Partial Molar Volumes of Amino Acids in Aqueous MgSO₄ 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

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Simão Pedro de Almeida Pinho

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
Acknowledgements

I would like to start by thanking my supervisor Professor Olga Ferreira for her teaching, availability, patience and support throughout the time that lasted the realization of this thesis. I also thank my co-supervisor Simão Pinho for all his help and guidance along the way.

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Finally I thank my parents because without them none of this would be possible, my brother, my boyfriend and all that somehow contributed to this work.
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⁻¹ 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 ($\text{NH}_3^+$, $\text{COO}^-$) 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 moleares 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\(^{-1}\) contendo os aminoácidos selecionados foram medidas a (278,15; 288,15; 298,15 e 308,15) K, usando um densímetro digital. Depois, os volumes moleares 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 \(\Delta_{tr}V^0_m\) sugerem que as interações íon/grupo hidrofílico (centros zeuteriónicos) são predominantes, e aplicando a metodologia proposta por Friedman e Krishnan (1973), concluiu-se que ocorrem principalmente entre pares. Relativamente ao efeito da cadeia alquila, o aumento da parte hidrofóbica dos aminoácidos (glicina > L-alanina > DL-2-ácido aminobutírico > L-valina) não resulta numa diminuição dos volumes moleares parciais de transferência.

Para analisar melhor o efeito da cadeia alquila dos aminoácidos, aplicou-se um método de contribuição de grupos para modelar os dados dos volumes moleares parciais. A contribuição dos grupos zeuteriónicos (\(\text{NH}_3^+\), \(\text{COO}^-\)) 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 alquila é muito menor, tendo uma tendência muito fraca de diminuição com o aumento da molalidade de sal.

Palavras-chave: Volumes Moleares 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 (mol\cdot kg^{-1})

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

\( M \)  Mass (g)

\( MM \)  Molar mass (g\cdot 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

\( V_{m,A}^o \)  Partial molar volume

\( w \)  Mass fraction

\( \Delta_{tr}V_{m,A}^o \)  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 (Mg$^{2+}$ and SO$_4^{2-}$), and the groups of proteins (NH$_3^+$, 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$_2$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):

$$ V = -\frac{q_{\text{Na}^+} \times q_{\text{Cl}^-}}{4\pi\varepsilon_0 r} $$

(1.1)

where $q_{\text{Na}^+}$ and $q_{\text{Cl}^-}$ are the charges of the two ions, $r$ is the distance between ions and $\varepsilon_0$ is the permittivity of the vacuum ($8.854 \times 10^{-12}$ C$^2$N$^{-1}$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)).](image)

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

$$V = -\frac{2\mu_A\mu_B}{4\pi\varepsilon_0 r^3}$$

(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. Mg$^{2+}$ and 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).

$$V = -\frac{q\mu}{4\pi\varepsilon_0 r^2}$$

(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 \textit{polarizable}. The magnitude of the induced dipole moment is proportional to the strength of the electric field, $\varepsilon$:

$$\mu_{\text{ind}} = \alpha \varepsilon$$

(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:

$$V = -\frac{1}{2} \times \frac{\alpha q^2}{4\pi\varepsilon_0 r^4}$$

(1.5)

where $\alpha = \alpha' / 4\pi\varepsilon_0$ (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:

$$V = -\frac{\alpha \mu^2}{4\pi\varepsilon_0 r^6}$$

(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:

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

(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.
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, $\Delta_{\text{hydr}}S$, and Gibbs free energy of hydration, $\Delta_{\text{hydr}}G$, at 298.15 K (Marcus 1991; Marcus 1997).

<table>
<thead>
<tr>
<th>Ion</th>
<th>$\Delta_{\text{hydr}}G$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN$^-$</td>
<td>-280</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>-300</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>-340</td>
</tr>
<tr>
<td>CH$_3$COO$^-$</td>
<td>-365</td>
</tr>
<tr>
<td>SO$_2^-$</td>
<td>-1080</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>-285</td>
</tr>
<tr>
<td>K$^+$</td>
<td>-304</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>-365</td>
</tr>
<tr>
<td>Li$^+$</td>
<td>-475</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>-1515</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>-1755</td>
</tr>
</tbody>
</table>
The cation (Mg\(^{2+}\)) and anion (SO\(_4^{2-}\)) studied in this work are highlighted in Figure 1.2 and Table 1.1. As can be seen both Mg\(^{2+}\) and SO\(_4^{2-}\) 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\(_2\)SO\(_4\), CH\(_3\)COONa and MgSO\(_4\) stabilize proteins whereas salts such as MgCl\(_2\), CaCl\(_2\) 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.

<table>
<thead>
<tr>
<th>Ion</th>
<th>(\Delta_{\text{hyd}}G) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(^{2+})</td>
<td>-1760</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>-1830</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>-1955</td>
</tr>
</tbody>
</table>
First, the concept of apparent molar volume \( V_{A,\phi} \) in aqueous saline solutions is defined as:

\[
V_{A,\phi} = \frac{V_T - V_B}{n_A}
\]

(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.

\[
M_T = M_1 + M_A + M_3
\]

(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:

\[
m = \frac{n_i}{M_1} = \frac{M_i/MM_i}{M_1} (=) M_i = m \times MM_i \times M_1
\]

(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:

\[
M_T = M_1 + M_A \times MM_A \times M_1 + M_3 \times MM_3 \times M_1
\]

(1.11)

By analogy:

\[
M_B = M_1 + M_3 \times MM_3 \times M_1
\]

(1.12)

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

\[
V_T = \frac{M_T}{\rho_T} = \frac{M_1 \times (1 + M_A \times MM_A + M_3 \times MM_3)}{\rho_T}
\]

(1.13)

\[
V_B = \frac{M_B}{\rho_B} = \frac{M_1 \times (1 + M_3 \times MM_3)}{\rho_B}
\]

(1.14)

By replacing \( V_T \) and \( V_B \) in Equation 1.8:

\[
V_{A,\phi} = \frac{\frac{M_1 \times (1 + M_A \times MM_A + M_3 \times MM_3)}{\rho_T} - \frac{M_1 \times (1 + M_3 \times MM_3)}{\rho_B}}{n_A}
\]

(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, $m_A \to 0$, $\rho_T \to \rho_B$, 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:

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

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 \((\partial V_{mA}^o/\partial T)_p > 0\) and \((\partial^2 V_{mA}^o/\partial T^2)_p < 0\), the solute is hydrophilic;

- If \((\partial V_{mA}^o/\partial T)_p < 0\) and \((\partial^2 V_{mA}^o/\partial T^2)_p > 0\), the solute is hydrophobic;

where \((\partial V_{mA}^o/\partial T)_p\) 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 (-NH\(_3^+\), - 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.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Temperature (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>288.15 to 318.15</td>
<td>Rima et al. (2013), Dhir (2012) and Banipal et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>298 to 443</td>
<td>Cibulka et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>Munde and Kishore (2003)</td>
</tr>
<tr>
<td></td>
<td>278.15 to 318.15</td>
<td>Martins et al. (2014)</td>
</tr>
<tr>
<td><strong>alanine</strong></td>
<td>397 to 521</td>
<td>Hakin et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>298 to 443</td>
<td>Cibulka et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>Munde and Kishore (2003)</td>
</tr>
<tr>
<td></td>
<td>288.15 to 318.15</td>
<td>Rima et al. (2013), Dhir (2012) and Banipal et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>278.15 to 318.15</td>
<td>Martins et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>288.15 to 303.15</td>
<td>Romero and Negrete (2004)</td>
</tr>
<tr>
<td></td>
<td>288.15 to 318.15</td>
<td>Rima et al. (2013), Dhir (2012) and Banipal et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>288.15 to 318.15</td>
<td>Banipal et al. (2008) and Dhir (2012)</td>
</tr>
<tr>
<td></td>
<td>288.15, 298.15 and 308.15</td>
<td>Islam and Wadi (2003)</td>
</tr>
<tr>
<td></td>
<td>297.15</td>
<td>Dipaola and Belleau (1978)</td>
</tr>
</tbody>
</table>
Table 2.2. References for the partial molar volumes and other properties for the ternary system glycine + salt + water published in the literature.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>298.15</td>
<td>Bhat and Ahluwalia (1985)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Yuan et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Ogawa et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>278.15, 288.15, 298.15 and 308.15</td>
<td>Shen et al. (2000)</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>288.15, 298.15 and 308.15</td>
<td>Islam and Wadi (2003)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Wadi and Ramasami (1997)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>288.15, 298.15, and 308.15</td>
<td>Lark et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>288.15</td>
<td>Pal and Kumar (2005b)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Pal and Kumar (2005a)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Badarayani and Kumar (2003a)</td>
</tr>
<tr>
<td>NaBr and KBr</td>
<td>298.15</td>
<td>Badarayani and Kumar (2003a)</td>
</tr>
<tr>
<td>(CH₃)₄NBr, (C₅H₁₀)₄NBr and (C₅H₁₀)₄NBr</td>
<td>Badarayani and Kumar (2004a)</td>
<td></td>
</tr>
<tr>
<td>C₄H₁₀BrN</td>
<td>298.15</td>
<td>Banerjee and Kishore (2005)</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>298.15</td>
<td>Mallick and Kishore (2006)</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>298.15</td>
<td>Natarajan et al. (1990)</td>
</tr>
<tr>
<td>LiCl</td>
<td>298.15</td>
<td>Ogawa et al. (1984)</td>
</tr>
<tr>
<td>NaSCN</td>
<td>298.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td>NaCH₃(CH₂)₃(OSO₃ and BrC₁₀H₁₃N(CH₃)₃)</td>
<td>Singh et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>298.15</td>
<td>Badarayani and Kumar (2003a)</td>
</tr>
<tr>
<td>Mg(CH₃COO)₂</td>
<td>308.15</td>
<td>Ogawa et al. (1984)</td>
</tr>
<tr>
<td>C₁₅H₁₄BrN and C₁₇H₃₈BrN</td>
<td>298.15</td>
<td>Banipal et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>Talele and Kishore (2013)</td>
</tr>
<tr>
<td>NaCH₃COO</td>
<td>298.15</td>
<td>Banipal et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>298.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>Wang et al. (1999)</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>278.15, 288.15, 298.15 and 308.15</td>
<td>Martins et al. (2014)</td>
</tr>
<tr>
<td>CH₃N₂.HCl</td>
<td>288.15, 298.15, and 308.15</td>
<td>Yan et al. (1998)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>288.15, 298.15, 308.15 and 318.15</td>
<td>Banipal et al. (2008)</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>288.15, 298.15, 308.15 and 318.15</td>
<td>Banipal et al. (2008)</td>
</tr>
<tr>
<td>KSCN</td>
<td>288.15, 298.15 and 308.15</td>
<td>Wadi and Goyal (1992)</td>
</tr>
</tbody>
</table>
Table 2.3. References for the partial molar volumes and other properties for the ternary system alanine + salt + water published in the literature.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>283.15 to 313.15, 298.15</td>
<td>Badarayani and Kumar (2003b), Ogawa et al. (1984)</td>
</tr>
<tr>
<td>Mg(CH(_3)COO)(_2)</td>
<td>298.15</td>
<td>Banipal et al. (2006)</td>
</tr>
<tr>
<td>C(_2)H(_3)NaO(_2)</td>
<td>298.15</td>
<td>Banipal et al. (2004)</td>
</tr>
<tr>
<td>(CH(_3))(_2)NBr, (C(_2)H(_3))(_2)NBr and (C(_3)H(_3))(_2)NBr</td>
<td>298.15</td>
<td>Badarayani and Kumar (2004a)</td>
</tr>
<tr>
<td>Ca(_2)H(_2)BrN</td>
<td>298.15</td>
<td>Banerjee and Kishore (2005)</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>298.15</td>
<td>Mallick and Kishore (2006)</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>298.15</td>
<td>Natarajan et al. (1990)</td>
</tr>
<tr>
<td>LiCl</td>
<td>298.15</td>
<td>Ogawa et al. (1984)</td>
</tr>
<tr>
<td>NaSCN</td>
<td>298.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td>NaCH(_3)(CH(_2))(_3)OSO(_3) and BrC(_6)H(_3)N(CH(_3))(_3)</td>
<td>298.15</td>
<td>Singh et al. (2004)</td>
</tr>
<tr>
<td>NaCl</td>
<td>298.15, 298.15 and 308.15</td>
<td>Ogawa et al. (1984), Bhat and Ahluwalia (1985), Rodriguez et al. (2003), Yuan et al. (2006), Shen et al. (2000)</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>288.15, 298.15, and 308.15, 288.15 and 308.15, 298.15</td>
<td>Lark et al. (2004), Pal and Kumar (2005b), Pal and Kumar (2005a)</td>
</tr>
<tr>
<td>KSCN</td>
<td>288.15, 298.15 and 308.15</td>
<td>Wadi and Goyal (1992)</td>
</tr>
<tr>
<td>(NH(_4))(_2)SO(_4)</td>
<td>288.15, 298.15, and 308.15</td>
<td>Martins et al. (2014)</td>
</tr>
<tr>
<td>CH(_3)N(_2),HCl</td>
<td>278.15, 288.15, 298.15 and 308.15</td>
<td>Yan et al. (1998)</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>298.15</td>
<td>Yan et al. (2004)</td>
</tr>
<tr>
<td>NaCH(_3)COO</td>
<td>298.15, 308.15</td>
<td>Singh and Kishore (2003), Wang et al. (1999)</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈H₁₆BrN</td>
<td></td>
<td>Banerjee and Kishore (2005)</td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>Bhat and Ahluwalia (1985)</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td></td>
<td>Natarajan et al. (1990)</td>
</tr>
<tr>
<td>C₂H₅NaO₂</td>
<td>298.15</td>
<td>Banipal et al. (2004)</td>
</tr>
<tr>
<td>Mg(CH₃COO)₂</td>
<td></td>
<td>Banipal et al. (2006)</td>
</tr>
<tr>
<td>MgSO₄</td>
<td></td>
<td>Mallick and Kishore (2006)</td>
</tr>
<tr>
<td>C₁₅H₃₄BrN and C₁₇H₁₈BrN</td>
<td></td>
<td>Talele and Kishore (2013)</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>288.15, 298.15, and 308.15</td>
<td>Islam and Wadi (2003)</td>
</tr>
<tr>
<td>NaCH₃COO</td>
<td>308.15</td>
<td>Wang et al. (1999)</td>
</tr>
<tr>
<td>CH₃N₂HCl</td>
<td>278.15, 288.15, 298.15, and 308.15</td>
<td>Yan et al. (1998)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>288.15, 298.15, 308.15, and 318.15</td>
<td>Yan et al. (2004)</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>288.15, 298.15, 308.15, and 318.15</td>
<td>Banipal et al. (2008)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature (K)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaCl₂</td>
<td></td>
<td>Roy et al. (2013)</td>
</tr>
<tr>
<td>C₁₈H₃₄BrN</td>
<td></td>
<td>Banerjee and Kishore (2005)</td>
</tr>
<tr>
<td>NaCl</td>
<td>298.15</td>
<td>Bhat and Ahluwalia (1985)</td>
</tr>
<tr>
<td>MgSO₄</td>
<td></td>
<td>Mallick and Kishore (2006)</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td></td>
<td>Natarajan et al. (1990)</td>
</tr>
<tr>
<td>NaCH₃(CH₂)₁₁OSO₃ and BrC₁₈H₃₄N(CH₃)₃</td>
<td>298.15</td>
<td>Singh et al. (2004)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>298.15</td>
<td>Pal and Kumar (2005a)</td>
</tr>
<tr>
<td></td>
<td>298.15 and 308.15</td>
<td>Pal and Kumar (2005b)</td>
</tr>
<tr>
<td>KCl and KNO₃</td>
<td>298.15, 303.15, 308.15, 313.15, 318.15, and 323.15</td>
<td>Riyazuddeen and Khan (2009)</td>
</tr>
<tr>
<td>NaCH₃COO</td>
<td>298.15, 308.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td></td>
<td>308.15</td>
<td>Wang et al. (1999)</td>
</tr>
<tr>
<td>CH₃N₂HCl</td>
<td>278.15, 288.15, 298.15, and 308.15</td>
<td>Yan et al. (1998)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>278.15, 288.15, 298.15, and 308.15</td>
<td>Yan et al. (2004)</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>298.15</td>
<td>Singh and Kishore (2003)</td>
</tr>
<tr>
<td></td>
<td>288.15, 298.15, 308.15, and 318.15</td>
<td>Islam and Wadi (2003)</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>288.15, 298.15, 308.15, and 318.15</td>
<td>Banipal et al. (2012)</td>
</tr>
</tbody>
</table>
Chapter 2. State of the Art
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>
<thead>
<tr>
<th>Chemical name</th>
<th>Supplier</th>
<th>Mass fraction purity (%)</th>
<th>Molecular mass (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>magnesium chloride hexahydrate</td>
<td>Panreac</td>
<td>≥ 99.0</td>
<td>203.29910</td>
</tr>
<tr>
<td>magnesium sulphate heptahydrate</td>
<td>Merck</td>
<td>≥ 99.0</td>
<td>246.48000</td>
</tr>
<tr>
<td>L-valine</td>
<td>Merck</td>
<td>≥ 99.0</td>
<td>117.14638</td>
</tr>
<tr>
<td>DL-2-aminobutyric acid</td>
<td>Merck</td>
<td>≥ 99.0</td>
<td>103.11983</td>
</tr>
<tr>
<td>L-alanine</td>
<td>Merck</td>
<td>≥ 99.0</td>
<td>89.09328</td>
</tr>
<tr>
<td>glycine</td>
<td>Merck</td>
<td>≥ 99.7</td>
<td>75.06673</td>
</tr>
</tbody>
</table>

*Declared by the supplier
Chapter 3. Experimental Part

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 ± 0.1 mg). The uncertainty in the solute molality was estimated to be ± 2×10⁻⁵ mol⋅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™ 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.

![Analytical balance used throughout this work.](image)

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\(^{-3}\) (error ± 0.000005 g cm\(^{-3}\)), with temperature varying between 273.15 and 368.15 K (error ± 0.01 K) and pressure range from 0 to 10 bar (Paar 2011). The measurement repeatability is ±1×10\(^{-5}\) g cm\(^{-3}\).
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.

![Graphs showing density versus amino acid molality](image)

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 $a_\rho$:

$$\frac{\rho - \rho_0}{m_A} = a_\rho + b_\rho m_A$$  \hspace{1cm} (3.1)$$

where $\rho$ is the solution density, $m_A$ is the amino acid molality and $b_\rho$ 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 (cm³mol⁻¹) of amino acids in aqueous solutions obtained in this work and found in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>278.15 K</th>
<th>288.15 K</th>
<th>298.15 K</th>
<th>308.15 K</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>glycine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This work</td>
<td>41.13 (0.05)</td>
<td>42.39 (0.03)</td>
<td>43.17 (0.07)</td>
<td>43.82 (0.07)</td>
</tr>
<tr>
<td>Zhao (2006)</td>
<td>41.33</td>
<td>42.42</td>
<td>43.18</td>
<td>43.86</td>
</tr>
<tr>
<td>Martins <em>et al.</em> (2014)</td>
<td>41.17 (0.02)</td>
<td>42.39 (0.01)</td>
<td>43.20 (0.01)</td>
<td>43.76 (0.02)</td>
</tr>
<tr>
<td>Dhir (2012)</td>
<td>--</td>
<td>42.81 (0.05)</td>
<td>43.17 (0.02)</td>
<td>44.34 (0.05)</td>
</tr>
<tr>
<td>Talele and Kishore (2013)</td>
<td>--</td>
<td>--</td>
<td>43.14</td>
<td>--</td>
</tr>
<tr>
<td>Rima et al. (2013)</td>
<td>--</td>
<td>41.69</td>
<td>42.43</td>
<td>43.00</td>
</tr>
<tr>
<td><strong>alanine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This work</td>
<td>58.99 (0.03)</td>
<td>59.91 (0.03)</td>
<td>60.51 (0.03)</td>
<td>60.98 (0.04)</td>
</tr>
<tr>
<td>Zhao (2006)</td>
<td>58.95</td>
<td>59.84</td>
<td>60.48</td>
<td>61.09</td>
</tr>
<tr>
<td>Martins <em>et al.</em> (2014)</td>
<td>58.91 (0.01)</td>
<td>59.83 (0.01)</td>
<td>60.46 (0.02)</td>
<td>60.92 (0.02)</td>
</tr>
<tr>
<td>Dhir (2012)</td>
<td>--</td>
<td>59.89 (0.04)</td>
<td>60.40 (0.03)</td>
<td>61.31 (0.12)</td>
</tr>
<tr>
<td>Talele and Kishore (2013)</td>
<td>--</td>
<td>--</td>
<td>60.43</td>
<td>--</td>
</tr>
<tr>
<td>Rima et al. (2013)</td>
<td>--</td>
<td>59.28</td>
<td>60.07</td>
<td>60.70</td>
</tr>
<tr>
<td><strong>DL-2-aminobutyric acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This work</td>
<td>74.07 (0.05)</td>
<td>74.97 (0.03)</td>
<td>75.64 (0.02)</td>
<td>76.18 (0.04)</td>
</tr>
<tr>
<td>Zhao (2006)</td>
<td>74.40</td>
<td>74.75</td>
<td>75.60</td>
<td>76.44</td>
</tr>
<tr>
<td>Dhir (2012)</td>
<td>--</td>
<td>75.62 (0.09)</td>
<td>75.97 (0.03)</td>
<td>76.47 (0.12)</td>
</tr>
<tr>
<td>Mallick and Kishore (2006)</td>
<td>--</td>
<td>--</td>
<td>75.51</td>
<td>--</td>
</tr>
<tr>
<td>Talele and Kishore (2013)</td>
<td>--</td>
<td>--</td>
<td>75.51</td>
<td>--</td>
</tr>
<tr>
<td>Rima et al. (2013)</td>
<td>--</td>
<td>74.35</td>
<td>75.18</td>
<td>75.84</td>
</tr>
<tr>
<td><strong>valine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This work</td>
<td>89.08 (0.03)</td>
<td>90.03 (0.02)</td>
<td>90.71 (0.05)</td>
<td>91.37 (0.02)</td>
</tr>
<tr>
<td>Zhao (2006)</td>
<td>89.42</td>
<td>89.92</td>
<td>90.87</td>
<td>91.56</td>
</tr>
<tr>
<td>Dhir (2012)</td>
<td>--</td>
<td>90.02 (0.05)</td>
<td>90.76 (0.02)</td>
<td>91.22 (0.07)</td>
</tr>
<tr>
<td>Talele and Kishore (2013)</td>
<td>--</td>
<td>--</td>
<td>90.39</td>
<td>--</td>
</tr>
<tr>
<td>Banipal <em>et al.</em> (2008)</td>
<td>--</td>
<td>90.05</td>
<td>90.74</td>
<td>91.52</td>
</tr>
<tr>
<td>Mallick and Kishore (2006)</td>
<td>--</td>
<td>--</td>
<td>90.39</td>
<td>--</td>
</tr>
</tbody>
</table>
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 cm$^3$ mol$^{-1}$ (for DL-2-aminobutyric acid at 278.15 K) and the minimum is 0.01 cm$^3$ 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, $E_m^o = \left( \frac{\partial V_m^o}{\partial T} \right)_p$. For that, the partial molar volumes at infinite dilution in water were fitted to:

$$V_m^o = a + bT + cT^2$$

(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.](image-url)
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: □ This work; × (Martins et al. 2014); ◊ (Banipal et al. 2008); ○ (Shen et al. 2000); △ (Yan et al. 1999); + (Wadi et al. 1990); – (Chalikian et al. 1993); * (Rima et al. 2013); □ (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>
<thead>
<tr>
<th>T (K)</th>
<th>Gly</th>
<th>L-Ala</th>
<th>Aaba</th>
<th>L-Val</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\frac{\partial V_m^{\circ}}{\partial T})</td>
<td>(\frac{\partial^2 V_m^{\circ}}{\partial T^2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>278.15</td>
<td>0.13373</td>
<td>0.09923</td>
<td>0.09748</td>
<td>0.09782</td>
</tr>
<tr>
<td>288.15</td>
<td>0.10364</td>
<td>0.07688</td>
<td>0.07909</td>
<td>0.08297</td>
</tr>
<tr>
<td>298.15</td>
<td>0.07354</td>
<td>0.05452</td>
<td>0.06067</td>
<td>0.06812</td>
</tr>
<tr>
<td>308.15</td>
<td>0.04344</td>
<td>0.03217</td>
<td>0.04224</td>
<td>0.05327</td>
</tr>
<tr>
<td></td>
<td>-0.00301</td>
<td>-0.00224</td>
<td>-0.00184</td>
<td>-0.00149</td>
</tr>
</tbody>
</table>

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 MgCl$_2$6H$_2$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.

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 MgSO$_4$7H$_2$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 independents 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).

<table>
<thead>
<tr>
<th>$m_S$ (mol kg$^{-1}$)</th>
<th>$V_m^o$ (cm$^3$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>278.15 K</td>
</tr>
<tr>
<td>glycine</td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>41.13 (0.05)</td>
</tr>
<tr>
<td>0.1000</td>
<td>42.15 (0.05)</td>
</tr>
<tr>
<td>0.3000</td>
<td>43.43 (0.06)</td>
</tr>
<tr>
<td>0.7000</td>
<td>45.25 (0.05)</td>
</tr>
<tr>
<td>1.0000</td>
<td>46.51 (0.05)</td>
</tr>
<tr>
<td>L-alanine</td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>58.99 (0.03)</td>
</tr>
<tr>
<td>0.1000</td>
<td>59.71 (0.04)</td>
</tr>
<tr>
<td>0.3000</td>
<td>60.73 (0.03)</td>
</tr>
<tr>
<td>0.7000</td>
<td>62.61 (0.04)</td>
</tr>
<tr>
<td>1.0000</td>
<td>63.27 (0.02)</td>
</tr>
<tr>
<td>DL-2-aminobutyric acid</td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>74.07 (0.05)</td>
</tr>
<tr>
<td>0.1000</td>
<td>74.69 (0.04)</td>
</tr>
<tr>
<td>0.3000</td>
<td>76.00 (0.09)</td>
</tr>
<tr>
<td>0.7000</td>
<td>77.71 (0.06)</td>
</tr>
<tr>
<td>1.0000</td>
<td>78.57 (0.03)</td>
</tr>
<tr>
<td>L-valine</td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>89.08 (0.03)</td>
</tr>
<tr>
<td>0.1000</td>
<td>89.87 (0.03)</td>
</tr>
<tr>
<td>0.3000</td>
<td>91.02 (0.03)</td>
</tr>
<tr>
<td>0.7000</td>
<td>92.94 (0.07)</td>
</tr>
<tr>
<td>1.0000</td>
<td>93.62 (0.03)</td>
</tr>
</tbody>
</table>
For all the systems, partial molar volumes increase when increasing salt molality or temperature. The estimated uncertainties are relatively low, being 0.13 cm$^3$ 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.
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:

\[
n_H = \frac{(V_{m,A}^o - V_{m,A,\text{int}}^o)}{(V_e^o - V_b^o)}
\]  

(4.1)

where \( V_e^o \) is the molar volume of electrostricted water, \( V_b^o \) is the molar volume of bulk water and \( V_{m,A,\text{int}}^o \) 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 cm\(^3\)mol\(^{-1}\) at 278.15, 288.15, 298.15 and 308.15 K, respectively. To calculate the intrinsic volume of the amino acid \( (V_{m,A,\text{int}}^o) \), the following relationship was used (Millero et al. 1978):
Chapter 4. Discussion of Results

\[ V_{m,A,int}^o = \frac{0.7}{0.634}V_{m,A,cryst}^o \]  

where \( V_{m,A,cryst}^o \) 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 \( V_{m,Aab,cryst}^o \).

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

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

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

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 ≈ 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).
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 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 \(\nu\), 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:

\[
\Delta n_H = a + b \sum_{i} \nu_i \frac{z_i}{r_i} + c \frac{\Delta G_{hyd, \text{cation}}}{\Delta G_{hyd, \text{potassium}}} \tag{4.3}
\]

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>
<thead>
<tr>
<th>(\Delta n_H) (Gly)</th>
<th>(\Delta n_H) (Ala)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.27</td>
<td>0.78</td>
<td>Mallick and Kishore (2006)</td>
</tr>
<tr>
<td>1.28</td>
<td>1.00</td>
<td>This work</td>
</tr>
<tr>
<td>1.68</td>
<td>1.30</td>
<td>Correlation (Martins et al. 2014)</td>
</tr>
</tbody>
</table>

### 4.2. Partial Molar Volumes of Transfer

The partial molar volumes of transfer at infinite dilution (\(\Delta_{tr} V_{m,A}^o\)) 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.

\[
\Delta_{tr} V_{m,A}^o = V_{m,A}^o \text{ (in aqueous salt solution)} - V_{m,A}^o \text{ (in water)} \tag{4.4}
\]
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.

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

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 ($\cdot\text{COO}^-$ and $\cdot\text{NH}_3^+$) 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 $\Delta_{tr}V_{m,a}^0$ values, while the interactions ion/hydrophobic group give negative $\Delta_{tr}V_{m,a}^0$ 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 – CH$_2$– group in glycine to form alanine, causes a reduction on $\Delta_{\text{tr}}V_{m,A}^o$. However, when comparing the four amino acids studied in solutions containing magnesium sulphate, the magnitude of $\Delta_{\text{tr}}V_{m,A}^o$ 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 $\Delta_{\text{tr}}V_{m,A}^o$ 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) ($m_{\text{calcium chloride}} = 3.0$ mol kg$^{-1}$ and $T = 298.15$ K) the magnitude of $\Delta_{\text{tr}}V_{m,A}^o$ follows DL-alanine > glycine > DL-valine > DL-2-aminobutyric acid; in the work published by Banipal et al. (2008) ($m_{\text{zinc chloride}} = 3.0$ mol kg$^{-1}$ and $T = 298.15$ K) the magnitude of $\Delta_{\text{tr}}V_{m,A}^o$ 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 $\Delta_{\text{tr}}V_{m,A}^o$ 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: \( \text{CH}_2-(\text{Gly}), \text{CH}_3\text{CH}-(\text{Ala}), \text{CH}_3\text{CH}_2\text{CH}-(\text{Aaba}) \) and \( \text{CH}_3\text{CH}_2\text{CHCH}-(\text{Val}) \). 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.

<table>
<thead>
<tr>
<th>( m_g/\text{mol kg}^{-1} )</th>
<th>( \text{NH}_3^+, \text{COO}^- )</th>
<th>( \text{CH}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>278.15 K</td>
<td>288.15 K</td>
</tr>
<tr>
<td>0.0</td>
<td>26.085</td>
<td>27.330</td>
</tr>
<tr>
<td>0.1</td>
<td>27.070</td>
<td>28.125</td>
</tr>
<tr>
<td>0.3</td>
<td>28.285</td>
<td>29.195</td>
</tr>
<tr>
<td>0.7</td>
<td>30.085</td>
<td>30.905</td>
</tr>
<tr>
<td>1.0</td>
<td>31.335</td>
<td>31.835</td>
</tr>
</tbody>
</table>
It is interesting to note that $V_{mA}^0$ 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](image)

Figure 4.1. $V_{mA}^0 (\text{\textbullet NH}_3^+,\text{COO}^-; \text{\textbullet CH}_2)/\text{cm}^3 \text{mol}^{-1}$: 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 (NH$_3^+$, COO$^-$) and 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.

<table>
<thead>
<tr>
<th>$m_g$/mol kg$^{-1}$</th>
<th>NH$_3^+$, COO$^-$</th>
<th>CH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>278.15 K</td>
<td>288.15 K</td>
</tr>
<tr>
<td>0.1</td>
<td>0.98</td>
<td>0.80</td>
</tr>
<tr>
<td>0.3</td>
<td>2.20</td>
<td>1.87</td>
</tr>
<tr>
<td>0.7</td>
<td>4.00</td>
<td>3.58</td>
</tr>
<tr>
<td>1.0</td>
<td>5.25</td>
<td>4.51</td>
</tr>
</tbody>
</table>

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):

$$\Delta_{tr}V_m^o = 2V_{AS}m_S + 3V_{ASS}m_S^2 + \ldots$$ (4.8)

where constants $V_{AS}$ and $V_{ASS}$ 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. $V_{AS}$ (cm$^3$ mol$^{-2}$ kg$^{-1}$) and $V_{ASS}$ (cm$^3$ mol$^{-3}$ kg$^2$) interaction coefficients for glycine, L-alanine, DL-2-aminobutyric acid and L-valine in aqueous magnesium sulphate solutions at different temperatures.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$V_{AS}$</th>
<th>$V_{ASS}$</th>
<th>$V_{AS}$</th>
<th>$V_{ASS}$</th>
<th>$V_{AS}$</th>
<th>$V_{ASS}$</th>
<th>$V_{AS}$</th>
<th>$V_{ASS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glycine</td>
<td>L-alanine</td>
<td>DL-2-aminobutyric acid</td>
<td>L-valine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>278.15</td>
<td>4.061</td>
<td>-0.942</td>
<td>3.424</td>
<td>-0.848</td>
<td>3.271</td>
<td>-0.681</td>
<td>3.803</td>
<td>-1.019</td>
</tr>
<tr>
<td>288.15</td>
<td>3.569</td>
<td>-0.855</td>
<td>3.092</td>
<td>-0.803</td>
<td>2.861</td>
<td>-0.596</td>
<td>3.330</td>
<td>-0.895</td>
</tr>
<tr>
<td>298.15</td>
<td>3.233</td>
<td>-0.753</td>
<td>2.846</td>
<td>-0.700</td>
<td>2.693</td>
<td>-0.577</td>
<td>3.010</td>
<td>-0.756</td>
</tr>
<tr>
<td>308.15</td>
<td>2.918</td>
<td>-0.642</td>
<td>2.608</td>
<td>-0.585</td>
<td>2.832</td>
<td>-0.703</td>
<td>2.873</td>
<td>-0.714</td>
</tr>
</tbody>
</table>
For all systems, the $V_{AS}$ coefficient is positive while $V_{ASS}$ 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 4. Discussion of Results
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 \( \Delta_{tr} V_m^o \) 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 –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.
References


References


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.

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<th>$m_S = 0.70000$ mol kg$^{-1}$</th>
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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.

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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.

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$ρ$ (kg m$^{-3}$)
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.

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Appendix B

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

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

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

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## Appendix D

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

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DTAB: dodecyltrimethylammonium bromide  
TTAB: tetradecyltrimethylammonium bromide
### Supporting Information

#### Continued

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### Supporting Information

**Islam and Wadi (2003)**

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**Wang et al. (1999)**

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## Appendix E

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

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