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Partial Molar Volumes of L-Serine and L-Threonine in Aqueous Ammonium Sulfate Solutions at (278.15, 288.15, 298.15, and 308.15) K

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Abstract In this study, the partial molar volumes of L-serine and L-threonine in aqueous solutions of ammonium sulfate at (0.0, 0.1, 0.3, 0.7, and 1.0) mol·kg^{-1} are reported between 278.15 and 308.15 K. Transfer volumes and hydration numbers were obtained, which are larger in L-serine than in L-threonine. Dehydration of the amino acids is observed, rising with the temperature and salt molality. The data suggest that interactions between ions and charged/hydrophilic groups are predominant, and by applying the McMillan and Mayer formalism, it was concluded that they are mainly pair wise. The combination of the data presented in this study with solubility and molecular dynamics data suggests a stronger interaction of the ammonium cation with the zwitterionic centers of the amino acids when compared to the interactions of those centers with the sulfate anion.

Keywords Partial molar volume · Hydration · Serine · Threonine · Ammonium sulfate

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1 Introduction

Hydrophobic and/or charged atomic groups and ions are components of almost every biologically important system. It is generally recognized that the hydration of such atomic groups plays an important role in the conformational stability of biopolymers. Consequently, characterization of the hydration properties of both hydrophobic and charged groups should provide insights into the role of solute–solvent interactions associated with fundamental protein phenomena such as folding/unfolding transitions, solubility, and denaturation. Because proteins are large complex molecules, the direct study of protein–electrolyte interactions is difficult. It is therefore, useful to investigate the interaction of model compounds such as amino acids, peptides, and their derivatives that constitute part of the protein structures [1].

Following this idea, a large amount of work concerning the study of thermodynamic properties of aqueous electrolyte solutions containing amino acids have been published in the last decade. An extensive number of measurements on electrolyte activity coefficients in the presence of an amino acid or a peptide have been listed by Ferreira et al. [2], but less work has been published on solubility [2–10] or water activity [11, 12]. Additionally, several researchers studied partial molar volumes or other partial molar quantities to understand the interactions of the amino acids in electrolyte solutions, aiming to provide insights into the conformational stability and unfolding behavior of globular proteins [13]. In this respect, the review by Zhao [14] and the compilation book by Dhir [15] are starting points to find important references on the subject and to attest its relevance. The set of selected studies published very recently [16–21] shows that this is a very active research topic.

In this study, we selected ammonium sulfate, since it is often used for protein precipitation [22]. Despite the influence of ammonium sulfate on amino acid solubility data that has been published recently [10], no information was found on the volumetric properties of amino acids in such electrolyte solutions. That information can be used for a better understanding of its interactions with different amino acid functional groups, giving new contributions for studies on protein purification and precipitation.

In this study we report the density of L-serine and L-threonine in aqueous ammonium sulfate solutions in the temperature range between 278.15 and 308.15 K. The partial molar volumes of amino acids at infinite dilution were calculated, and some physico-chemical parameters of those solutions were also calculated and interpreted. This is the first part of a more extended study where structural changes of the amino acids are taken into account, and will be completed by publication of the same type of experimental information for ammonium sulfate systems containing glycine or alanine.

2 Experimental

2.1 Chemicals

The source, purity, and specifications of the chemicals used are given in Table 1. The amino acids were kept at room temperature and were used without further purification, but ammonium sulfate was oven-dried (about 380 K) for at least 24 h and used after cooling at room temperature in a dehydrator with silica gel to avoid contamination by moisture from air. The distilled water was purified in a Milli-Q ultra-pure water system from Millipore, and was degassed before use by boiling it for 1 h to avoid micro air bubbles in the solutions.

Table 1 Specifications of the chemicals used

Chemical name	Formula	CAS RN	Supplier	Mass fraction purity[a]
L-Serine ((*S*)-2-amino-3-hydroxypropanoic acid)	$C_3H_7O_3N$	56-45-1	Sigma Aldrich	0.99
L-Threonine ((2*S*,3*R*)-2-amino-3-hydroxybutanoic acid)	$C_4H_9O_3N$	72-19-5	Fluka	0.99
Ammonium sulfate	$(NH_4)_2SO_4$	7783-20-2	Sigma Aldrich	0.99

[a] Declared by the supplier

2.2 Preparation of the Solutions

First, the ammonium sulfate solutions were prepared by mass (Precisa 2200C SCS balance; resolution = 10 mg, estimated uncertainty = ±0.02 %) in a dried flask of 2,000 cm^3. Ammonium sulfate solutions were prepared at the molalities of (0.1, 0.3, 0.7, and 1.0) $mol \cdot kg^{-1}$. After this, the ternary solutions were prepared by weighing (Precisa 40SM-200A balance, resolution = 0.01 mg, uncertainty = ±0.1 mg) the required amounts of the amino acid and the sulfate solution, to achieve the desired amino acid molalities ranging from 0.10 to 0.50 $mol \cdot kg^{-1}$. It is important to emphasize at this point that all amino acid or ammonium sulfate molalities are expressed as number of moles of solute per kilogram of water. The uncertainty in the solute molality is estimated to be $\pm 2 \times 10^{-5}$ $mol \cdot kg^{-1}$.

2.3 Density Measurements

The density measurements of aqueous ammonium sulfate solutions containing amino acids were performed using a vibrating-tube densimeter with a U-shaped glass tube (DSA 5000, Anton Paar, Graz, Austria). For each set of measurements, the densimeter was calibrated at 20 °C with dry air and water, and at each temperature a water check was performed and compared to the literature values (NIST), which must be within a few units of the order 10^{-3} $kg \cdot m^{-3}$. The measurement repeatability of density is $\pm 1 \times 10^{-2}$ $kg \cdot m^{-3}$ and 1 mK for temperature. To prevent condensation of moisture from air on the parts of the device at lower temperatures, 278.15 and 288.15 K, a flow of nitrogen was passed through the equipment. Finally, it is also important to indicate that pH measurements were also carried out (digital pH meter PHI 04) confirming that the amino acids in ammonium sulfate solutions are in their zwitterionic form as the pH varied from 5.36 to 5.88, which is within the pH band around the isoelectric point.

3 Experimental Results

The density data measured in this study at four different temperatures and five salt molalities are presented in Tables 2 and 3 for solutions of L-serine and L-threonine, respectively.

From the density data, the partial molar volume ($V^{o}_{m,A}$) of the amino acid at infinite dilution is calculated by:

$$V^{o}_{m,A} = \frac{M_A}{\rho_0} - \frac{1 + m_S M_S}{\rho_0^2} a_v \quad (1)$$

Table 2 Densities of aqueous ammonium sulfate solutions containing L-serine at different temperatures, and molalities of amino acid (m_A) and salt (m_S)

m_A (mol·kg^{-1})	ρ (kg·m^{-3})			
	278.15 K	288.15 K	298.15 K	308.15 K
$m_S = 0$ mol·kg^{-1}				
0.00000	999.983	999.107	997.049	994.036
0.09994	1,004.607	1,003.606	1,001.458	998.383
0.14999	1,006.895	1,005.839	1,003.647	1,000.541
0.19999	1,009.164	1,008.037	1,005.803	1,002.670
0.29999	1,013.632	1,012.385	1,010.068	1,006.878
0.40009	1,018.010	1,016.655	1,014.256	1,011.009
0.49997	1,022.307	1,020.843	1,018.370	1,015.071
$m_S = 0.10000$ mol·kg^{-1}				
0.00000	1,008.180	1,007.060	1,004.846	1,001.742
0.10007	1,012.645	1,011.413	1,009.119	1,005.962
0.14996	1,014.847	1,013.557	1,011.222	1,008.036
0.19991	1,017.025	1,015.681	1,013.308	1,010.092
0.30000	1,021.343	1,019.888	1,017.442	1,014.174
0.39991	1,025.574	1,024.017	1,021.497	1,018.184
0.50000	1,029.742	1,028.091	1,025.500	1,022.133
$m_S = 0.30000$ mol·kg^{-1}				
0.00000	1,023.469	1,021.953	1,019.468	1,016.209
0.10005	1,027.661	1,026.052	1,023.499	1,020.191
0.14988	1,029.730	1,028.066	1,025.475	1,022.153
0.20014	1,031.789	1,030.080	1,027.460	1,024.106
0.30010	1,035.857	1,034.041	1,031.356	1,027.955
0.39983	1,039.868	1,037.934	1,035.187	1,031.742
0.50239	1,043.827	1,041.865	1,039.054	1,035.568
$m_S = 0.70000$ mol·kg^{-1}				
0.00000	1,051.334	1,049.196	1,046.307	1,042.815
0.09999	1,055.067	1,052.862	1,049.923	1,046.394
0.14990	1,056.925	1,054.688	1,051.705	1,048.156
0.20015	1,058.767	1,056.487	1,053.486	1,049.920
0.29994	1,062.379	1,060.037	1,056.983	1,053.381
0.40005	1,065.917	1,063.524	1,060.447	1,056.807
0.49922	1,069.397	1,066.932	1,063.812	1,060.141
$m_S = 1.00000$ mol·kg^{-1}				
0.00000	1,070.282	1,067.802	1,064.702	1,061.066
0.10003	1,073.775	1,071.226	1,068.078	1,064.408
0.14997	1,075.494	1,072.913	1,069.743	1,066.059
0.20031	1,077.213	1,074.599	1,071.407	1,067.712
0.30012	1,080.565	1,077.909	1,074.674	1,070.950
0.39973	1,083.896	1,081.170	1,077.889	1,074.138
0.50034	1,087.163	1,084.400	1,081.087	1,077.305

Table 3 Densities of aqueous ammonium sulfate solutions containing L-threonine at different temperatures, and molalities of amino acid (m_A) and salt (m_S)

m_A (mol·kg^{-1})	ρ (kg·m^{-3})			
	278.15 K	288.15 K	298.15 K	308.15 K
m_S = 0.00000 mol·kg^{-1}				
0.00000	999.983	999.107	997.049	994.036
0.09910	1,004.318	1,003.335	1,001.202	998.133
0.15000	1,006.518	1,005.475	1,003.303	1,000.208
0.19995	1,008.652	1,007.558	1,005.344	1,002.221
0.29997	1,012.856	1,011.657	1,009.371	1,006.196
0.39985	1,016.973	1,015.674	1,013.315	1,010.090
0.49996	1,021.039	1,019.635	1,017.203	1,013.920
m_S = 0.10000 mol·kg^{-1}				
0.00000	1,008.180	1,007.060	1,004.846	1,001.742
0.09997	1,012.397	1,011.177	1,008.889	1,005.742
0.14422	1,014.237	1,012.978	1,010.656	1,007.486
0.19998	1,016.530	1,015.214	1,012.855	1,009.660
0.29999	1,020.601	1,019.180	1,016.753	1,013.507
0.39998	1,024.564	1,023.071	1,020.571	1,017.276
0.49999	1,028.457	1,026.876	1,024.316	1,020.986
m_S = 0.30000 mol·kg^{-1}				
0.00000	1,023.469	1,021.953	1,019.468	1,016.209
0.10000	1,027.401	1,025.795	1,023.265	1,019.958
0.15000	1,029.346	1,027.702	1,025.143	1,021.814
0.19999	1,031.273	1,029.586	1,026.990	1,023.647
0.29999	1,035.061	1,033.297	1,030.645	1,027.263
0.40000	1,038.791	1,036.947	1,034.239	1,030.816
0.50000	1,042.443	1,040.531	1,037.767	1,034.309
m_S = 0.70000 mol·kg^{-1}				
0.00000	1,051.334	1,049.196	1,046.307	1,042.815
0.10000	1,054.810	1,052.615	1,049.679	1,046.156
0.15000	1,056.524	1,054.303	1,051.345	1,047.799
0.20000	1,058.221	1,055.962	1,052.989	1,049.426
0.30000	1,061.572	1,059.262	1,056.239	1,052.653
0.40000	1,064.858	1,062.504	1,059.445	1,055.824
0.49999	1,068.102	1,065.686	1,062.590	1,058.938
m_S = 1.00000 mol·kg^{-1}				
0.00000	1,070.282	1,067.802	1,064.702	1,061.066
0.10000	1,073.464	1,070.935	1,067.798	1,064.137
0.14999	1,075.040	1,072.491	1,069.333	1,065.662
0.20000	1,076.607	1,074.030	1,070.860	1,067.171
0.30000	1,079.670	1,077.059	1,073.849	1,070.140
0.40000	1,082.676	1,080.021	1,076.784	1,073.048
0.49995	1,085.656	1,082.961	1,079.695	1,075.932

where M_A and M_S are the molar mass of the amino acid and salt, respectively, m_S is the molality of the salt, and ρ_0 is the density of solvent (pure water or binary water + salt solvent). The parameter a_v is obtained by representing the experimental density data in the linear form:

$$\frac{\rho - \rho_0}{m_A} = a_v + b_v m_A \tag{2}$$

where ρ is the density of the solution, m_A is the amino acid molality, and b_v is the slope of the linear equation. The procedure adopted in this study is different from that usually found in the literature, for which the apparent molar volumes are first calculated at each solute molality and then fitted to a linear equation. This procedure often shows the lack of a functional dependence between the apparent molar volume and the solute molality, and the extrapolation to infinite dilution is only obtained by taking an average of all of the data points [13, 19, 23, 24]. Following the approach presented here, at all salt molalities and temperatures, a very good linear fit was found using Eq. (2) (minimum $R^2 = 0.990$), for which a typical representation is given in Fig. 1 for L-serine in aqueous 0.3 mol·kg^{-1} ammonium sulfate solutions at different temperatures. The calculated partial molar volumes at infinite dilution of L-serine and L-threonine are compiled in Table 4, including the margin of uncertainty for a 98 % confidence interval, which is given in parentheses. Generally, the uncertainties are greater at 278.15 K, which is in accordance with the larger experimental difficulties found, with a maximum of 0.19 cm^3·mol^{-1}. The maximum uncertainties of the fitting parameters are found for the data measured in systems containing L-serine, at 278.15 K and 0.3 mol·kg^{-1} salt molality, for which the fit is presented in Fig. 1. The maximum margin of uncertainty for a 98 % confidence interval is 0.195 kg^2·m^{-3}·mol^{-1}, for an intercept a_v of 42.256 kg^2·m^{-3}·mol^{-1}, while for the slope b_v the corresponding values are 0.630 kg^3·m^{-3}·mol^{-2} and −3.315 kg^3·m^{-3}·mol^{-2}, respectively.

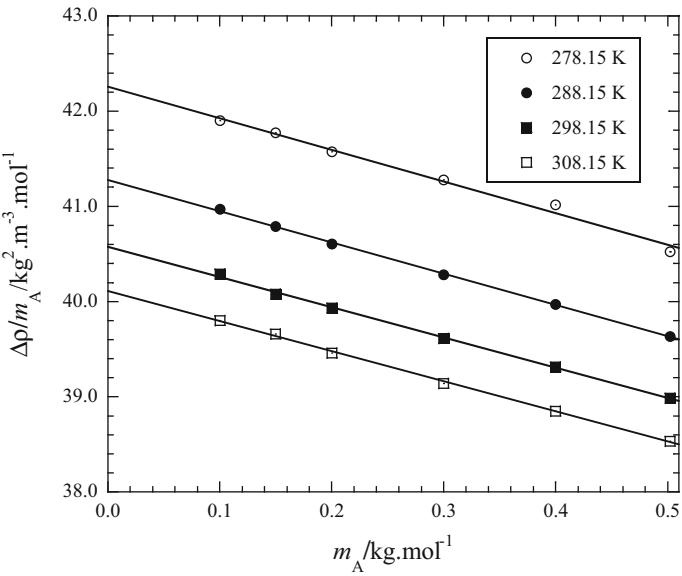

Fig. 1 Typical representation of function (2) for the calculation of partial molar volumes of aqueous 0.3 mol·kg^{-1} ammonium sulfate solutions containing L-serine

Table 4 Partial molar volumes at infinite dilution of L-serine and L-threonine at different temperatures and ammonium sulfate molalities (values in parentheses are estimated uncertainties)

m_S (mol·kg^{-1})	$V^o_{m,A}$ (cm^3·mol^{-1})			
	278.15 K	288.15 K	298.15 K	308.15 K
L-Serine				
0.0000	58.39 (0.07)	59.67 (0.08)	60.62 (0.08)	61.30 (0.07)
0.1000	59.38 (0.04)	60.54 (0.04)	61.40 (0.04)	62.01 (0.08)
0.3000	60.74 (0.19)	61.74 (0.05)	62.50 (0.07)	63.04 (0.07)
0.7000	62.69 (0.17)	63.42 (0.15)	64.09 (0.04)	64.57 (0.04)
1.0000	63.41 (0.11)	64.19 (0.05)	64.76 (0.04)	65.20 (0.01)
L-Threonine				
0.0000	74.95 (0.07)	76.09 (0.05)	76.93 (0.05)	77.61 (0.03)
0.1000	75.71 (0.07)	76.75 (0.06)	77.59 (0.03)	78.15 (0.06)
0.3000	76.99 (0.06)	77.95 (0.10)	78.51 (0.07)	79.12 (0.05)
0.7000	78.65 (0.05)	79.32 (0.09)	79.91 (0.07)	80.41 (0.09)
1.0000	79.54 (0.12)	80.14 (0.11)	80.67 (0.11)	81.09 (0.10)

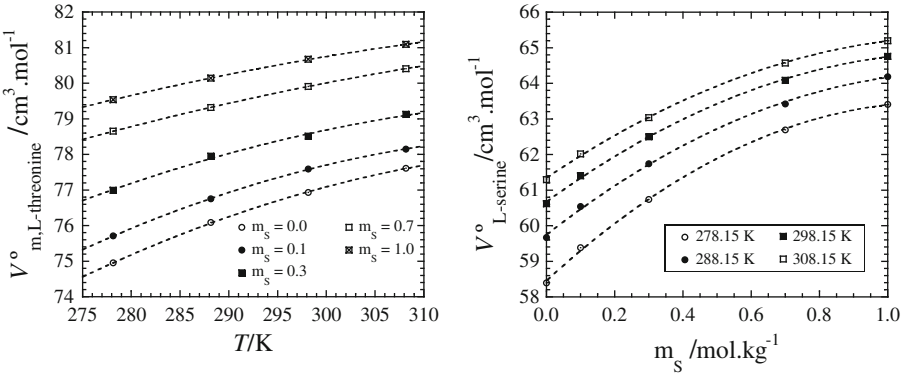

Fig. 2 Partial molar volumes at infinite dilution for L-serine and L-threonine at different temperatures and salt molalities (the *lines* are to aid the eye)

As shown in Fig. 2, the partial molar volume of the amino acid increases with the salt molality and temperature, which is in line with the interpretation that both factors reduce the electrostriction of water around the amino acid molecules. In particular, in the vicinity of the charged groups COO^- and NH_3^+, those water molecules are more like bulk water, giving an increase in the volume of the amino acids.

4 Discussion

4.1 Data Analysis

To check the quality of our data, the calculated partial molar volumes in water are compared to the average values calculated by Zhao [14] at 298.15 K. These reported values are

60.57 and 76.80 $cm^3 \cdot mol^{-1}$ for serine (average of 13 data points) and threonine (average of 8 data points), respectively, in good agreement with those found in this study. For other temperatures, the number of available data is much less, but excluding the volumes at 278.15 K, the mean deviation is 0.09 $cm^3 \cdot mol^{-1}$, which indicates high consistency with the data measured in this study. In fact, there are insufficient data to calculate averages at 278.15 K and, frequently, they present large differences. For L-serine the values found are 59.6 $cm^3 \cdot mol^{-1}$ [25] and 58.37 $cm^3 \cdot mol^{-1}$ [26]. The latter value is, however, much closer to the one found in this study.

A stringent test of these data is obtained by a comparison between the values of the partial molar expansion, $E^{o}_{m,A} = \left(\partial V^{o}_{m,A}/\partial T\right)_p$. The partial molar volumes at infinite dilution, in water, calculated in this study were fitted to:

$$V^{o}_{m,A} = a + bT + cT^2 \tag{3}$$

where T is the absolute temperature. The same procedure was applied to the data published by Kharakoz [27], Hakin et al. [28], and Yan et al. [25] allowing the comparison in Fig. 3 for aqueous systems containing L-serine at different temperatures. The partial molar expansions found in this study show very good agreement with those obtained by Kharakoz [27] and Hakin et al. [28], but show a totally opposite trend to the information published by Yan et al. [25]. Cabani et al. [29], using a dilatometric apparatus, measured the expansibilities that also show high agreement to the values calculated in this study. For L-threonine, data on partial molar expansion was also found [28, 30] showing good agreement among all sources, with the values from this study falling between those of Hakin et al. [28] and Mizuguchi et al. [30]. No information was found in the open literature for the partial molar volumes of amino acids in the presence of ammonium sulfate.

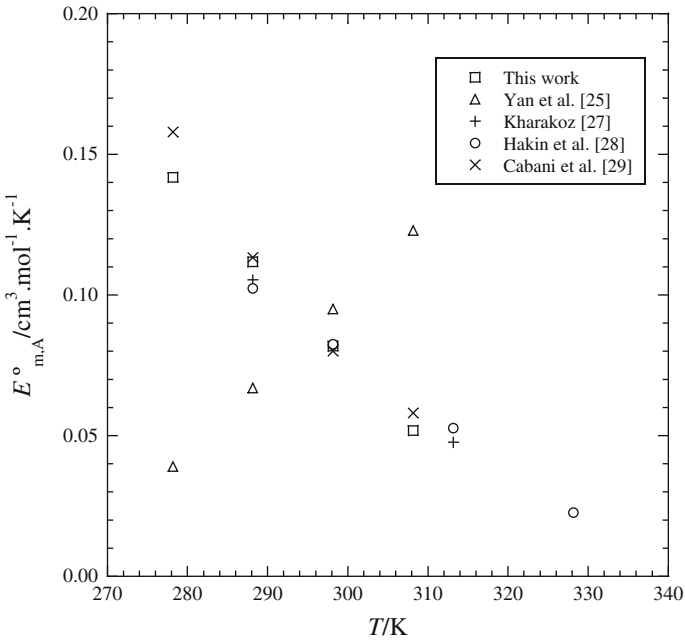

Fig. 3 Comparison of the partial molar expansion of L-serine in aqueous solutions

4.2 Partial Molar Volumes of Transfer

The partial molar volumes of transfer at infinite dilution ($\Delta_{tr}V^{o}_{m,A}$) from water to aqueous ammonium sulfate solutions have been calculated using Eq. (4) and are listed in Table 5, together with the corresponding errors that are given in parentheses.

$$\Delta_{tr}V^{o}_{m,A} = V^{o}_{m,A}(\text{in aqueous salt solution}) - V^{o}_{m,A}(\text{in water}) \quad (4)$$

The partial molar volumes at infinite dilution are free of interactions among amino acid molecules. Therefore, the values obtained in this study can be interpreted considering the interactions between the ions (NH_4^+ and SO_4^{2-}) and the hydrophilic groups of the amino acids (–COO^-, –NH_3^+, and –OH), or between the ions and the hydrophobic nonpolar parts of the amino acids. According to the co-sphere overlap model [31], the first type of interactions results in positive $\Delta_{tr}V^{o}_{m,A}$ values, whereas, the ion/hydrophobic group interactions give negative $\Delta_{tr}V^{o}_{m,A}$ values. The positive transfer volumes obtained in this study indicate the predominance of interactions between the ions and the zwitterionic centers of the amino acids, in agreement with very recent theoretical studies using molecular dynamics [32, 33]. Qualitatively, the data are also in agreement with the co-sphere overlap model when a comparison is made between the transfer volumes in L-serine and L-threonine. In fact, the introduction of a hydrophobic CH_2 group into threonine, relative to serine, causes a reduction on the transfer volumes.

Quantitatively, the formalism proposed by McMillan and Mayer [34] allows the representation of the thermodynamic transfer function at infinite dilution in terms of the interaction of the solute with different numbers of co-solute species. In this regard, the transfer partial molar volume of the dilute amino acid can be expressed as [35]:

$$\Delta_{tr}V^{o}_{m,A} = 2V_{A,S}m_S + 3V_{A,SS}m_S^2 + \ldots \quad (5)$$

where the constants $V_{A,S}$ and $V_{A,SS}$ denote pair and triplet interactions, which were found by fitting of the transfer volumes data at each temperature. Considering both the number of available data points at each temperature and the quality of the curves obtained (see Fig. 4, with a minimum $R^2 = 0.998$), only two parameters were estimated that are reported in

Table 5 Partial molar volumes of transfer at infinite dilution for L-serine and L-threonine at different temperatures and ammonium sulfate molalities

m_S (mol·kg^{-1})	$\Delta_{tr}V^{o}_{m,A}$ (cm^3·mol^{-1})			
	278.15 K	288.15 K	298.15 K	308.15 K
L-Serine				
0.1000	0.99 (0.08)	0.87 (0.09)	0.78 (0.09)	0.71 (0.11)
0.3000	2.35 (0.20)	2.07 (0.09)	1.88 (0.11)	1.74 (0.10)
0.7000	4.30 (0.18)	3.75 (0.17)	3.47 (0.09)	3.27 (0.08)
1.0000	5.02 (0.13)	4.52 (0.09)	4.14 (0.09)	3.90 (0.07)
L-Threonine				
0.1000	0.76 (0.10)	0.66 (0.08)	0.66 (0.06)	0.54 (0.07)
0.3000	2.04 (0.09)	1.86 (0.11)	1.58 (0.09)	1.51 (0.06)
0.7000	3.70 (0.09)	3.23 (0.10)	2.98 (0.09)	2.80 (0.08)
1.0000	4.59 (0.14)	4.05 (0.12)	3.74 (0.12)	3.48 (0.10)

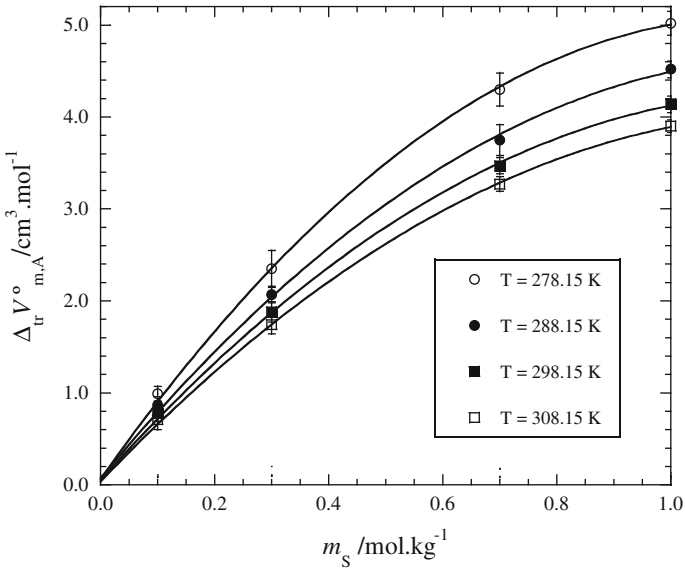

Fig. 4 Partial molar volumes of transfer versus salt molality in aqueous L-serine–ammonium sulfate solutions at different temperatures

Table 6 $V_{A,S}$ (m^3·mol^{-2}·kg) and $V_{A,SS}$ (m^3·mol^{-3}·kg^2) interaction coefficients for L-serine and L-threonine in aqueous ammonium sulfate solutions at different temperatures

T (K)	L-Serine		L-Threonine	
	$V_{A,S}$	$V_{A,SS}$	$V_{A,S}$	$V_{A,SS}$
278.15	4.522	−1.350	3.723	−0.964
288.15	3.897	−1.103	3.315	−0.874
298.15	3.572	−1.007	2.921	−0.709
308.15	3.308	−0.909	2.754	−0.683

Table 6. An interesting suggestion by one reviewer was to check the truncation of Eq. (5) after the second term by representing $\Delta_{tr}V^{o}_{m,A}/m_S$ versus m_S. If the two parameters analysis is valid, then a linear line must be found. For systems containing L-threonine, the linear fit is clearly observed while for L-serine the representation is given in Fig. 5. At 288.15 K the largest deviation from linearity is found, but at all the other temperatures linear behavior can be assumed once the experimental errors are considered.

In both 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 ammonium sulfate and the amino acid are dominated by pairwise interactions, while comparing the correspondent coefficients between the two amino acids shows a stronger interaction of L-serine with the salt. It is also very interesting to verify that the pair interaction coefficient decreases and the triplet interaction coefficient increases with a small increase in the hydrophobic part of the amino acid. This is also the trend found by Banipal et al. [24] who studied the interactions of sodium and magnesium acetates with the same amino acids at 298.15 K.

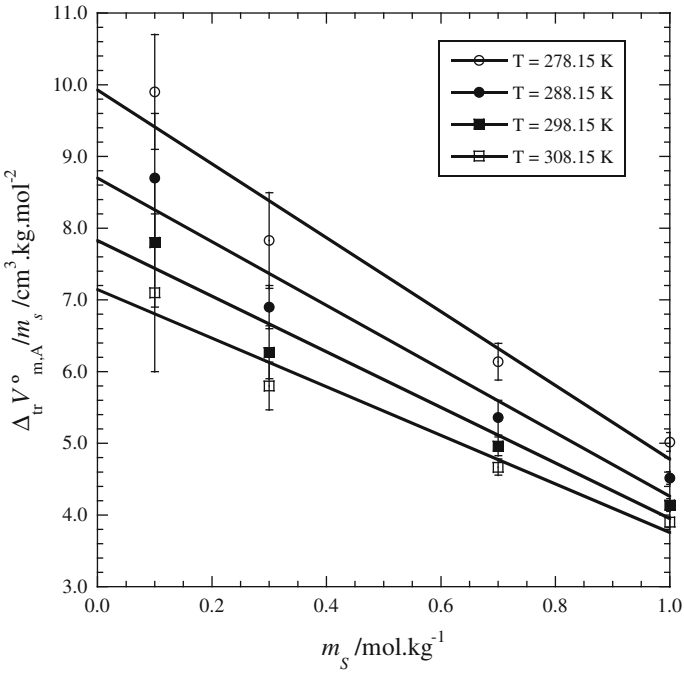

Fig. 5 Representation of $\Delta_{tr}V^{o}_{m,A}/m_S$ versus salt molality in aqueous L-serine–ammonium sulfate solutions at different temperatures

4.3 Hydration Numbers

The calculation of the hydration numbers of nonelectrolytes, namely for amino acids and proteins, has recently been the subject of a detailed review by Burakowski and Gliński [36]. The Millero method [37] was specifically developed for amino acid solutions using both density and speed of sound data, and has been the most used approach to interpret the dehydration of proteins and amino acids by electrolytes [13, 19, 38]. According to the Millero method, the hydration number (n_H) is given by:

$$n_H = (V^{o}_{m,A} - V^{o}_{m,A,int})/(V^{o}_{e} - V^{o}_{b}) \tag{6}$$

where V^{o}_{e} is the partial molar volume of electrostricted water and V^{o}_{b} is the molar volume of bulk water. Yan et al. [13] published $V^{o}_{e}-V^{o}_{b}$ values for the temperatures of interest, which were calculated by the procedure described by Millero et al. [37]. Similarly, among the different alternatives tested [37] to obtain the intrinsic partial molar volume of the amino acid ($V^{o}_{m,A,int}$), the use of the following relationship has been recommended:

$$V^{o}_{m,A,int} = (0.7/0.634)V^{o}_{m,A,cryst} \tag{7}$$

where $V^{o}_{m,A,cryst}$ are the crystal molar volumes determined from the study of Berlin and Pallansh [39]. Table 7 presents the hydration numbers of L-serine and L-threonine at all experimental conditions, which are larger for L-serine. The dehydration of the amino acids increases both with salt molality and temperature, but for L-serine the decrease in the

Table 7 Hydration numbers of L-serine and L-threonine at different temperatures and salt molalities

m_S (mol·kg^{-1})	n_H			
	278.15 K	288.15 K	298.15 K	308.15 K
L-Serine				
0.0000	5.75	4.72	3.86	3.01
0.1000	5.37	4.42	3.62	2.83
0.3000	4.85	4.00	3.29	2.58
0.7000	4.10	3.42	2.80	2.19
1.0000	3.82	3.16	2.60	2.04
L-Threonine				
0.0000	4.92	4.02	3.28	2.53
0.1000	4.63	3.79	3.08	2.40
0.3000	4.13	3.38	2.80	2.15
0.7000	3.49	2.90	2.37	1.83
1.0000	3.15	2.62	2.14	1.66

hydration number with the electrolyte concentration is larger than for L-threonine. This is in agreement with the findings of Banipal et al. [38], who studied the effect of zinc chloride in the dehydration of a series of five amino acids and reported that the decrease in the hydration number becomes progressively smaller as the hydrophobic part of amino acids increases. The same feature is observed when considering the effect of the temperature on the dehydration of the amino acids.

For L-serine it was possible to compile a reasonable set of comparable experimental information for the effect of the electrolyte on the dehydration of the amino acids. In Table 8, the magnitude of the dehydration effect is represented as the difference (Δn_H) between the hydration numbers of the amino acid in pure water at 298.15 K to those in an aqueous solution containing an electrolyte with 1 mol·kg^{-1} concentration at the same temperature.

Analyzing the relative magnitude of Δn_H for 1:1 type electrolytes, the dehydration effect increases for less-hydrated cations ($K^+ < Na^+ < Li^+$) and more-hydrated anions ($SCN^- < Cl^- < CH_3COO^-$). The last trend is also satisfied for the 1:2 electrolyte Na_2SO_4, containing the highly hydrated anion (SO_4^{2-}), which presents a greater dehydration effect. Even where the information available is scarce, this consistency observed for the sodium and chloride salts is of most interest to clarify molecular level interpretations of the Hofmeister series. In fact, such observations seem to suggest strong interactions between the cation and the COO^- group of the amino acid causing its dehydration, while the anions seem to cause dehydration by a different mechanism. Supporting these ideas, molecular dynamics simulation studies performed by Tomé et al. [33] in systems containing alanine, isoleucine, or valine showed a mechanism of salting-in based on the direct interaction of anions with the nonpolar moieties of the amino acids, while for cations these interactions are not as important; instead cations interact more strongly with the carboxylate group of the amino acids [34].

However, looking to sulfate salts (electrolytes of type 1:2), the salt with the less hydrated cation ($NH_4^+ < Na^+$) shows a smaller dehydration effect. In this way, it would be expected to find a more intense salting-out effect of Na_2SO_4 on the solubility of serine than $(NH_4)_2SO_4$. El-Dossoki [8] studied the effect of several salts on the solubility of DL amino acids, showing a very strong salting-in effect; at 1 mol·kg^{-1} salt concentration the

Table 8 Hydration number change in aqueous 1 mol·kg^{-1} electrolyte solutions at 298.15 K

Salt	Δn_H	Reference
$Mg(CH_3COO)_2$	1.27	Banipal et al. [24]
$NaCH_3COO$	0.88	Banipal et al. [24]
$NaCH_3COO$	0.74	Singh and Kishore [40]
NaSCN	0.44	Singh and Kishore [40]
Na_2SO_4	1.54	Singh and Kishore [40]
Na_2SO_4	1.52	Liu and Ren [41]
LiCl	0.43	Ogawa et al. [42]
NaCl	0.61	Ogawa et al. [42]
KCl	0.62	Ogawa et al. [42]
$(NH_4)_2SO_4$	1.25	This study

serine solubility is almost 2.6 times higher than its value in pure water. Since no other data were found for the solubility of serine in sodium sulfate solutions, these contradictory results suggested a comparison for the effect of Na_2SO_4 on the solubility of DL-alanine. While El-Dossoki [8] observed at the same salt molality a moderate salting in (1.7 times higher than the values in pure water), Ferreira et al. [2] and Ramasami [7] observed a salting-out, which is in accordance with the information gathered from partial molar volumes. A comparison of the salt effect on the solubility of glycine and alanine [2, 10] also showed the higher salting-out character of sodium sulfate when compared to ammonium sulfate. In this way, due also to the presence of an OH group on the aliphatic chain of serine that introduces specific interactions in the system, it would be interesting to compare the behavior of serine and alanine aqueous solutions containing sulfates, combining molecular dynamics simulations with solubility and partial molar volume data.

Ionic polarizabilities and ion size play central roles in the understanding the Hofmeister effects [43], and the classical ideas about changes in bulk water structure are being overturned [33, 44]. Indeed, Batchelor et al. [45] showed that the notion of stabilizers and denaturants as bulk water structure makers, and breakers is disproved on thermodynamic grounds, which inhibit theoretical interpretations based on the second derivative of the amino acids partial molar volumes with the temperature, often found in similar studies [38, 46].

5 Conclusions

Partial molar volumes at infinite dilution of L-serine and L-threonine were obtained from density measurements between 278.15 and 308.15 K. The good quality of the data was proved either by using directly the partial molar volumes in pure water or the partial molar expansibilities.

These data were used to calculate the partial molar volumes of transfer and hydration numbers. From the transfer volumes, it was concluded that the most predominant interactions are pairwise between the ions and the zwitterionic centers of the amino acids. Both increasing temperature and salt concentration dehydrate the amino acids, making it possible to rationalize the hydration numbers information in terms of stronger interactions between the cation with the $–COO^-$ group of L-serine when compared to the interactions of the anions with the $–NH_3^+$ group of L-serine, in agreement with the experimental solubility data.

The analysis presented in this study could help in understanding the behavior of more complex biomolecules in aqueous electrolyte solutions, and thus be relevant in areas such as biotechnology and life sciences.

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