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Research paper

Effect of NH_4^+ and SO_4^{2-} ions on the solubility and solvation thermodynamics of L-proline at different temperatures

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ABSTRACT

The solubility in most of the amino acids in electrolyte-containing water has a significant role on their solvation thermodynamics, which in turn affects their biophysical actions within the human body. Our current research is focused on the solubility and thermodynamic behaviour of an important amino acid L-Proline, given its diverse range of applications. For L-Proline, the solubility is influenced in distinct ways by various electrolytes. In this work we have reported for the first time the influence of ammonium sulphate [$(NH_4)_2SO_4$] on the solubility and related transfer thermodynamics for better understanding of solvation characteristics of L-Proline in electrolytic solvent systems. It is found that the presence of polyvalent anion and dissociation character of $(NH_4)_2SO_4$ control the solubility of L-Proline in both aqueous and electrolytic media which is justified by various thermodynamic parameters.

1. Introduction

Amino acids are considered as most promising building units in human body due to their diversified biological functions whereas the metabolism is controlled mainly by the protein molecules [1–3]. Besides, the diverse areas are covered by this biomolecule such as chemicals, food industry, cosmetic and polymer industry since a long time [4]. Solubility of materials is one of the most important properties that control the working principle in modern industries. The accumulation of amino acids in the human body is always accepted by the continuous interactions with other biomolecules present within the body system [1,4–6]. The behavioural pattern of protein molecules in electrolytic solution can be clearly understood with the help of thermodynamic properties [4–6]. Thus, the exploration of solubility and related dissolution thermodynamics like transfer free energies of biomolecules like amino acids in mixed different solution is must be studied to correlate those mechanism arises in the human body.

The variation of solubility in aqueous solution of amino acids can be seen due to the involvement of common chemical factors like H-bonding, hydrophobic-hydrophilic interactions, zwitterionic forms, shape and size, charge density etc. [7]. Generally, amino acids are synthesized using the hydrolysis of protein or fermentation method, but inorganic salt is necessary in both cases [8]. The proper knowledge is required on the effect of salt ions in amino acids solubility in order to explain the behaviour of other biomolecules in complex solvent system. Several research have been done on amino acids solubility in pure aqueous [8,9], organo-aqueous [10–12], organo-non-aqueous solvent [13] and electrolytic aqueous solvent system [14,15]. The presence of electrolyte solution influences the structural pattern of amino acids including van der Waals interactions [16].This interesting behaviour is explained in ternary electrolyte-amino acids-water solutions [14,17].

Proline is a non-essential multifunctional amino acid having heterocyclic ring with an exceptional conformational rigid characteristic [18]. This is an essential component for primary metabolic process in plant and the other roles are construction biomolecules under osmotic stress [19], controlling enzyme activity [20], antioxidant feature [21],

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plant growth & development [22] etc. Previously, we studied the effect of KNO_3, $NaNO_3$, KCl and NaCl on the solubility of L-Proline and its transfer free energetics and stability order was found as NaCl > KCl > $NaNO_3$ > KNO_3. Therefore, the aim of the current study is to investigate the behaviour of L-Proline in the presence of NH_4^+ and SO_4^{2-} ions. The solubility and thermodynamical properties of L-Proline in aqueous solution of $(NH_4)_2SO_4$ at different concentrations and temperature are studied here and given explanation.

2. Experimental part

2.1. Chemicals purification

We utilized L-Proline (99.8%, GR) procured from E. Merck, Bombay, India, which was vacuum-dried prior to use. Additionally, we obtained 99.9% pure $(NH_4)_2SO_4$ from E. Merck Co. Ltd, which was dried out for 5 days at 380.15 K in a temperature-controlled dryer oven, subsequently reached at room temperature, and stored again for 5 days in a void desiccator before use. For the preparation of aqueous solutions, we employed completely triple-distilled water for whole experiments.

2.2. Preparations of saturated solutions

To determine the solubility of L-Proline in aqueous mixtures of $(NH_4)_2SO_4$, we prepared mixtures with concentrations of electrolyte ranging from 0.0 to 3.0 (mol/kg) in well-fitting stoppered glass tubes and partially filled to encourage proper addition. All measurements were conducted in a temperature-controlled thermostat with high accuracy (0.10 K). Here, the equilibrated saturated solubility of L-Proline was determined through analytical gravimetric analysis [4,22]. To do this, 5 cm^3 samples were extracted from the supernatant upper phase using warmed pipettes, filtered with 0.22 m HPLC disposable filters, and then weighed promptly. The material was dried for three days in an oven set to 380.15 K, and subsequently weighed again after cooling for two days in a dehydrator with silica gel. The process was iterated until a consistent weight of amino acid was acquired. Subsequently, the weight of the empty glass container was used to determine the quantity of the dissolved amino acid in the dehydrated specimen. Four sets of measurements were conducted for all mixtures at temperature range 288.15 to 308.15 K with an uncertainty ± 0.10 K by equilibrating the solutions via shaking. The obtained solubilities were found to be within ± 2.6 % of each other. We used a simple gravimetric analysis method to determine the solubility of L-Proline in aqueous $(NH_4)_2SO_4$ solutions, as well as the relative solubilities (S_S/S_R) i.e., the ration of L-Proline solubility in presence of electrolyte (S_S) and the solubility of L-Proline in pure water (S_R). The values are presented in Tables 1 and 2, respectively. To calculate the amount of amino acid present in the mixture, we measure the weight of the empty glass vessel and well dried sample from the saturated solution, taking into account the embryonic electrolytic concentration in the solution. A very light amount of electrolyte was also dried together with the amino acid which was further determined by simple mathematical process. Considering the molality of the electrolyte $(NH_4)_2SO_4$ in aqueous solution as 'm', the weight of electrolyte in 'x' g (≈ x mL, since density of water ≈ 1 g/mL at 298.15 K) evaporated water would be $w_1 = (M \times x \times m/1000)$ g; where, M = molar mass of $(NH_4)_2SO_4$. If you take the weight of dry glass container (completely empty) as W_2 g and the glass vessel with amino acid and little amount of electrolyte (completely dried) as W_3 g, then the amount of the exact amount of dissolved amino acid in 'x' mL or x g of water would be, $W = (W_3–W_2–W_1)$ g [7,12]. From this we measured the solubility of L-Proline in mole per kg of water. Previous studies have demonstrated that this gravimetric approach does not result in any significant adsorption or precipitation of electrolyte onto amino acids [12].

In order to assess the cations present in the solution and determine whether there was any potential incorporation or adsorption of electrolytes onto the solid-phase of amino acid, we employed atomic absorption. As previous research has reported [12], we found that the electrolytes were not absorbed by or integrated onto the solid amino acids. During our investigation, we analyzed the levels of cations present in both the electrolyte-water solution and the amino acid-electrolyte-water solution. For these comparisons, amino acid concentrations of 5%, 10%, 30%, and 50% over saturation were applied, and the cation concentration from the supernatant phase was determined. Even with varying additions of the amino acid, the largest variance in the findings was just 0.005 mol·kg^{-1}.

3. Theoretical part

3.1. Determination of solubility

By applying Eq. (1), we were able to calculate the mole fraction solubility of L-Proline (S) in pure water in both absence and presence of ammonium sulfate across a range of mole fraction compositions of ammonium sulfate and temperatures ranging from 288.15 K to 308.15 K. The results in mole fraction scale are cited in Table 1 and the data are shown in Figs. 1 and 2. Eq. (2) provided the mole fraction of electrolyte in solution.

$$S = (m_1/M_1)/(m_1/M_1 + m_2/M_2 + m_3/M_3) \quad (1)$$

$$Zs = (m_2/M_2)/(m_2/M_2 + m_3/M_3) \quad (2)$$

Here 1, 2, and 3 correspond to L-Proline, ammonium sulfate, and water, respectively. In the equation, "m" represents the weight taken for each substance, and "M" represents the molar masses of solute L-Proline, ammonium sulfate, and water, respectively.

3.2. Calculation of transfer Gibbs free energetic

The equation (3) was used in this study to measure the standard Gibbs free energies of solutions ($\Delta G_S^0(i)$) as done in previous studies [12,13,17]. The results are shown in Table 3.

Table 1
. Saturated solubility of L-Proline in water and water + $(NH_4)_2SO_4$ solvent systems at five different temperatures (288.15 to 308.15 K) under 0.1MPa[a] atmospheric pressure.

Molality of salt (mol/kg)	Solubility of L-Proline in mol/kg of water & mole fraction scale													
	0.0		0.5		1.0		1.5		2.0		2.5		3.0	
Temperature (K)	mol/kg	Mole Fraction	mol/kg	Mole Fraction	mol/kg	Mole Fraction	mol/kg	Mole Fraction	mol/kg	Mole Fraction	mol/kg	Mole Fraction	mol/kg	Mole Fraction
288.18 K	13.80	0.1991	13.54	0.1961	13.23	0.1925	12.96	0.1893	12.54	0.1843	11.98	0.1775	11.63	0.1732
293.15 K	14.55	0.2077	14.20	0.2037	13.77	0.1988	13.41	0.1946	13.06	0.1905	12.61	0.1851	12.25	0.1808
298.15 K	15.32	0.2163	14.90	0.2116	14.48	0.2069	14.05	0.2019	13.65	0.1974	13.15	0.1915	12.77	0.1870
303.15 K	16.06	0.2244	15.46	0.2178	15.05	0.2133	14.53	0.2075	14.13	0.2029	13.73	0.1983	13.37	0.1941
308.15 K	16.85	0.2329	16.02	0.2239	15.52	0.2185	14.99	0.2126	14.69	0.2093	14.17	0.2034	13.88	0.2000

Standard uncertainties (u): u(T) = 0.10 K[#] and u(m) = 0.01 mol/kg; relative uncertainties (u_r): u_r(S) = 0.0007(in mole fraction ratio) and u_r(p) [a] = 0.02.

Table 2
Values of relative Solubility i.e., (L-Proline solubility in presence of electrolyte (S_S)/ solubility of L-Proline in pure water (S_R) and log (S_S/S_R) of L-Proline in water and water + $(NH_4)_2SO_4$ systems at five different temperatures (288.15 to 308.15 K) under 0.1MPa[a] atmospheric pressure.

Molarity of salt (mol/kg)	Relative Solubility (S_S/S_R) and log (S_S/S_R)									
	(S_S/S_R) 288.15 K	log (S_S/S_R) 288.15 K	(S_S/S_R) 293.15 K	log (S_S/S_R) 293.15 K	(S_S/S_R) 298.15 K	log (S_S/S_R) 298.15 K	(S_S/S_R) 303.15 K	log (S_S/S_R) 303.15 K	(S_S/S_R) 308.15 K	log (S_S/S_R) 308.15 K
0.5	0.9812	−0.0082	0.9759	−0.0106	0.9726	−0.0121	0.9626	−0.0166	0.9507	−0.0219
1.0	0.9587	−0.0183	0.9464	−0.0239	0.9451	−0.0245	0.9371	−0.0282	0.9211	−0.0357
1.5	0.9391	−0.0273	0.9216	−0.0355	0.9171	−0.0376	0.9047	−0.0435	0.8896	−0.0508
2.0	0.9087	−0.0416	0.8976	−0.0469	0.8910	−0.0501	0.8798	−0.0556	0.8718	−0.0596
2.5	0.8681	−0.0614	0.8667	−0.0621	0.8584	−0.0663	0.8549	−0.0681	0.8409	−0.0753
3.0	0.8428	−0.0743	0.8419	−0.0747	0.8336	−0.0789	0.8325	−0.0796	0.8237	−0.0842

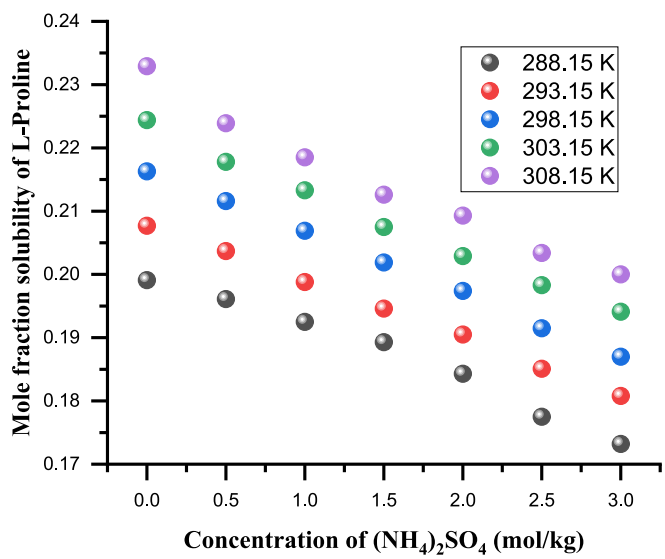

Fig. 1. Solubility (in mol fraction scale) of L-Proline in aqueous ammonium sulfate solutions at different composition of electrolyte ammonium sulfate and temperatures (288.15–308.15 K).

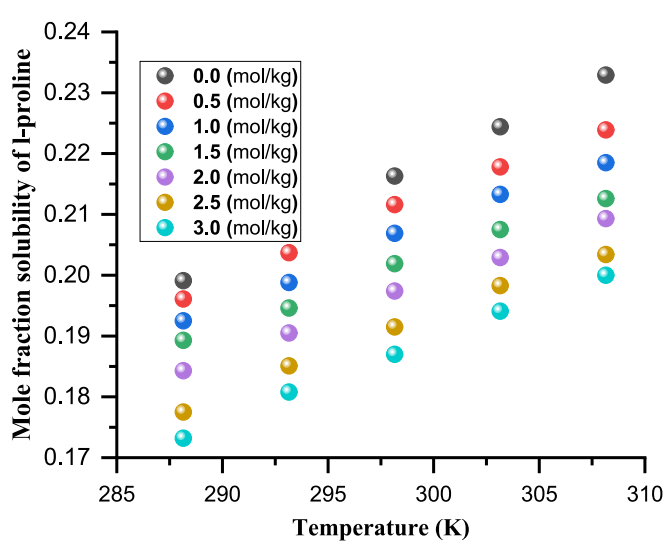

Fig. 2. Solubility (mole fraction scale) of L-Proline in aqueous ammonium sulphate solutions with temperatures (K).

$$\Delta G_s^0(i) = -RT\ln S\gamma \approx -RT\ln S \tag{3}$$

The equation includes the molal activity coefficient (γ) and the saturated equilibrated solubility of L-Proline in mol/kg (S). As zwitterions are present in aqueous solutions of amino acids, strong dipole–dipole interactions are expected to occur, which may affect the activity coefficient factor. Hence, prior research has concentrated on determining the activity coefficients of amino acids and dipeptides in solvent systems containing a mixture of aqueous electrolytes and has found that the activity coefficient values (γ) for these biomolecules are nearly unity at lower concentrations [23–26]. In this study, the saturated solubility values in Table 1 indicate that the mole fractions of L-Proline in the various compositions of the aqueous electrolyte solutions though comparatively not negligible. But for avoiding complexity, we make the assumption that the activity coefficient (γ) is equal to one in the current experimental solvent systems when computing the standard Gibbs energies of solutions ($\Delta G_S^0(i)$).

Our main objective is to find the transfer Gibbs free energy ($\Delta G_t^0(i)$) value for L-Proline from aqueous electrolyte solution to pure aqueous solution. Similar goals were reflected in earlier investigations [17,27,28,29]. The relationship between $\Delta G_t^0(i)$ and is given by [$\Delta G_t^0(i) = \Delta G_S^0(i) - \Delta G_R^0(i)$], where $\Delta G_S^0(i)$ and $\Delta G_R^0(i)$ stand for the involved free energies of the L-Proline aqueous ammonium sulphate and in water respectively. Therefore, $\Delta G_t^0(i)$ carries the activity coefficient factor ratio –RT $\ln\gamma_S/\gamma_R$, which is probably very modest ('s' for aqueous-$(NH_4)_2SO_4$ and 'R' for H_2O).

Equation (4) is used for fitting of the measured free energies of solutions, [$\Delta G_S^0(i)$] at the five equidistant temperatures. Table 4 presents the values and units of coefficients a, b, and c, which are determined using the method of least squares. The temperature (T) is measured in Kelvin. The equation is expressed as follows:

$$\Delta G_s^0 = a + bT + cT\ln T \tag{4}$$

The values obtained from the equation are found in good agreement to each other with a deviation of less than ± 0.03 units.

4. Discussion

Equation (5) was employed to measure the standard transfer Gibbs energies of amino acids between aqueous solutions with and without $(NH_4)_2SO_4$ at 298.15 K, on a mole fraction scale.

$$\Delta G_t^0(i) = {}_s\Delta G_{sol}^0(i) - {}_R\Delta G_{sol}^0(i)$$

$$\Delta G_t^0(i) = (a_s - a_R) + (b_s - b_R)T + (c_s - c_R)T\ln T - RT\ln(M_s/M_R) \tag{5}$$

In this context, the subscript's' denotes aqueous ammonium sulfate, while 'R' represents for H_2O. The molar masses of the pure and electrolyte-containing solvents are denoted as M_R and Ms, respectively. The values of standard transfer Gibbs energies for L-Proline are computed and shown in Table 5. The standard uncertainties associated with determining these values ($\Delta G_t^0(i)$) are determined to be 0.04 kJ/mol.

Table 3
Values of Gibbs energies of solution ΔG_S^0(i) in molal scale (kJ/mol) with the respective solubilities (S) of L-Proline in water + $(NH_4)_2SO_4$ systems at different temperatures (K).

288.15 K		293.15 K		298.15 K		303.15 K		308.15 K	
S (mol/kg)	$\Delta G_s^0(i)$	S (mol/kg)	$\Delta G_s^0(i)$	S (mol/kg)	$\Delta G_s^0(i)$	S (mol/kg)	$\Delta G_s^0(i)$	S (mol/kg)	$\Delta G_s^0(i)$
13.80	−6.2879	14.55	−6.5259	15.32	−6.7651	16.06	−6.9974	16.85	−7.2359
13.54	−6.2423	14.20	−6.4666	14.90	−6.6962	15.46	−6.9015	16.02	−7.1065
13.23	−6.1868	13.77	−6.3917	14.48	−6.6253	15.05	−6.8337	15.52	−7.0252
12.96	−6.1374	13.41	−6.3271	14.05	−6.5506	14.53	−6.7451	14.99	−6.9362
12.54	−6.0585	13.06	−6.2626	13.65	−6.4789	14.13	−6.6748	14.69	−6.8844
11.98	−5.9490	12.61	−6.1772	13.15	−6.3865	13.73	−6.6024	14.17	−6.7921
11.63	−5.8780	12.25	−6.1066	12.77	−6.3138	13.37	−6.5354	13.88	−6.7391

Table 4
The values of a, b and c coefficients; Gibbs energies $\Delta G_t^0(i)$, and entropies $T\Delta S_t^0(i)$ of transfer L-Proline in water + $(NH_4)_2SO_4$ systems at 298.15 K.

Molality of salt (mol/kg)	a (kJ/mol)	b (kJ/mol/K)	c (kJ/mol/K)	$\Delta G_t^0(i)$(kJ/mol)	$T\Delta S_t^0(i)$(kJ/mol)
0.0	10.35	−0.1147	0.01005	0.000	0.000
0.5	38.16	−0.7610	0.10716	−0.056	−1.087
1.0	32.48	−0.6369	0.08876	−0.119	−1.218
1.5	18.78	−0.3393	0.04464	−0.157	−1.726
2.0	18.83	−0.3314	0.04327	−0.191	−1.234
2.5	41.96	−0.8456	0.11994	−0.232	−0.922
3.0	24.49	−0.4469	0.06030	−0.241	−0.601

Tables 4 and 6 display the theoretically calculated values of standard transfer Gibbs energies on a mole fraction scale. These values are complex because they take into account various factors that affect the transfer of solutes from aqueous to various aqueous electrolyte solvents. The standard transfer Gibbs energy includes the free energy required to create cavities for the solutes in the solvent (denoted by $\Delta G_{t,cav}^0(i)$). The combination of bipolar zwitterionic amino acids and solvent water molecules results in dipole–dipole interactions, which contribute to the free energy (denoted by $\Delta G_{t,d-d}^0(i)$). In addition to $(NH_4)_2SO_4$, other chemical interactions such as acid-base, dispersion, hydrophilic or hydrophobic hydration, and the structural characteristics of the solute and solvent molecules also play a role in the chemical transfer free energy (represented by $\Delta G_{t,ch}^0(i)$). However, in this particular scenario, the contribution of dipole-induced dipole interaction is not taken into account, which aligns with previous research studies [23,29].

The standard transfer Gibbs energy value is comprised of several individual terms, which are listed below:

$$\Delta G_t^0(i) = \Delta G_{t,cav}^0(i) + \Delta G_{t,d-d}^0(i) + \Delta G_{t,ch}^0(i) \tag{6}$$

To estimate the standard transfer Gibbs energy values, we employed

Table 5
Mole fraction: co-solvent (Z_S), water (Z_R); mean mol. weight of co-solvent (M_S), density (d_s), hard sphere diameter (σ_s) of H_2O+ $(NH_4)_2SO_4$) and $\sigma_{s-x} = 1/2(\sigma_S + \sigma_x)$, dipole-moment of mixed solvent (μ_s), isobaric thermal coefficient (α) of H_2O + $(NH_4)_2SO_4$ solution at 298.15 K.

Molality of salt (mol/kg)	Mole fraction of $(NH_4)_2SO_4$) (Z_S)	Mole % salt	Mole fraction (z_R)	Molar mass (M_S) (g/mol)	d_s (g/cm^3)	Molar Volume (V_s) (cm^3/mol)	Dipole Moment (μ_s) (Debye)	σ_s (nm)	σ_{s-x} (nm) (Proline)	α × 10^3 (K^{-1}) (isobaric thermal coefficient)
H_2O+ $(NH_4)_2SO_4$-solvent system										
0.0	0.0000	0.00	1.000	18.015	0.9970	18.069	1.831	0.2740	0.3855	0.257
0.5	0.0089	0.89	0.9911	19.031	1.0039	18.966	1.815	0.2746	0.3858	0.257
1.0	0.0177	1.77	0.9823	20.035	1.0107	19.851	1.799	0.2752	0.3861	0.257
1.5	0.0263	2.63	0.9937	21.017	1.0172	20.632	1.783	0.2757	0.3867	0.257
2.0	0.0348	3.48	0.9652	21.987	1.0239	21.494	1.767	0.2763	0.3867	0.257
2.5	0.0431	4.31	0.9569	22.934	1.0304	22.266	1.752	0.2768	0.3869	0.257
3.0	0.0513	5.13	0.9487	23.869	1.0367	23.025	1.737	0.2773	0.3872	0.257

u(m) = ± 0.008**(mol/kg)**. u(M_s) = ± 0.0005**(g/mol)**. u(d_s) = ± 0.0003 **g /cm^3)**. u(σ_s) = ± 0.0001. u(μ_s) = ± 0.0003 Debye.[u for uncertainty]; Dipole moment of $(NH_4)_2SO_4$) is 0.003D (from DFT study); *For reference [30] Calculated diameter of $(NH_4)_2SO_4$) is 3.385 Å (DFT study).

Table 6
The values of Gibbs free energy $\Delta G_t^0(i)$, Gibbs free energy for cavity interaction $\Delta G_{t,cav}^0(i)$,Gibbs free energy for dipole–dipole interaction $\Delta G_{t,d-d}^0(i)$,enthalpy for cavity formation $\Delta H_{t,cav}^0(i)$ and entropy transfer $T\Delta S_t^0(i)$,$T\Delta S_{t,cav}^0(i)$, $T\Delta S_{t,d-d}^0(i)$ and $T\Delta S_{t,ch}^0(i)$ of solute L-Proline in different composition of H_2O + $(NH_4)_2SO_4$ at 298.15 K in kJ/mol.

Molality of salt (mol/kg)	$\Delta G_t^0(i)$ kJ/mol	$\Delta G_{t,cav}^0(i)$ kJ/mol	$\Delta G_{t,d-d}^0(i)$ kJ/mol	$\Delta G_{t,ch}^0(i)$ kJ/mol	$T\Delta S_t^0(i)$ kJ/mol	$\Delta H_{t,cav}^0(i)$ kJ/mol	$T\Delta S_{t,cav}^0(i)$ kJ/mol	$T\Delta S_{t,d-d}^0(i)$ kJ/mol	$T\Delta S_{t,ch}^0(i)$ kJ/mol
0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	−0.056	−0.404	0.016	0.332	−1.087	−0.376	0.028	0.017	−1.132
1.0	−0.119	−0.767	0.058	0.590	−1.218	−0.686	0.081	0.063	−1.362
1.5	−0.157	−1.090	0.123	0.810	−1.726	−0.940	0.150	0.132	−2.008
2.0	−0.191	−1.390	0.201	0.998	−1.234	−1.150	0.240	0.216	−1.690
2.5	−0.232	−1.660	0.290	1.138	−0.922	−1.330	0.330	0.312	−1.564
3.0	−0.241	−1.910	0.390	1.279	−0.601	−1.490	0.420	0.420	−1.441

Diameter of L-Proline 0.497 nm [35], and dipole moment of L-Proline 5.86D [35].

the scaled particle theory. In this method, we regarded both solute and solvent molecules as hard spheres, each having their respective diameters (specified in Table 5). The interaction between the solute and solvent cavities was calculated using Equation (7), which was previously described in other research studies [13,29]:

$$\Delta G^0_{cav}(i) = G_C + RT\ln(RT/V_S) \tag{7}$$

where,

$$G_C = RT[-\ln(1-Z) + \{3X/(1-Z)\}\sigma_x + \{3Y/(1-Z)\}\sigma_x^2 + \{9X^2/2(1-Z)^2\}\sigma_x^2]$$
$$Z = \pi N_A/6V_s(z_R\sigma_R^3 + z_s\sigma_s^3)$$
$$X = \pi N_A/6V_s(z_R\sigma_R^2 + z_s\sigma_s^2)$$
$$Y = \pi N_A/6V_s(z_R\sigma_R + z_s\sigma_s)$$
$$V_s = \mathrm{M}_s/d_s$$

Avogadro's number is denoted by N_A, Z_R and Zs represent the mole fraction of pure water and electrolytes, respectively. The symbol 'σ_x' represents the hard sphere diameter of amino acid, 'σ_R' refers to the hard sphere diameter of water, and 'σ_s' denotes for co-solvent.

The molar volume is denoted by V_S, whereas Ms, represents the molar mass, and d*s* represents the molar density of aqueous electrolyte solutions. The values of these parameters can be found in Table 5.

Therefore, $\Delta G^0_{t,cav}(i)$ signifies the difference as Eq. (8) [17,29].

$$\Delta G^0_{t,cav}(i) = {}_s\Delta G_t(cav) - {}_R\Delta G_t(cav) = ({}_sG_c - {}_RG_c) + RT\ln(V_R/V_s). \tag{8}$$

In this study $\Delta G^0_{t,d-d}(i)$ values were solved by Keesom-orientation expression (Eq. (9)) as mentioned below:

$$\Delta G^0_{t,d-d}(i) = ({}_s\Delta G^0_{d-d}(i) - {}_R\Delta G^0_{d-d}(i)) \tag{9}$$

In solution, ${}_S\Delta G^0_{t,d-d}(i)$ is presented as:

$$\begin{gathered} {}_s\Delta G^0_{d-d}(i) = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{s-x}^{-3}(kT)^{-1}V_x^{-1} = A/TV_s; \\ where\, A = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{s-x}^{-3}(k)^{-1} \\ and \\ V_s = M_s/d_s \end{gathered} \tag{10}$$

where, $\sigma_{S\text{-}x}$ = diameter in between solute and solvent interaction occurs and it is equal to ½($\sigma_S + \sigma_x$) [17].

The values obtained for the parameters mentioned above were multiplied by to obtain the values of in mole fraction scale, as was done in earlier studies [23,29]. The expression for can be written as follows:

$$X_{s1} = X_s(\mu_s/\sigma_s^3)/(\mu_R/\sigma_R^3) \tag{11}$$

As mentioned earlier, represents the definite mole fraction contribution resulting from the effect of dipole–dipole interactions. To obtain, one needs to subtract the values of and from, which can be found in Table 5.

4.1. Calculation of entropy

To calculate the transfer entropies, several factors need to be considered, including cavity formation, dipole–dipole interactions, and enthalpy. The transfer free energy can be expressed as the sum of the following contributions [17].

$$\text{Where} \quad \Delta P^0_t(i) = \Delta P^0_{t,cav}(i) + \Delta P^0_{t,d-d}(i) + \Delta P^0_{t,ch}(i) \tag{12}$$

Where $\Delta P^0_{t,cav}(i)$ represents the contribution of cavity formation, $\Delta P^0_{t,d-d}(i)$ represents the contribution of dipole–dipole interactions, $\Delta P^0_{t,ch}(i)$ represents the contribution of various chemical interactions like H-bonding, acid base interaction, etc.

The cavity formation contribution ($\Delta P^0_{t,cav}(i)$) can be calculated using the Scaled Particle Theory (SPT) [30,31] which assumes that both solute and co-solvent molecules are hard spheres. The expression for can be written as:Again,

$$\Delta G^0_{t,d-d}(i) = ({}_s\Delta G^0_{d-d}(i) - {}_R\Delta G^0_{d-d}(i) \tag{13}$$

and $\Delta S^0_{t,d-d}(i) = ({}_s\Delta S^0_{d-d}(i) - {}_R\Delta S^0_{d-d}(i))$ were calculated by using Keesom-orientation expression [13,32], for ${}_s\Delta G^0_{d-d}(i)$ in a solvent S, as given below:

$${}_s\Delta G^0_{d-d}(i) = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{s-x}^{-3}(kT)^{-1}V_s^{-1} = \mathrm{A/T\,V_s} \tag{14}$$

Where $\mathrm{A} = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{s-x}^{-3}(k)^{-1}$ and $\mathrm{V_s} = \mathrm{M_s/d_s}$ and that of $\Delta S^0_{d-d}(i)$ as follows–

$${}_s\Delta S^0_{d-d}(i) = -\{\delta\,{}_s\Delta G^0_{d-d}(i)/\delta\mathrm{T}\}_p \tag{15}$$

i.e., $\mathrm{T}\,{}_s\Delta S^0_{d-d}(i) = {}_s\Delta G^0_{d-d}(i)[1 + \mathrm{T}\alpha]\alpha$ = isobaric thermal coefficient [Table 2] of the solvents and calculated by the Eq. (16) [33].

$$\alpha = (\delta\ln V_s/\delta\mathrm{T})_P = -(\delta\ln d_s/\delta\mathrm{T})_P \tag{16}$$

Marcus [30] and Kim et al. [32] state that to determine the true contribution of dipole–dipole interactions to the mole fraction scale's (i) value must be multiplied by like the previous X_{s1}.Where

$$X_{s1} = X_s(\mu_s/\sigma_s^3)/(\mu_R/\sigma_R^3) \tag{17}$$

After subtraction of $\Delta P^0_{t,cav}(i)$ and $\Delta P^0_{t,d-d}(i)$ from the $\Delta P^0_t(i)\Delta$ we can get the value of $\Delta P^0_{t,ch}(i)$ which are presented in Table 6.

4.2. Solubility

The solubility of L-Proline decreases as the concentration of electrolyte (ammonium sulfate) increases at a given temperature, as shown in Table 1 and Fig. 1. This trend is associated with the salting-out effect [16,34]. As ammonium sulfate is strong electrolyte that undergoes dissociation in aqueous solution. The hard divalent anions of sulphate produced during dissociation have a crucial role for salting out process. The water molecules in the aqueous solution interact with the charged portion of L-Proline that reduces as the salt concentration rises due to the formation of hydrated ions.

The interaction of water and the dissociated ion from salt enhances when the nonpolar group contain amino acids is introduced in the solution. The study revealed that L-Proline has a greater solubility in pure water than in $(NH_4)_2SO_4$ aqueous solutions at a specific temperature. On addition of L-Proline in aqueous - $(NH_4)_2SO_4$, leads to an increase interaction between nonpolar group of solute and cosolvent molecules that interaction is more rather than in pure water molecules. This nonpolar group interaction is mainly associated with van der Waals force, and this helps to agglomerate the L-Proline molecules and thus decrease the solubility. Consequently, solubility decreases in water with increasing $(NH_4)_2SO_4$ concentration at constant temperature.

Moreover, the trend of increasing negative results for Setchenow constant (K_{si}) supports the fact, since negative value of K*si* indicates salting-out effect resulting solubility of the L-Proline decreases with electrolyte concentration. Additionally, this can be interpreted based on the covalent nature of proline. In presence of $(NH_4)_2SO_4$,water-proline (dipole–dipole/H-bonding) interaction diminish in a greater extent due to greater covalent character present in proline compared with $(NH_4)_2SO_4$ exhibiting strong ion–dipole interaction with polar water molecule. This result lowers the available solvent molecule for interaction with L-Proline and resulting lowering of solubility.

Solubility of the solute rises with raising temperature in both aqueous and electrolytic solution for a particular concentration (Table 1 & Fig. 2). L-Proline has an ability to generate strong field around it due to its zwitterionic structure. Thus, strong solute–solute interactions (inter ionic) exist in the solution. With increasing temperature, the solute–solute interactions getting weaker and solute–solvent interactions

become predominant due to formation of H-bonding. Proton NMR study supports the formation of this H-bonding [33] Therefore, the solubility of solute (L-Proline) in pure water increases with rising temperature. A complex interaction can be expected between L-Proline and $(NH_4)_2SO_4$ in a binary solvent system but with temperature enhancement this complex interaction becomes weak and consequently prolines adhere more to water molecules, and thus increases solubility.

The value of K*si* (Setchenow constant) was obtained from graph of $\log(Ss/S_R)$ *vs.* C [mol/kg concentration of electrolyte] (Fig. 3). The precise value of the constant K*si* (Setchenow constant) for the L-Proline was calculated from the inverse slope of the $\log(Ss/S_R)$ *vs.* C (Fig. 3) plot. The experimental solubility is substantially supported by the predicted K*si* values for the L-Proline at different concentrations of $(NH_4)_2SO_4$. The salting-out and salting-in effects are inversely correlated with the positive and negative values of K*si*, respectively. Here, essentially the larger size of L-Proline (0.497 nm) fence to form strong ion pair complexes reduces the salting effects and decreases solubility in aqueous $(NH_4)_2SO_4$ solution.

4.3. Free energy

A negative increment is observed for $\Delta G_t^0(i)$ with the increasing concentration of $(NH_4)_2SO_4$ at a particular temperature (298.15 K) (Table 6 & Fig. 4). There is an inter-relation exhibited between solubility, stability & total Gibbs energy of transfer qualitatively. Greater the negative value of $(\Delta G_t^0(i))$ leads to higher the stability & greater the stability leads to the lower solubility i.e.; Table 6 demonstrates the lowering solubility and increasing stability in presence of electrolyte. In the pure water system, L-Proline involves with dipole–dipole interaction effect with H_2O molecules but the addition of $(NH_4)_2SO_4$ creates comparatively strong ion–dipole interaction with polar water molecules, ammonium and sulphate ions. This enhances the tendency of aggregation and therefore, L-Proline attain more stability due to increasing hydrophobic interaction among themselves and attain higher negative $(\Delta G_t^0(i)$ values with growing electrolyte concentration. Besides, L-Proline forms a better complex with ammonium ion compared with H^+ ion due to comparable size. Thus, in presence of $(NH_4)_2SO_4$, L-Proline produces more effective interaction with them compared to water molecules. This also reduces the available proline for making interaction with polar water molecules and result in decreasing solubility and increasing stability.

$\Delta G_{t,ch}^0(i)$ can be assessed after the subtraction of $\Delta G_{t,cav}^0(i)$&$\Delta G_{t,d-d}^0(i)$

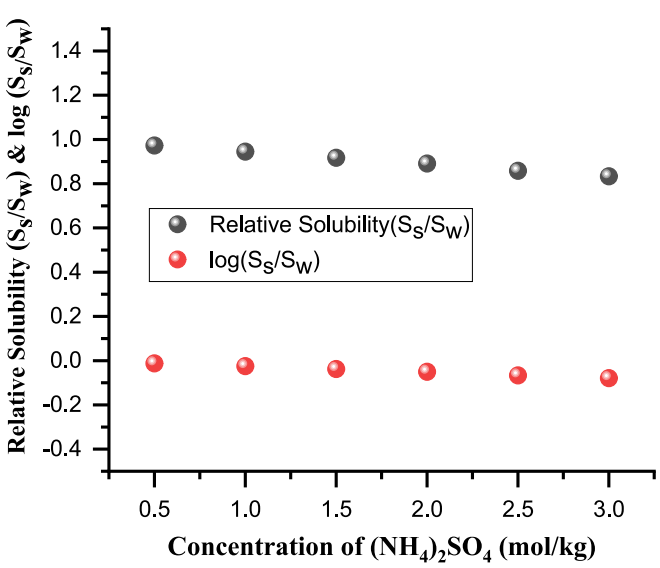

Fig. 3. A plot of relative solubility of L-Proline [(S_s/S_w)] Vs Concentration of electrolyte in mol/kg at 298.15 K.

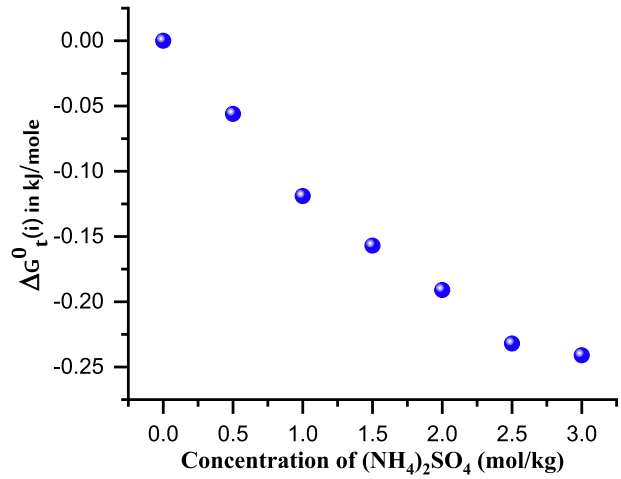

Fig. 4. $\Delta G_t^0(i)$ variation in kJ/mol of L-Proline in absence and present of ammonium sulphate in aqueous solution at 298.15 K.

from $\Delta G_t^0(i)$. The fact that total chemical transfer free energy change $[\Delta G_{t,ch}^0(i)]$ progressively increases with an increase electrolyte $(NH_4)_2SO_4$ content for L-Proline indicates instability of L-Proline, as shown by Table 6 and Fig. 5. It has been noted that the formation of cavities and interactions between dipoles provide clues for the stabilization of solute molecules. However, the collective impact of these two interactions and the additional elements influencing total transfer free energy, $\Delta G_t^0(i)$ led to a positive $\Delta G_{t,ch}^0(i)$ for the L-Proline. With the increasing concentration of $(NH_4)_2SO_4$, fewer water molecules are available to interact with the charged component of L-Proline because some of them moves to the salt ions to produce hydrated ions. Therefore, L-Proline is not able to enter the solution very effectively. This process is also accelerated by the polyvalent sulphate ions present within the electrolytes. The rising value of this $[\Delta G_{t,ch}^0(i)]$ also supports the lower solubility of solute L-Proline in solution with ammonium sulphate.

4.4. Entropy

The negative values of both total entropy of transfer $[\Delta P_t^0(i)]$ and total chemical entropy of transfer $[\Delta P_{t,ch}^0(i)]$ increase with increasing concentration up to 1.5 mol/kg and then decrease as found in Figs. 6, 7 & Table 6.

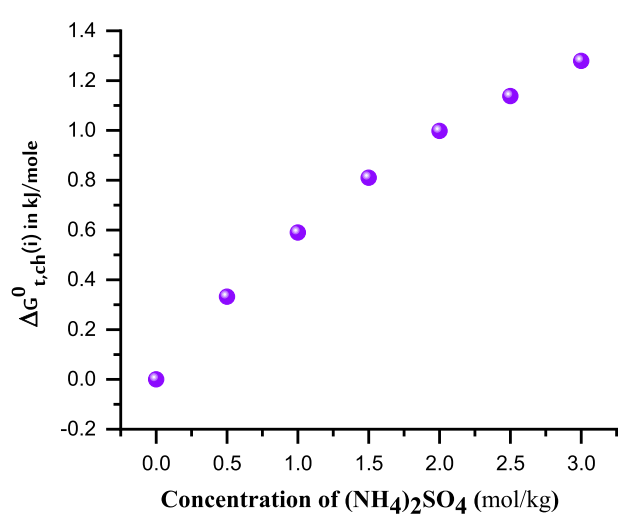

Fig. 5. Values of $\Delta G_{t,ch}^0(i)$ [kJ/mole] of L-Proline in aqueous ammonium sulphate solution at 298.15 K.

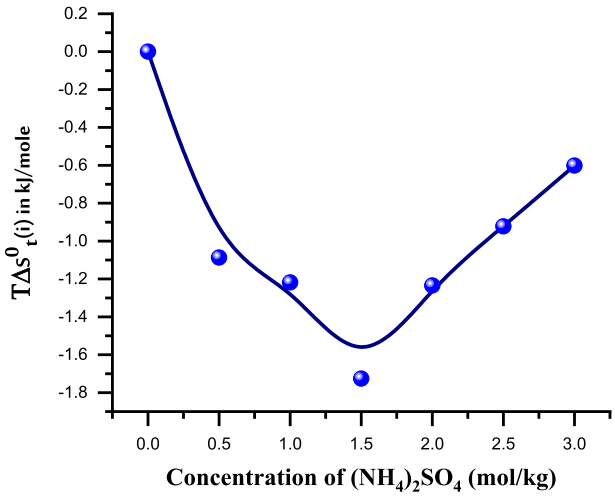

Fig. 6. $T\Delta S_t^0(i)$ values in kJ/mole of L-Proline in aqueous ammonium sulfate solution at 298.15 K.

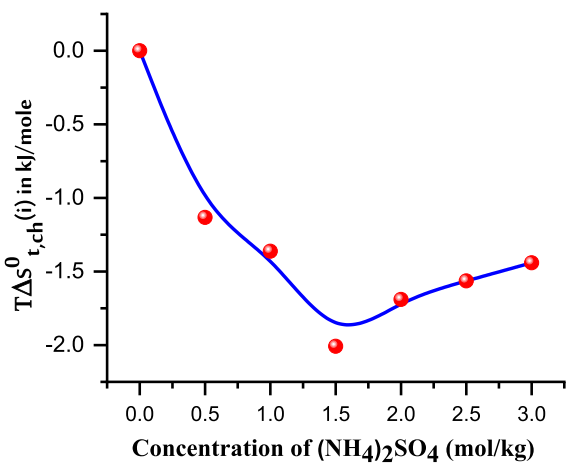

Fig. 7. Variation of $T\Delta S_{t,ch}^0(i)$ values of L-Proline in kJ/mole with molality (mol/kg) of electrolyte concentration at 298.15 K.

Entropy can be considered here as diorderness of the molecule and ions in the solution and that is decreased with increasing aggregation. In case of L-Proline, the closeness between them rises up with increasing $(NH_4)_2SO_4$ concentration due to the unavailable number of solvent water molecules for creating dipole–dipole interaction between L-Proline and water. In the presence of $(NH_4)_2SO_4$, water hydrates both ions of salt in a better way. After reaching a certain concentration of $(NH_4)_2SO_4$, ion-ion interaction predominates and then again water-proline interaction establishes fruitfully and as a result positive increments of total entropy transfer and total chemical entropy transfer can be observed.

5. Conclusion

When the temperature remains constant, the solubility of L-Proline decreases with an increase in electrolyte concentration, owing to the salting-out effect. Nonetheless, if the temperature rises, the solubility of L-Proline increases in both electrolytic and aqueous solutions, at a specific electrolyte concentration. The solubility of L-Proline is closely linked to its free energy ($\Delta G_{t,ch}^0(i)$), and the positive increase in this value in the current study supports this behaviour. The fluctuation in solubility can be attributed to dipole–dipole and ion-ion interactions, which affect both the total entropy transfer and total chemical entropy transfer. The solubility and thermodynamic properties of L-Proline in solution are significantly affected by the electrolyte $(NH_4)_2SO_4$ used in this study.

CRediT authorship contribution statement

Avishek Saha: Investigation. **Kalachand Mahali:** Validation. **Puspal Mukherjee:** Validation. **Sintu Ganai:** Investigation. **Aslam Hossain:** Methodology, Writing – original draft. **A.M.A. Henaish:** Conceptualization, Writing – review & editing. **Sanjay Roy:** Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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