: glycine > L-alanine  $\approx$  L-valine.

In Table 4.2 the magnitude of the dehydration is represented as the difference ( $\Delta n_H$ ) between the hydration numbers of the amino acid in pure water and in an aqueous solution containing an electrolyte with 1.0 molal concentration, at 298.15 K. The values obtained for L-valine were added here to the values of glycine and L-alanine that have been published previously (Martins *et al.* 2014).



Table 4.2. Hydration number change in aqueous 1.0 molal electrolyte solutions at 298.15 K.

Salt	$\Delta n_H$ (Gly)	$\Delta n_H$ (Ala)	$\Delta n_H$ (Val)	Reference
LiNO <sub>3</sub>	0.39	---	---	Liu <i>et al.</i> (2008)
NaNO <sub>3</sub>	0.52	---	---	Liu <i>et al.</i> (2008)
KNO <sub>3</sub>	0.51	---	---	Liu <i>et al.</i> (2008)
NaSCN	0.35	0.36*	0.55*	Singh and Kishore (2003)
KSCN	0.42	0.40	---	Wadi and Goyal (1992)
LiCl	0.44	0.33*	---	Ogawa <i>et al.</i> (1984)
NaCl	0.55	0.48*	---	Ogawa <i>et al.</i> (1984)
NaCl	0.41	0.24	0.26*	Bhat and Ahluwalia (1985)
NaCl	0.60	0.54*	---	Yuan <i>et al.</i> (2006)
KCl	0.50	0.45*	---	Ogawa <i>et al.</i> (1984)
NH <sub>4</sub> Cl	0.33	---	---	Natarajan <i>et al.</i> (1990)
NaCH <sub>3</sub> COO	0.88	0.59	---	Banipal <i>et al.</i> (2004)
NaCH <sub>3</sub> COO	0.66	0.58*	0.50*	Singh and Kishore (2003)
Mg(CH <sub>3</sub> COO) <sub>2</sub>	1.18	0.90	---	Banipal <i>et al.</i> (2006)
MgSO <sub>4</sub>	1.27	0.78	0.87*	Mallick and Kishore (2006)
MgSO <sub>4</sub>	1.28	1.00*	1.13*	This work
Na <sub>2</sub> SO <sub>4</sub>	1.36	1.23	---	Wadi and Ramasami (1997)
Na <sub>2</sub> SO <sub>4</sub>	1.39	1.23*	1.30*	Singh and Kishore (2003)
Na <sub>2</sub> SO <sub>4</sub>	1.29	1.17*	---	Liu and Ren (2009)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.13	0.98	---	Martins <i>et al.</i> (2014)
MnCl <sub>2</sub>	1.78	1.54	1.15	Banipal <i>et al.</i> (2012)
ZnCl <sub>2</sub>	1.29	1.20	0.89	Banipal <i>et al.</i> (2008)
CdCl <sub>2</sub>	1.06	1.01	0.73	Banipal <i>et al.</i> (2011)

\*The value refers to L-alanine or L-valine.

If we compare glycine with L-alanine, the decrease in the hydration number becomes progressively smaller as the hydrophobic part of the amino acids increases (Martins *et al.* 2014). However, in the case of L-valine, both in this work and in the works of Bhat and Ahluwalia (1985), Mallick and Kishore (2006), Singh and Kishore (2003), this behaviour is not obeyed. In fact, apparently, for some salts, the increase of the hydrophobic chain doesn't significantly affect the amino acids hydration. We will go back to this question later.

Martins *et al.* (2014), using  $\Delta n_H$  data available for glycine and L-alanine, discussed the dehydration effect caused by several types of electrolytes. For L-valine, the information is scarce and, therefore, tendencies are much harder to find.

For electrolytes of the type 1:1 the dehydration effect, measured by  $\Delta n_H$ , generally increases for less hydrated cations ( $K^+ < Na^+ < Li^+$ ) (see Table 1.1, for hydration energies) and for more hydrated anions ( $SCN^- < NO_3^- < Cl^- < CH_3COO^-$ ), in the case of glycine and L-alanine. For L-valine, only the effect of the anions could be discussed, based on the data of sodium salts. As can be seen, the  $SCN^-$  anion does not follow the previous trend.

For 1:2 type electrolytes such as  $(NH_4)_2SO_4$  and  $Na_2SO_4$ , containing the highly hydrated sulphate anion, the dehydration effect is much larger when compared to  $NH_4Cl$  or  $NaCl$ , respectively. The information available for L-Val, regarding  $Na_2SO_4$  and  $NaCl$  salts, also follows this trend. However, since the ammonium cation is much less hydrated than sodium, the dehydration effect of ammonium sulphate is smaller than sodium sulphate (Martins *et al.* 2014). Exactly the same is observed when comparing the salts containing the also highly hydrated anion  $CH_3COO^-$ , in which the salt containing the less hydrated cation ( $Na^+ < Mg^{2+}$ ) is the less effective dehydrating agent. An interpretation was proposed based on the recent conclusions found by combining molecular dynamics and solubility studies (Tome *et al.* 2013), where for less hydrated anions the magnitude of the salting-in phenomena is governed by the nature of the cation, but if the anion has significant hydration, a complex competition between cation and anion effects determines the behaviour of the system.

However, when considering the salts containing the  $SO_4^{2-}$  anion, the reverse effect is observed for the three amino acids as the salt containing the less hydrated cation ( $Na^+ < Mg^{2+}$ ) is the more effective dehydrating agent.

Finally, Martins *et al.* (2014) have also considered 2:1 electrolytes with the chloride anion. The results obtained there analysing  $\Delta n_H$  for L-alanine and glycine, can be extended to L-valine. The effect of the cations on dehydration can be ranked according to  $Mn^{2+} > Zn^{2+} > Cd^{2+}$ . As pointed previously (Martins *et al.* 2014), the number of systems studied is small, but  $Mn^{2+}$  and  $Cd^{2+}$  being close in terms of hydration and both much

less hydrated than  $\text{Zn}^{2+}$ , the rank given before cannot, at this time, be connected only to the cation degree of hydration. The molecular dynamics studies indicate some molecular mechanisms beyond the Hofmeister series (Tome *et al.* 2013).

Martins *et al.* (2014) have proposed the following linear relationship, in which  $\Delta n_H$  is a function of the ratio  $z/r$  of both ions of a given electrolyte, considering the stoichiometric coefficients  $\nu_i$ , and the normalized  $\Delta G_{hyd}$  of the cation in the same electrolyte, taking the Gibbs energy of hydration of potassium, a salt considered in the middle of the Hofmeister series, as the reference:

$$\Delta n_H = a + b \sum_{\forall i} \nu_i \frac{z_i}{r_i} + c \frac{\Delta G_{hyd, \text{cation}}}{\Delta G_{hyd, \text{potassium}}} \quad (4.3)$$

where  $a$ ,  $b$  and  $c$  are empirical constants, the summation is over the two ions present in an electrolyte and  $\nu_i$  is the stoichiometric coefficient of ion  $i$ .

As can be seen in Table 4.3, the calculated values by the correlation also indicate a decrease in  $\Delta n_H$  from glycine to alanine. Quantitatively, the values obtained in this work are closer to the correlation values.

Table 4.3. Hydration number change in aqueous 1.0 molal electrolyte solutions at 298.15 K.

$\Delta n_H$ (Gly)	$\Delta n_H$ (Ala)	Reference
1.27	0.78	Mallick and Kishore (2006)
1.28	1.00	This work
1.68	1.30	Correlation (Martins <i>et al.</i> 2014)

## 4.2. Partial Molar Volumes of Transfer

The partial molar volumes of transfer at infinite dilution ( $\Delta_{tr}V_{m,A}^0$ ) from water to aqueous magnesium sulphate solutions have been calculated by Equation 4.4 and are listed in Table 4.4 together with the uncertainties given in parentheses.

$$\Delta_{tr}V_{m,A}^0 = V_{m,A}^0(\text{in aqueous salt solution}) - V_{m,A}^0(\text{in water}) \quad (4.4)$$

where  $V_{m,A}^0$  (in aqueous salt solution) is the partial molar volume of amino acid in aqueous salt solution and  $V_{m,A}^0$  (in water) is the partial molar volume of amino acid in water.

Table 4.4. Partial molar volumes of transfer at infinite dilution for Gly, L-Ala, Aaba and L-Val at different temperatures and magnesium sulphate molalities.

$m_s/\text{mol}\cdot\text{kg}^{-1}$	$\Delta_{\text{tr}}V_{m,A}^0$ ( $\text{cm}^3\cdot\text{mol}^{-1}$ )			
	278.15 K	288.15 K	298.15 K	308.15 K
glycine				
0.1000	0.96 (0.10)	0.83 (0.08)	0.76 (0.09)	0.60 (0.18)
0.3000	2.29 (0.11)	1.90 (0.06)	1.73 (0.12)	1.58 (0.11)
0.7000	4.12 (0.10)	3.69 (0.09)	3.36 (0.09)	3.12 (0.17)
1.0000	5.38 (0.10)	4.60 (0.07)	4.23 (0.15)	3.92 (0.15)
L-alanine				
0.1000	0.72 (0.07)	0.59 (0.07)	0.58 (0.07)	0.54 (0.10)
0.3000	1.73 (0.06)	1.63 (0.06)	1.53 (0.07)	1.36 (0.05)
0.7000	3.61 (0.07)	3.16 (0.12)	2.93 (0.10)	2.83 (0.09)
1.0000	4.28 (0.05)	3.77 (0.09)	3.60 (0.08)	3.45 (0.10)
DL-2-aminobutyric acid				
0.1000	0.62 (0.09)	0.48 (0.08)	0.48 (0.04)	0.45 (0.06)
0.3000	1.93 (0.14)	1.69 (0.08)	1.56 (0.03)	1.53 (0.07)
0.7000	3.64 (0.11)	3.15 (0.08)	2.84 (0.06)	2.79 (0.06)
1.0000	4.50 (0.08)	3.94 (0.06)	3.69 (0.04)	3.59 (0.09)
L-valine				
0.1000	0.79 (0.06)	0.66 (0.05)	0.65 (0.10)	0.60 (0.04)
0.3000	1.95 (0.06)	1.70 (0.06)	1.54 (0.10)	1.50 (0.04)
0.7000	3.86 (0.10)	3.39 (0.09)	3.14 (0.10)	2.98 (0.09)
1.0000	4.54 (0.06)	3.96 (0.07)	3.74 (0.10)	3.60 (0.13)

Generally, the partial molar volumes of transfer at infinite dilution increase when increasing salt molality or decreasing temperature. The values obtained in this work can be interpreted considering the interactions between the ions ( $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ ) and the hydrophilic groups of the amino acids ( $-\text{COO}^-$  and  $-\text{NH}_3^+$ ) or between the ions and the hydrophobic nonpolar parts of the amino acids. According to the co-sphere overlap model (Frank and Evans 1945), the first type of interactions results in positive  $\Delta_{\text{tr}}V_{m,A}^0$  values, while the interactions ion/hydrophobic group give negative  $\Delta_{\text{tr}}V_{m,A}^0$  values. The positive transfer volumes obtained in this work indicate the predominance of the interactions between the ions and the zwitterionic centers of the amino acids, in

agreement with very recent theoretical studies using molecular dynamics (Tome *et al.* 2010; Tome *et al.* 2013) in systems containing alanine, isoleucine or valine and electrolytes such as ammonium chloride, ammonium sulphate, magnesium chloride or magnesium sulphate.

Qualitatively, the data of glycine and L-alanine are in agreement with the co-sphere overlap model when a comparison is made between their transfer volumes. In fact, the introduction of a hydrophobic  $-\text{CH}_2-$  group in glycine to form alanine, causes a reduction on  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$ . However, when comparing the four amino acids studied in solutions containing magnesium sulphate, the magnitude of  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$  follows, glycine > L-valine > L-alanine > DL-2-aminobutyric acid except in case of the  $m_s = 0.3$  and  $m_s = 1.0$  molal where the magnitude of  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$  is glycine > L-valine > DL-2-aminobutyric acid > L-alanine.

Therefore, the increase of the hydrophobic part of amino acids (glycine > L-alanine > DL-2-aminobutyric acid > L-valine) does not result in a decrease in the partial molar volumes of transfer; moreover, for L-valine (amino acid with the higher hydrophobic part) the values of partial molar volumes of transfer are higher than L-alanine.

Comparing similar information for the same amino acids at 298.15 K, but in the presence of different electrolytes, the same trends were observed for solutions containing sodium sulphate (Islam and Wadi 2003), magnesium sulphate (Mallick and Kishore 2006), among others (see Appendix D).

However, different trends also exist, for example in the work published by Yan *et al.* (2004) ( $m_{\text{calcium chloride}} = 3.0 \text{ mol}\cdot\text{kg}^{-1}$  and  $T = 298.15 \text{ K}$ ) the magnitude of  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$  follows DL-alanine > glycine > DL-valine > DL-2-aminobutyric acid; in the work published by Banipal *et al.* (2008) ( $m_{\text{zinc chloride}} = 3.0 \text{ mol}\cdot\text{kg}^{-1}$  and  $T = 298.15 \text{ K}$ ) the magnitude of  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$  is glycine > DL-alanine > DL-2-aminobutyric acid > L-valine.

A review about the partial molar volumes of transfer at different electrolytes and at various temperatures was made and is presented in the supporting information, Appendix D. In Table D.1 we can observe that the behaviour of  $\Delta_{\text{tr}}V_{\text{m,A}}^{\circ}$  is not always the same for the amino acids studied in this work.

### 4.3. Group Contribution Methods

To further analyse the alkyl chain effect of the amino acids, in the next section, group contribution methods were applied in an attempt to model the partial molar volumes data.

#### 4.3.1. Partial Molar Volumes

The alkyl chain of the homologous series of amino acids investigated in this work is:  $\text{CH}_2$  – (Gly),  $\text{CH}_3\text{CH}$  – (Ala),  $\text{CH}_3\text{CH}_2\text{CH}$  – (Aaba) and  $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}$  – (Val). If we accept the assumptions:

$$V_{A,\phi}(\text{CH}_3) = 1.5V_{A,\phi}(\text{CH}_2) \quad (4.5)$$

$$V_{A,\phi}(\text{CH}) = 0.5V_{A,\phi}(\text{CH}_2) \quad (4.6)$$

proposed by Hakin *et al.* (1994) for the infinite dilution apparent molar volumes of amino acids at all temperatures, this linear variation can be represented by:

$$V_{A,\phi} = V_{A,\phi}(\text{NH}_3^+, \text{COO}^-) + n_C V_{A,\phi}(\text{CH}_2) \quad (4.7)$$

where  $n_C$  is the number of carbon atoms in the alkyl chain of the amino acids. A linear regression analysis of  $V_{A,\phi}$  values at any given temperature using Equation 4.7 gives  $V_{A,\phi}(\text{NH}_3^+, \text{COO}^-)$ , the zwitterionic end group and  $V_{A,\phi}(\text{CH}_2)$ , the methylene group contributions. These results are shown in Table 4.5 and Figure 4.1.

Table 4.5. Contributions of the zwitterionic group ( $\text{NH}_3^+$ ,  $\text{COO}^-$ ) and  $\text{CH}_2$  groups to the partial molar volumes of the amino acids, in different aqueous magnesium sulphate solution between 278.15 and 308.15 K.

$m_s/\text{mol}\cdot\text{kg}^{-1}$	$\text{NH}_3^+, \text{COO}^-$				$\text{CH}_2$			
	278.15 K	288.15 K	298.15 K	308.15 K	278.15 K	288.15 K	298.15 K	308.15 K
0.0	26.085	27.330	28.070	28.625	15.893	15.798	15.775	15.785
0.1	27.070	28.125	28.815	29.195	15.814	15.736	15.726	15.775
0.3	28.285	29.195	29.795	30.145	15.804	15.745	15.721	15.774
0.7	30.085	30.905	31.325	31.670	15.817	15.708	15.699	15.739
1.0	31.335	31.835	32.230	32.470	15.663	15.623	15.636	15.702

It is interesting to note that  $V_{m,A}^o$  varies linearly with the number of carbon atoms in the alkyl chains of the amino acids, at the four studied temperatures, as reported in Appendix E (average coefficients of determination: 278.15 K,  $R^2 \geq 0.9987$ ; 288.15 K,  $R^2 \geq 0.9990$ ; 298.15 K,  $R^2 \geq 0.9991$  and at 308.15 K,  $R^2 \geq 0.9993$ ).

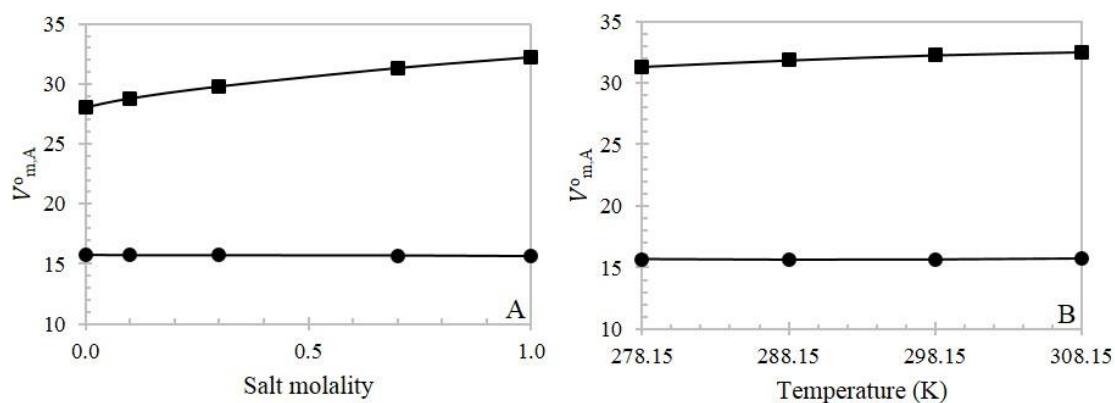


Figure 4.1.  $V_{m,A}^o$  (■ $\text{NH}_3^+, \text{COO}^-$ ; ● $\text{CH}_2$ )/ $\text{cm}^3 \cdot \text{mol}^{-1}$ : A. in function of the molality of magnesium sulphate at 298.15 K. B. in function of the temperature, for a solution 1.0 molal in magnesium sulphate.

As can be seen, in most cases, the contribution from the methylene group  $V_{A,\phi}(\text{CH}_2)$  decreases with increasing temperature or salt molality. The changes, however, are much smaller when compared to the ones suffered by the contribution from the zwitterionic groups. In the latter case,  $V_{A,\phi}(\text{NH}_3^+, \text{COO}^-)$  increases both with increasing temperature or salt molality, being the effect of the salt more pronounced.

Table 4.6 shows, for a given salt molality and temperature, the difference between the contribution of a given group in an aqueous solution containing salt and the contribution of that group in a solution containing pure water.

The contribution from the methylene group is always lower than 10% of the contribution of the zwitterionic group. This probably explains the difficulty in observing the effect of the alkyl chain length on the partial molar volumes data.

Table 4.6. Contributions of the zwitterionic group ( $\text{NH}_3^+$ ,  $\text{COO}^-$ ) and  $\text{CH}_2$  groups to the partial molar volumes of transfer of the amino acids, in different aqueous magnesium sulphate solution between 278.15 and 308.15 K.

$m_s/\text{mol}\cdot\text{kg}^{-1}$	$\text{NH}_3^+, \text{COO}^-$				$\text{CH}_2$			
	278.15 K	288.15 K	298.15 K	308.15 K	278.15 K	288.15 K	298.15 K	308.15 K
0.1	0.98	0.80	0.75	0.57	-0.08	-0.06	-0.05	-0.01
0.3	2.20	1.87	1.73	1.52	-0.09	-0.05	-0.05	-0.01
0.7	4.00	3.58	3.26	3.05	-0.08	-0.09	-0.08	-0.05
1.0	5.25	4.51	4.16	3.85	-0.23	-0.18	-0.14	-0.08

### 4.3.2. Partial Molar Volumes of Transfer

Qualitatively, the formalism proposed by Friedman and Krishnan (1973) allows the representation of the thermodynamic transfer function at infinite dilution in terms of the interaction of the solute with different number of co-solute species. In this regard, the partial molar volume of transfer of the diluted amino acid can be expressed as (Zhao *et al.* 2009):

$$\Delta_{\text{tr}}V_{\text{m,A}}^0 = 2V_{\text{A,S}}m_{\text{S}} + 3V_{\text{A,SS}}m_{\text{S}}^2 + \dots \quad (4.8)$$

where constants  $V_{\text{A,S}}$  and  $V_{\text{A,SS}}$  denote pair and triplet interactions, which were found by fitting the transfer volume data at each temperature. Only two parameters were estimated, which are reported in Table 4.7, considering both the number of available data at each temperature and the quality of the curves obtained (Figure 4.2).

Table 4.7.  $V_{\text{A,S}}$  ( $\text{cm}^3\cdot\text{mol}^{-2}\cdot\text{kg}$ ) and  $V_{\text{A,SS}}$  ( $\text{cm}^3\cdot\text{mol}^{-3}\cdot\text{kg}^2$ ) interaction coefficients for glycine, L-alanine, DL-2-aminobutyric acid and L-valine in aqueous magnesium sulphate solutions at different temperatures.

$T$ (K)	$V_{\text{A,S}}$	$V_{\text{A,SS}}$	$V_{\text{A,S}}$	$V_{\text{A,SS}}$	$V_{\text{A,S}}$	$V_{\text{A,SS}}$	$V_{\text{A,S}}$	$V_{\text{A,SS}}$
	glycine		L-alanine		DL-2-aminobutyric acid		L-valine	
278.15	4.061	-0.942	3.424	-0.848	3.271	-0.681	3.803	-1.019
288.15	3.569	-0.855	3.092	-0.803	2.861	-0.596	3.330	-0.895
298.15	3.233	-0.753	2.846	-0.700	2.693	-0.577	3.010	-0.756
308.15	2.918	-0.642	2.608	-0.585	2.832	-0.703	2.873	-0.714



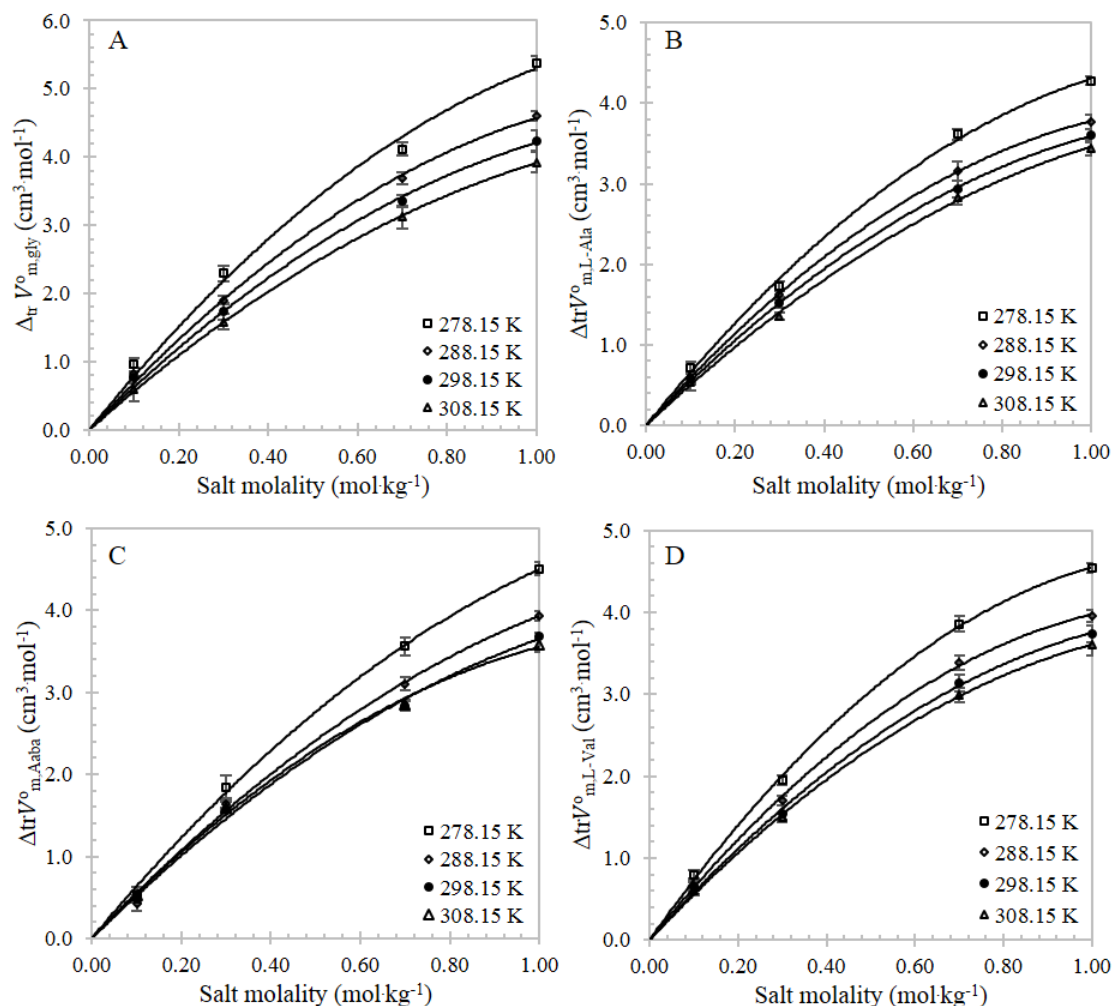


Figure 4.2. Partial molar volumes of transfer versus salt molality; A. Gly, B. L-Ala, C. Aaba and D. L-Val.

For all systems, the  $V_{A,S}$  coefficient is positive while  $V_{A,SS}$  is negative at all studied temperatures. For each amino acid, the relative magnitude of the coefficients indicates that interactions between magnesium sulphate and the amino acids are dominated by pairwise interaction, while comparing, at the same temperature, the correspondent pairwise interactions coefficients, the following sequence is observed between 278.15 K and 298.15 K, glycine > L-valine > L-alanine > DL-2-aminobutyric acid. At 308.15 K, the sequence is different: glycine > L-valine > DL-2-aminobutyric acid > L-alanine. These results preclude the application of group contribution formalism for the pairwise volumetric interaction coefficients. Again, this effect may result from the smaller impact of the alkyl groups relatively to the zwitterionic groups.



## Chapter 5

### Conclusions and Future Work

From density measurements between 278.15 and 308.15 K, partial molar volumes at infinite dilution of glycine, L-alanine, DL-2-aminobutyric acid and L-valine were calculated. Good quality of the data was proved either by using directly the partial molar volumes in pure water or partial molar expansibilities how is possible to see through of comparison with literature. These data were used to calculate the partial molar volumes of transfer, hydration numbers and group contributions.

From the transfer volumes, it was concluded that the predominant interactions are pairwise between the ions and the zwitterionic centers of the amino acids because the  $\Delta_{tr}V_{m,A}^0$  values are positive. The increase of the hydrophobic part of amino acids (glycine > L-alanine > DL-2-aminobutyric acid > L-valine) does not give a significant decrease in the partial molar volumes of transfer, furthermore, for L-valine (amino acid with the higher hydrophobic part) the values of partial molar volumes of transfer are higher than L-alanine.

For L-alanine and L-valine the hydration number is very similar and higher than glycine. If we compare glycine with L-alanine, the decrease in the hydration number becomes progressively smaller as the hydrophobic part of the amino acids increases but, in the case of L-valine, this conclusion can not be drawn. Therefore, seemingly, the increase of the hydrophobic chain does not significantly affect the amino acids hydration. An analysis over a large set of electrolytes confirmed, in a general way, that for electrolytes type 1:1 the dehydration increases in the presence of less hydrated cations and highly hydrated anions.

A group contribution method was successfully applied to describe the partial molar volumes of the studied amino acids. The contribution of the zwitterionic ( $\text{NH}_3^+$ ,  $\text{COO}^-$ )

group to the value of the standard partial molar volume increases with increasing concentration of magnesium sulphate. In general, the contribution of  $-\text{CH}_2$  and other alkyl groups has a very weak decreasing trend.

As future work, using the same conditions of this work, it is suggested the study with amino acids with largest hydrophobic chain to verify if the results in terms of partial molar volumes and hydration numbers are similar to those obtained here. In addition, it would be interesting to use other electrolytes such as the salts of the trivalent aluminium cation. It would also be desirable to extend this study to other amino acids containing multiple carboxylate or amine groups, and peptides.

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# **Supporting Information**

## **Appendix A**

Table A.1. Densities of aqueous magnesium sulphate solutions containing glycine at different temperatures, and amino acid ( $m_A$ ) and salt ( $m_S$ ) molalities.

$\rho$ (kg m <sup>-3</sup> )							
$m_S = 0.00000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K			
0.00000	999.964	999.099	997.043	994.029			
0.10001	1003.331	1002.345	1000.215	997.142			
0.15004	1004.992	1003.949	1001.778	998.677			
0.20001	1006.636	1005.541	1003.330	1000.210			
0.25002	1008.268	1007.121	1004.874	1001.731			
0.30005	1009.890	1008.691	1006.408	1003.240			
0.40000	1013.090	1011.795	1009.436	1006.220			
$m_S = 0.30000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K			
0.00000	1036.481	1034.981	1032.480	1029.195			
0.10000	1039.466	1037.881	1035.322	1031.991			
0.14998	1040.946	1039.319	1036.730	1033.380			
0.20003	1042.418	1040.747	1038.124	1034.755			
0.24999	1043.873	1042.161	1039.510	1036.121			
0.30004	1045.324	1043.573	1040.895	1037.486			
0.39880	1048.144	1046.323	1043.588	1040.144			
$m_S = 0.70000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K			
0.00000	1082.444	1080.308	1077.369	1073.761			
0.10000	1085.030	1082.814	1079.831	1076.189			
0.15001	1086.308	1084.051	1081.053	1077.387			
0.20002	1087.571	1085.293	1082.267	1078.594			
0.24998	1088.827	1086.515	1083.472	1079.777			
0.29999	1090.076	1087.732	1084.670	1080.967			
0.39966	1092.539	1090.144	1087.036	1083.285			
$m_S = 1.00000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	308.15 K		
0.00000	1115.536	1112.984	0.00000	1109.779	1105.989		
0.10001	1117.831	1115.233	0.10000	1111.991	1108.170		
0.14999	1116.345	1116.345	0.15000	1113.082	1109.258		
0.20002	1120.102	1117.450	0.20004	1114.164	1110.324		
0.25003	1118.547	1118.547	0.25001	1115.253	1111.395		
0.30005	1119.646	1119.646	0.30001	1116.324	1112.448		
0.40031	1124.554	1121.815	0.40016	1118.451	1114.551		
$m_S = 0.10000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	1012.442	0.00000	1011.348	0.00000	1009.139	0.00000	1006.030
0.10000	1015.659	0.09997	1014.457	0.10000	1012.180	0.09997	1009.031
0.15001	1017.245	0.15003	1016.003	0.15001	1013.685	0.15003	1010.506
0.19997	1018.822	0.19993	1017.526	0.19997	1015.176	0.19993	1011.975
0.25002	1020.388	0.25000	1019.045	0.25002	1016.662	0.25000	1013.438
0.30000	1021.942	0.30000	1020.549	0.30000	1018.134	0.30000	1014.892
0.39942	1024.997	0.39920	1023.508	0.39942	1021.033	0.39920	1017.739



Table A.2. Densities of aqueous magnesium sulphate solutions containing L-alanine at different temperatures, and amino acid ( $m_A$ ) and salt ( $m_S$ ) molalities.

$\rho$ (kg m <sup>-3</sup> )							
$m_S = 0.00000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	999.964	0.00000	999.099	0.00000	997.043	0.00000	994.029
0.10003	1002.950	0.10003	1001.998	0.10000	999.887	0.10000	996.836
0.15003	1004.425	0.15003	1003.429	0.15000	1001.292	0.15000	998.220
0.20002	1005.884	0.20002	1004.846	0.20000	1002.680	0.20000	999.598
0.25003	1007.330	0.25003	1006.256	0.25002	1004.064	0.25002	1000.958
0.30004	1008.770	0.30004	1007.656	0.30007	1005.433	0.30007	1002.307
0.40001	1011.605	0.40001	1010.409	0.40001	1008.130	0.40001	1004.974
$m_S = 0.10000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	1012.445	0.00000	1.011352	0.00000	1009.143	0.00000	1006.036
0.10002	1015.292	0.10000	1.014119	0.10001	1011.861	0.10000	1008.718
0.14998	1016.697	0.15001	1.015483	0.14999	1013.201	0.15001	1010.040
0.20004	1018.090	0.20004	1.016838	0.20004	1014.531	0.20004	1011.354
0.24997	1019.469	0.25005	1.018179	0.25001	1015.849	0.25005	1012.650
0.30003	1020.842	0.30005	1.019512	0.30004	1017.158	0.30005	1013.937
0.39961	1023.534	0.39907	1.022119	0.39934	1019.726	0.39907	1016.467
$m_S = 0.30000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	1036.481	0.00000	1034.981	0.00000	1032.480	0.00000	1029.195
0.09998	1039.076	0.10004	1037.500	0.10003	1034.957	0.09997	1031.652
0.15004	1040.358	0.15001	1038.743	0.15004	1036.180	0.14996	1032.865
0.19999	1041.627	0.19999	1039.981	0.20002	1037.394	0.20002	1034.069
0.24999	1042.885	0.24998	1041.206	0.25003	1038.600	0.24997	1035.260
0.30004	1044.139	0.29997	1042.421	0.30001	1039.795	0.30004	1036.449
0.39893	1046.582	0.39856	1044.788	0.39937	1042.139	0.39893	1038.764
$m_S = 0.70000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	1082.444	0.00000	1080.308	0.00000	1077.369	0.00000	1073.761
0.09999	1084.558	0.10002	1082.379	0.09999	1079.412	0.10002	1075.785
0.15001	1085.609	0.14998	1083.410	0.15001	1080.429	0.14998	1076.780
0.20004	1086.648	0.20005	1084.428	0.20004	1081.431	0.20005	1077.772
0.25005	1087.682	0.25001	1085.445	0.25005	1082.419	0.25001	1078.758
0.29998	1088.702	0.30000	1086.439	0.29998	1083.412	0.30000	1079.729
0.39954	1090.732	0.39954	1088.417	0.39954	1085.357	0.39954	1081.654
$m_S = 1.00000$ mol kg <sup>-1</sup>							
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	$m_A$ (mol kg <sup>-1</sup> )	308.15 K
0.00000	1115.536	0.00000	1112.984	0.00000	1109.779	0.00000	1105.989
0.10001	1117.366	0.10000	1114.779	0.10003	1111.542	0.10000	1107.739
0.15001	1118.270	0.15005	1115.673	0.15004	1112.416	0.15003	1108.598
0.20003	1119.168	0.20000	1116.551	0.20005	1113.283	0.19995	1109.455
0.25004	1120.060	0.25001	1117.419	0.25002	1114.146	0.25003	1110.308
0.29995	1120.944	0.30002	1118.294	0.30000	1114.995	0.30002	1111.157
0.40002	1122.687	0.40002	1120.008	0.39919	1116.663	0.39919	1112.811

Table A.3. Densities of aqueous magnesium sulphate solutions containing DL-2-aminobutyric acid at different temperatures, and amino acid ( $m_A$ ) and salt ( $m_S$ ) molalities.

$\rho$ (kg m <sup>-3</sup> )					
$m_S = 0.00000$ mol kg <sup>-1</sup>					
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K	
0.00000	999.964	999.099	997.043	994.029	
0.10002	1002.843	1001.894	999.781	996.729	
0.15003	1004.263	1003.270	1001.130	998.057	
0.20002	1005.664	1004.634	1002.465	999.376	
0.25005	1007.057	1005.986	1003.790	1000.679	
0.30004	1008.433	1007.326	1005.102	1001.969	
0.40001	1011.155	1009.968	1007.687	1004.520	
$m_S = 0.10000$ mol kg <sup>-1</sup>					
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K	
0.00000	1012.442	1011.348	1009.139	1006.030	
0.10001	1015.171	1014.005	1011.740	1008.597	
0.15000	1016.512	1015.310	1013.022	1009.860	
0.20001	1017.848	1016.609	1014.294	1011.115	
0.25000	1019.171	1017.895	1015.554	1012.357	
0.30004	1020.482	1019.168	1016.808	1013.590	
0.40026	1023.070	1021.685	1019.273	1016.021	
$m_S = 0.30000$ mol kg <sup>-1</sup>					
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K	
0.00000	1036.481	1034.981	1032.480	1029.195	
0.10000	1038.897	1037.337	1034.794	1031.474	
0.15002	1040.093	1038.495	1035.936	1032.602	
0.20000	1041.265	1039.651	1037.067	1033.719	
0.25002	1042.448	1040.795	1038.192	1034.824	
0.30004	1043.602	1041.923	1039.302	1035.920	
0.39972	1045.897	1044.151	1041.490	1038.077	
$m_S = 0.70000$ mol kg <sup>-1</sup>					
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	$m_A$ (mol kg <sup>-1</sup> )	288.15 K	298.15 K	308.15 K
0.00000	1082.444	0.00000	1080.308	1077.369	1073.761
0.10001	1084.329	0.10001	1082.161	1079.201	1075.563
0.14999	1085.261	0.15000	1083.073	1080.101	1076.450
0.20001	1086.184	0.20000	1083.988	1080.996	1077.335
0.25002	1087.112	0.25001	1084.887	1081.886	1078.209
0.30000	1088.029	0.30001	1085.785	1082.769	1079.078
0.39885	1089.809	0.40015	1087.557	1084.508	1080.797
$m_S = 1.00000$ mol kg <sup>-1</sup>					
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	$m_A$ (mol kg <sup>-1</sup> )	298.15 K	308.15 K
0.00000	1115.536	1112.984	0.00000	1109.779	1105.989
0.10000	1117.066	1114.493	0.09997	1111.262	1107.449
0.14999	1117.825	1115.241	0.14999	1111.995	1108.176
0.20001	1118.580	1115.984	0.20002	1112.723	1108.892
0.25001	1119.326	1116.725	0.24998	1113.444	1109.605
0.30002	1120.066	1117.458	0.29998	1114.165	1110.322
0.39950	1121.537	1118.897	0.40013	1115.580	1111.715

Table A.4. Densities of aqueous magnesium sulphate solutions containing L-valine at different temperatures, and amino acid ( $m_A$ ) and salt ( $m_S$ ) molalities.

$\rho$ (kg m <sup>-3</sup> )				
$m_S = 0.00000$ mol kg <sup>-1</sup>				
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K
0.00000	999.964	999.099	997.043	994.029
0.10004	1002.746	1001.792	999.6808	996.620
0.15005	1004.117	1003.119	1000.976	997.896
0.20004	1005.472	1004.435	1002.259	999.161
0.25004	1006.817	1005.735	1003.529	1000.408
0.30014	1008.150	1007.026	1004.791	1001.647
0.40000	1010.777	1009.569	1007.271	1004.082
$m_S = 0.10000$ mol kg <sup>-1</sup>				
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K
0.00000	1012.442	1011.348	1009.139	1006.030
0.10000	1015.035	1013.866	1011.603	1008.455
0.14998	1016.315	1015.110	1012.819	1009.648
0.19999	1017.585	1016.338	1014.018	1010.832
0.25001	1018.839	1017.556	1015.216	1012.005
0.30001	1020.085	1018.764	1016.392	1013.163
0.39963	1022.536	1021.137	1018.709	1015.436
$m_S = 0.30000$ mol kg <sup>-1</sup>				
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K
0.00000	1036.481	1034.981	1032.480	1029.195
0.10001	1038.744	1037.181	1034.644	1031.318
0.15000	1039.862	1038.270	1035.707	1032.364
0.20002	1040.972	1039.343	1036.760	1033.400
0.25004	1042.069	1040.414	1037.805	1034.430
0.30001	1043.164	1041.474	1038.842	1035.446
0.40067	1045.329	1043.577	1040.896	1037.466
$m_S = 0.70000$ mol kg <sup>-1</sup>				
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K
0.00000	1082.444	1080.308	1077.369	1073.761
0.10001	1084.086	1081.913	1078.949	1075.316
0.15002	1084.903	1082.713	1079.731	1076.086
0.20000	1085.704	1083.497	1080.501	1076.838
0.25003	1086.511	1084.278	1081.266	1077.593
0.30002	1087.317	1085.066	1082.033	1078.344
0.39999	1088.893	1086.598	1083.534	1079.815
$m_S = 1.00000$ mol kg <sup>-1</sup>				
$m_A$ (mol kg <sup>-1</sup> )	278.15 K	288.15 K	298.15 K	308.15 K
0.00000	1115.536	1112.984	1109.779	1105.989
0.10001	1116.795	1114.221	1110.987	1107.171
0.14996	1117.421	1114.836	1111.590	1107.765
0.20001	1118.044	1115.443	1112.192	1108.355
0.25001	1118.662	1116.051	1112.777	1108.927
0.29998	1119.278	1116.656	1113.362	1109.501
0.39992	1120.493	1117.836	1114.511	1110.627



## Appendix B

Table B.1. Comparison of PMV (glycine + water) from this work with literature's PMV.

Reference	278.15 K	288.15 K	298.15 K	308.15 K
This work	41.13 (0.05)	42.39 (0.03)	43.17 (0.07)	43.82 (0.07)
Zhao (2006)	41.33	42.42	43.18	43.86
Martins <i>et al.</i> (2014)	41.17 (0.02)	42.39 (0.01)	43.20 (0.01)	43.76 (0.02)
Dhir (2012)	--	42.81 (0.05)	43.17 (0.02)	44.34 (0.05)
Talele and Kishore (2013)	--	--	43.14	--
Rima <i>et al.</i> (2013)	--	41.69	42.43	43.00
Gucker <i>et al.</i> (1939)	--	--	43.20	--
Ellerton <i>et al.</i> (1964)	--	--	43.22	--
Tyrrell and Kennerle (1968)	41.10	42.30	--	--
Devine and Lowe (1971)	--	42.50	42.90	--
Gopal <i>et al.</i> (1973)	--	--	--	43.87
Shahidi and Farrell (1978)	--	--	43.50	--
Jolicoeur and Boileau (1978)	--	--	43.25	--
Millero <i>et al.</i> (1978)			43.19	
Lepori and Mollica (1980)	--	--	43.30	--
Wadi <i>et al.</i> (1981)	--	--	43.20	--
Lark and Bala (1983)	--	--	43.22	43.79
Mishra and Ahluwalia (1984)			43.19	
Ogawa <i>et al.</i> (1984)	--	--	43.23	--
Bhattacharya and Sengupta (1985)	--	--	--	41.7
Lark <i>et al.</i> (1986)	--	--	43.22	43.81
Kharakoz (1989)	--	42.40	43.30	43.80
Belibagli and Ayranci (1990)	--	--	43.18	--
Natarajan <i>et al.</i> (1990)	--	--	42.89	--
Iqbal and Mateullah (1990)	--	--	--	44.95
Wadi and Goyal (1992)	--	42.35	43.26	44.12
Chalikian <i>et al.</i> (1993)	--	--	43.20	--
Iqbal and Ahmed (1993)	--	--	--	44.52
Hakin <i>et al.</i> (1994)	--	42.48	43.26	--
Kikuchi <i>et al.</i> (1995)	41.07	42.29	43.19	43.81
Wadi and Ramasami (1997)	--	42.54	43.20	43.85
Ren <i>et al.</i> (1999)	--	--	42.48	--
Banipal and Kapoor (1999)	--	--	43.24	43.79
Yan <i>et al.</i> (1999)	41.90	42.60	43.50	44.20
Shen <i>et al.</i> (2000)	--	--	43.12	43.69
Banipal <i>et al.</i> (2000)	--	--	43.15	--
Li <i>et al.</i> (2001)	--	--	42.54	--
Islam and Wadi (2003)	--	42.35	--	44.12
Munde and Kishore (2003)	--	--	--	43.90
Singh and Kishore (2003)	--	--	43.14	--

continued

<b>Reference</b>	<b>278.15 K</b>	<b>288.15 K</b>	<b>298.15 K</b>	<b>308.15 K</b>
Pal and Kumar (2004)	--	--	43.19	--
Lark <i>et al.</i> (2004)	--	42.37	43.27	43.98
Badarayani and Kumar (2004a)	--	--	43.26	--
Banipal <i>et al.</i> (2004)	--	--	43.16	--
Mallick and Kishore (2006)	--	--	43.14	--

Table B.2. Comparison of partial molar volume (alanine + water) from this work with literature.

<b>Reference</b>	<b>278.15 K</b>	<b>288.15 K</b>	<b>298.15 K</b>	<b>308.15 K</b>
This work	58.99 (0.03)	59.91 (0.03)	60.51 (0.03)	60.98 (0.04)
Zhao (2006)	58.95	59.84	60.48	61.09
Dhir (2012)	--	59.89	60.40	61.31
Martins <i>et al.</i> (2014)	58.91 (0.01)	59.83 (0.01)	60.46 (0.02)	60.92 (0.02)
Talele and Kishore (2013)	--	--	60.43	--
Rima <i>et al.</i> (2013)	--	59.28	60.07	60.70
Millero <i>et al.</i> (1978)	--	--	60.47	--
Jolicoeur and Boileau (1978)	--	--	60.45	--
Ogawa <i>et al.</i> (1984)	--	--	60.50	--
Kharakoz (1989)	--	59.90	60.40	60.90
Hakin <i>et al.</i> (1994)	--	59.67	60.47	--
Kikuchi <i>et al.</i> (1995)	58.64	59.73	60.52	60.96
Ren <i>et al.</i> (1999)	--	--	60.92	--
Yan <i>et al.</i> (1999)	59.40	60.00	60.70	61.40
Li <i>et al.</i> (2001)	--	--	60.19	--
Munde and Kishore (2003)	--	--	--	60.44
Singh and Kishore (2003)	--	--	60.43	--
Pal and Kumar (2004)	--	--	60.43	--
Lark <i>et al.</i> (2004)	--	59.67	60.42	60.88
Mallick and Kishore (2006)	--	--	60.43	--

Table B.3. Comparison of PMV (DL-2-aminobutyric acid + water) from this work with literature's PMV.

<b>Reference</b>	<b>278.15 K</b>	<b>288.15 K</b>	<b>298.15 K</b>	<b>308.15 K</b>
This work	74.07 (0.05)	74.97 (0.03)	75.64 (0.02)	76.18 (0.04)
Zhao (2006)	74.4	74.75	75.60	76.44
Dhir (2012)	--	75.62	75.97	76.47
Mallick and Kishore (2006) Talele and Kishore (2013)	--	--	75.51	--
Rima <i>et al.</i> (2013)	--	74.35	75.18	75.84
Ellerton <i>et al.</i> (1964)	--	--	75.62	--

continued

<b>Reference</b>	<b>278.15 K</b>	<b>288.15 K</b>	<b>298.15 K</b>	<b>308.15 K</b>
Ahluwalia <i>et al.</i> (1977)	--	--	75.54	--
Millero <i>et al.</i> (1978) Lepori and Mollica (1980)	--	--	75.66	--
Ogawa <i>et al.</i> (1984)	--	--	84.45	--
Mishra and Ahluwalia (1984)	--	--	75.50	--
Bhattacharya and Sengupta (1985)	--	--	76.90	--
Bhat and Ahluwalia (1985)	--	--	75.92	--
Basumallick <i>et al.</i> (1986)	--	--	75.40	--
Natarajan <i>et al.</i> (1990)	--	--	75.24	--
Wadi <i>et al.</i> (1990)	--	74.78	75.64	76.03
Hakin <i>et al.</i> (1994)	--	74.67	75.51	--
Romero and Munar (1998)	--	--	75.38	--
Banipal and Kapoor (1999)	--	--	75.95	--
Yan <i>et al.</i> (1999)	74.40	75.10	75.80	76.20
Romero and Negrete (2004)	--	74.44	75.64	--
Mallick and Kishore (2006)	--	--	75.51	--

Table B.4. Comparison of PMV (valine + water) from this work with literature's PMV.

<b>Reference</b>	<b>278.15 K</b>	<b>288.15 K</b>	<b>298.15 K</b>	<b>308.15 K</b>
This work	89.08 (0.03)	90.03 (0.02)	90.71 (0.05)	91.37 (0.02)
Zhao (2006)	--	--	90.80	--
Dhir (2012)	--	90.02	90.76	91.22
Talele and Kishore (2013)	--	--	90.39	--
Banipal <i>et al.</i> (2008)	--	90.05	90.74	91.52
Mallick and Kishore (2006)	--	--	90.39	--
Yan <i>et al.</i> (1999)	89.79	90.22	90.97	91.64
Islam and Wadi (2003)	--	89.43	91.04	91.61
Singh and Kishore (2003)	--	--	90.39	--
Mallick and Kishore (2006)	--	--	90.39	--





## Appendix C

Table C.1. Densities of aqueous magnesium sulphate solutions (binary solutions) as a function of temperature and salt molality (values in parentheses represent standard deviation and number of independent measurements).

$m_s(\text{mol}\cdot\text{kg}^{-1})$	$\rho(\text{kg}\cdot\text{m}^{-3})$			
	278.15 K		288.15 K	
0.0000	999.964	--	999.099	--
0.1000	1012.442	(3.28x10 <sup>-2</sup> ; 12)	1011.348	(3.66x10 <sup>-2</sup> ; 9)
0.3000	1036.481	(5.08x10 <sup>-2</sup> ; 11)	1034.981	(4.69x10 <sup>-2</sup> ; 10)
0.7000	1082.444	(5.58x10 <sup>-2</sup> ; 9)	1080.308	(3.38x10 <sup>-2</sup> ; 8)
1.0000	1115.536	(3.11x10 <sup>-2</sup> ; 11)	1112.984	(3.72x10 <sup>-2</sup> ; 13)
$m_s(\text{mol}\cdot\text{kg}^{-1})$	298.15 K		308.15 K	
0.0000	997.043	--	994.029	--
0.1000	1009.139	(3.12x10 <sup>-2</sup> ; 11)	1006.030	(3.55x10 <sup>-2</sup> ; 9)
0.3000	1032.480	(4.20x10 <sup>-2</sup> ; x10)	1029.195	(4.10x10 <sup>-2</sup> ; 11)
0.7000	1077.369	(4.73x10 <sup>-2</sup> ; 9)	1073.761	(3.86x10 <sup>-2</sup> ; 8)
1.0000	1109.779	(3.31x10 <sup>-2</sup> ; 11)	1105.989	(2.62x10 <sup>-2</sup> ; 9)



# Appendix D

Table D.1. Partial molar volumes of transfer in different electrolytes and at various temperatures found in literature.

Reference	Amino acid					
Yan <i>et al.</i> (2004)		$m_{\text{CaCl}_2} = 3.0 \text{ mol kg}^{-1}$				
		278.15 K	288.15 K	298.15 K	308.15 K	
	glycine	8.24	7.83	7.39	7.01	
	DL-alanine	8.28	7.86	7.50	7.08	
	Aaba	6.39	5.70	5.47	5.15	
	DL-valine	5.82	6.35	6.19	6.08	
Banipal <i>et al.</i> (2004)		298.15 K				
	$m_{\text{NaCOOCH}_3} =$	0.5	1.0	2.0	4.0	5.0
	glycine	1.66	2.90	4.38	5.59	6.36
	DL-alanine	1.09	1.95	3.04	4.25	4.98
	Aaba	0.86	1.57	2.61	3.85	4.42
Banipal <i>et al.</i> (2006)		298.15 K				
	$m_{\text{Mg}(\text{CH}_3\text{COO})_2} =$	0.5	1.0	1.5	2.0	
	glycine	2.60	3.88	4.75	5.68	
	DL-alanine	1.96	2.98	3.65	4.23	
	Aaba	1.26	2.16	2.78	3.23	
Banipal <i>et al.</i> (2008)		288.15 K				
	$m_{\text{ZnCl}_2} =$	0.1	0.5	1.0	1.5	
	glycine	2.12	2.98	3.93	4.75	
	DL-alanine	1.44	2.59	3.16	4.11	
	Aaba	1.04	2.01	2.55	3.52	
	L-valine	0.83	1.64	1.96	2.95	
		298.15 K				
	$m_{\text{ZnCl}_2} =$	0.1	0.5	1.0	1.5	
	glycine	2.77	3.45	4.27	5.04	
	DL-alanine	2.01	3.96	2.72	4.84	
	Aaba	1.55	3.50	1.67	4.38	
	L-valine	1.04	2.01	2.95	3.74	
		308.15 K				
	$m_{\text{ZnCl}_2} =$	0.1	0.5	1.0	1.5	
	glycine	3.54	4.25	5.23	6.29	
DL-alanine	2.17	3.18	4.07	5.06		
Aaba	1.96	2.94	3.86	4.94		
L-valine	1.48	2.54	3.43	4.39		
Talele and Kishore (2013)		298.15 K				
		$m_{\text{DTAB}} = 0.1$			$m_{\text{TTAB}} = 0.1$	
	glycine	-0.07			-0.05	
	DL-alanine	-0.15			-0.32	
	Aaba	0.10			-0.23	
	L-valine	-0.38			-0.11	

**DTAB:** dodecyltrimethylammonium bromide

**TTAB:** tetradecyltrimethylammonium bromide

continued

Natarajan <i>et al.</i> (1990)	298.15 K						
	$m_{\text{NH}_4\text{Cl}} =$	0.02	0.10	1.00	2.00		
	glycine	0.39	0.22	1.09	2.82		
	$m_{\text{NH}_4\text{Cl}} =$	0.05	0.25	1.25	1.80		
	DL-alanine	0.03	0.38	1.72	2.08		
	$m_{\text{NH}_4\text{Cl}} =$	0.25	0.50	1.00	2.00		
	Aaba	0.50	0.97	1.31	2.42		
	$m_{\text{NH}_4\text{Cl}} =$	0.25	0.50	1.25	2.00		
L-valine	0.62	0.75	1.76	1.77			
Ogawa <i>et al.</i> (1984)	298.15 K						
		LiCl		NaCl		KCl	
	$m_{\text{salt}} =$	1.0	2.0	1.0	2.0	1.0	2.0
	glycine	1.45	2.40	1.82	3.01	1.66	2.89
	alanine	1.09	1.98	1.58	2.69	1.47	2.64
	Iso-Aaba	0.91	1.64	1.32	2.43	1.24	2.44
Wadi and Goyal (1992)	288.15 K						
	$m_{\text{KSCN}} =$	1.0		3.0		5.0	
	glycine	1.98		3.78		4.03	
	DL-alanine	1.38		2.72		4.08	
	$\gamma$ -Aaba	2.86		4.28		5.10	
	298.15 K						
	$m_{\text{KSCN}} =$	1.0		3.0		5.0	
	glycine	1.37		3.52		3.81	
	DL-alanine	1.33		2.38		3.69	
	$\gamma$ -Aaba	1.61		3.08		4.13	
	308.15 K						
	$m_{\text{KSCN}} =$	1.0		3.0		5.0	
glycine	1.04		2.76		3.22		
DL-alanine	1.02		2.39		3.24		
$\gamma$ -Aaba	1.36		2.63		3.53		
Wadi and Ramasami (1997)	288.15 K						
	$m_{\text{Na}_2\text{SO}_4} =$	0.5		1.0		1.5	
	glycine	3.04		4.67		5.89	
	DL-alanine	2.51		2.84		5.01	
	298.15 K						
	$m_{\text{Na}_2\text{SO}_4} =$	0.5		1.0		1.5	
	glycine	2.95		4.50		5.35	
	DL-alanine	2.60		4.06		5.04	
	308.15 K						
	$m_{\text{Na}_2\text{SO}_4} =$	0.5		1.0		1.5	
glycine	2.74		3.99		5.03		
DL-alanine	2.24		3.69		4.74		

continued

Islam and Wadi (2003)	288.15 K				
	$m_{\text{NaSO}_4^-}$	0.5	1.0	1.5	
	glycine	3.40	4.80	6.00	
	DL-alanine	3.10	4.00	5.40	
	Aaba	3.00	4.20	5.50	
	DL-valine	3.20	4.40	6.50	
	298.15 K				
	$m_{\text{NaSO}_4^-}$	0.5	1.0	1.5	
	glycine	3.20	4.20	5.70	
	DL-alanine	2.70	3.90	4.90	
	Aaba	2.40	3.60	4.80	
	DL-valine	2.70	4.10	5.20	
	308.15 K				
	$m_{\text{NaSO}_4^-}$	0.5	1.0	1.5	
	glycine	2.50	3.70	4.90	
DL-alanine	2.20	3.40	4.50		
Aaba	2.10	3.30	4.50		
DL-valine	2.40	3.60	4.80		
Wang <i>et al.</i> (1999)	308.15 K				
	$m_{\text{NaCOOCH}_3^-}$	0.5	1.0	1.5	2.0
	glycine	0.70	1.71	2.25	3.16
	DL-alanine	0.51	1.35	1.89	2.52
	Aaba	0.45	1.29	1.59	1.97
	DL-valine	0.34	0.77	1.08	1.65



## Appendix E

Table E.1. Correlation coefficients ( $R^2$ ) of all salt molalities and at all temperatures.

$m_s(\text{mol}\cdot\text{kg}^{-1})$	278.15 K	288.15 K	298.15 K	308.15 K
0.0000	0.9981	0.9985	0.9988	0.9991
0.1000	0.9986	0.9989	0.9991	0.9992
0.3000	0.9988	0.9989	0.9990	0.9993
0.7000	0.9989	0.9992	0.9993	0.9994
1.0000	0.9993	0.9994	0.9994	0.9995