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Article

Solubility and solvation thermodynamics of L-tryptophan in aqueous methanol solvent mixtures

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Abstract

This article presents mole fraction solubilities of L-tryptophan in binary aqueous methanol solvent systems and applied them to estimate thermodynamical parameters like transfer Gibbs free energetics and entropies of solutions. The estimated thermodynamic energies were linked with interaction parameters like dipole–dipole interactions, enthalpy due to transfer of L-tryptophan from water to binary water–methanol solvent mixtures. The transfer Gibbs free energetics for chemical interactions and entropies for solvent–solvent interaction were calculated by mathematical relations. The chemical transfer energetics was applied to explain the probable stability of L-tryptophan in the mixed solvents. Many associated thermophysical parameters were also calculated at standard temperature (298.15 K).

Key words: L-tryptophan, aqueous methanol solution, solubility, chemical free energy, chemical entropy

1. Introduction

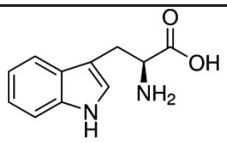
The function of biomolecules (like amino acid) in life and natural systems is very significant for their unusual properties. The side chains of different amino acids are responsible for different properties. The strength of the interaction of these molecules with many other molecules like proteins[1] and with other solvent systems like aqueous electrolyte,[2–5] aqueous organic,[6,7] pure organic,[8] pure solvent,[9] and ionic liquid[10] is reliant on the nature of side chains. In recent past, we described all such interactions in terms of thermodynamical effects in a review.[11] It is true that the chemical nature of amino acids is vital for the construction and extraction of many proteins from natural resources. On the other hand, the solvent atmosphere is an important factor in which the amino acids exist.

Generally, the supersaturated solution has a direct effect in improved crystallization process. The saturated solubility and solvation thermodynamics of the amino acids are vital for the purification and in designing crystallization methods.[12] In this regard, the resolving of stability nature of biomolecules like amino acids in various solvent systems and understanding of solubility nature and solvation related thermodynamical factors are most important.

To resolve the problems, many research groups worldwide are trying many solvent systems mainly for the biomolecules as well as amino acids.[2–5,7,13,14] Nozaki, Tanford, and other many scientists[10,13–17] resolved many solute–solvent interactions primarily in terms of thermodynamics parameters of many amino acids from aqueous glycerol, urea, acetonitrile, ethylene glycol, dimethylformamide, and 1-butyl-2,3-dimethylimidazolium bromide from their equilibrium solubility. In their cases, the experiments were planned to get a thought about the various interactions and the conclusions were made on the relative stability of the amino acids in pure, binary, and ternary solvent systems.

The existence of bulkier groups in L-tryptophan makes it less soluble in several solvent systems. In that case, some interesting behaviors have been accounted through comparative studies of many amino acids in aqueous organic solvent system.[18] Even in salt water system, amino acids like DL-phenylalanine exhibit some interesting characteristics.[19,20] Still, there are numerous successful research that were done with many amino acids, but not on the topic of solubility and the solvation thermodynamic of L-tryptophan. For this reason, in the present study we have selected L-tryptophan to do the same. Methanol was preferred as solvent in the present study as its boiling point is low and it may accelerate the crystallization process of L-tryptophan. The intent of this investigation was to measure the saturated solubilities of L-tryptophan in water and aqueous methanol from 288.15 to 308.15 K using analytical gravimetric method, and the results must offer thoughtful knowledge on solvation energetic of L-tryptophan in explaining its relative stability in aqueous methanol systems. This is helpful in the separation and crystallization methods of L-tryptophan from aqueous methanol systems or in pure methanol. The correlation of thermodynamical data and solubility may be a trusty and cost-effective way in separat-

Table 1. Specification of chemical samples.

Chemical name	Chemical structure	Source	Initial purity[a] (fraction)	Purification method	Final purity (fraction)
L-tryptophan		Merck, India	98.5% (mass)	Drying in a dehydrator with silica gel	—
Water	H_2O	—	—	Distillation	Triple distilled
MeOH	CH_3OH	Spectrochem	99.0% (mass)	Vacuum distillation	—

[a]Stated by the supplier.

ing and differentiating L-tryptophan from various natural sources.

2. Experimental methods

2.1. Chemicals and their purifications

L-tryptophan (>98.5.0%, melting point ≈563.15 K) was purchased from Merck, India, and it was used after drying in a vacuum desiccator without extra refinement. Methanol (99.0%) was purchased from Spectrochem, Mumbai, India. Solutions were prepared and experiments were performed with pure distilled water. The detailed specifications of the chemical samples are presented in Table 1.

2.2. Preparation of saturated solutions and method of measurement

The gravimetric method is inherently more accurate than the other volumetric or titration methods. Therefore, in this study, we used this method to measure the solubility of L-tryptophan in aqueous methanol solvent mixtures. In this method, the first step is the preparation of a saturated solution at required temperature. Hence, definite amount of MeOH was dissolved to make the solutions of MeOH 0.00, 0.2038, 0.4050, 0.6045, 0.8092, and 1.00 in mole fraction scale. The solutions were prepared with triple distilled water; stoppered measuring flask was used for good mixing of solutions. Excess solid L-tryptophan was added to sealed glass tubes and thoroughly mixed for 2 days. The experiment were conducted using a low-cum-high thermostat with a temperature accuracy of ±0.10 K. The sealed glass tubes were allowed to settle for a minimum of 7 h at the desired temperature prior to being sampled for gravimetric analysis.[21] The measurements were taken at the specified temperatures. For this method, 5 mL of the mixed saturated solution was collected using a dry pipette, filtered through a 0.22 μm HPLC disposable filter, transferred to a glass vessel, and quickly weighed. The solution was evaporated until it was completely dry, and then dried completely in a drying oven at a temperature of 400.15 K[22] (since the thermal decomposition temperature of free tryptophan falls within the range of 491.15–913.15 K).[22] The samples were then cooled in a dehydrator filled with silica gel for 24 h and weighed, with the process being repeated until a constant mass was achieved. The solubility of L-tryptophan in water in the presence of methanol was measured on a mole fraction scale.[21] Four sets of measurements were taken for all of the mixtures at temperatures of 288.15, 293.15, 298.15, 303.15, and 308.15 K (±0.10 K) by vigorously shaking the solutions to reach equilibrium. A solution was considered saturated or in equilibrium when the concentrations measured at 2-day intervals were in agreement.

3. Theoretical section

3.1. Calculation of solubility in mole fraction scale

For calculation of various thermodynamical factors, the necessary parameters like mole fraction of methanol (Z_s), water (Z_R), molar mass of aqueous methanol system (M_S), density (d_s), molar volume of co-solvent (V_s), hard sphere diameter of co-solvent (σ_s) and σ_{s-x} (=1/2($\sigma_s + \sigma_x$)), apparent co-solvent dipole moment (μ_s), and thermal expansibility constant (α, isobaric) of co-solvents at standard temperature 298.15 K are cited in Table 2.

The mole fraction solubilities of L-tryptophan (S) in pure water and water–methanol solvent system at different mole fraction compositions of methanol from 288.15 to 308.15 K were calculated using eq. 1 and the results are recorded in Table 3 and data are presented in Figs. 1 and 2. The initial mole fraction composition of the binary solvent (Z_S) is defined by eq. 2:

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

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

where 1, 2, and 3 refer to L-tryptophan, water, and methanol solvents, respectively. Here, m and M are the weight taken and the molar masses of L-tryptophan, water, and methanol solvents, respectively.

For better understanding the mole fraction solubility, data are multiplied by 10^3 and the resultant solubilities (solubility (S) × 10^3) are reported in Table 3.

3.2. Computation of total transfer Gibbs free energy and entropy of solution from solubility

The Gibbs energy of solutions of L-tryptophan on molal scale was calculated at the experimental temperatures using eq. 3[11,13,17] and shown in Table 4.

Table 2. Values of solvent parameters: mole fraction (Z_S), water (Z_R), mean mol. weight (M_S), density (d_s), hard sphere diameter of co-solvent (σ_s) (MeOH + H_2O) and $\sigma_{s-x} = 1/2(\sigma_s + \sigma_x)$, dipole moment of co-solvent (μ_s), and thermal expansibility constant (α) of H_2O and MeOH solution at 298.15 K.

Mole fraction of MeOH (Z_S)	Mole fraction of water (Z_R)	Molar mass (M_S)	10^3 d_s (Kg/m^3)	Molar volume (V_s)	σ_s (nm)	σ_{s-x} (nm) for L-tryptophan	Dipole moment (μ_S)(D)	α ($\times 10^3$) (K^{-1})
0	1	18.015	0.997	18.0692	0.274	0.436	1.831*	0.257*
0.2038	0.7962	20.874	0.957	21.8119	0.292	0.445	1.804	0.419
0.4050	0.5950	23.696	0.914	25.9261	0.309	0.454	1.778	0.579
0.6045	0.3955	26.494	0.871	30.4182	0.326	0.462	1.752	0.738
0.8092	0.1908	29.366	0.831	35.3380	0.344	0.471	1.725	0.900
1	0	32.042	0.792	40.4571	0.360	0.479	1.700	1.052

*For ref. 25: density, molar mass, thermal expansibility constant, size, and dipole moment values are taken from ref. 25. Dipole moment of L-tryptophan is 1.378 D (calculated by DFT as per ref. 42). Hard sphere diameter, σ_x, of L-tryptophan is 0.598 nm.[25] Dipole moment of MeOH is 1.70 D.[25,26] Hard sphere diameter of MeOH is 0.360 nm.[25,26]

Table 3. Mole fraction solubilities (S) $\times$ 10^3 of L-tryptophan in aqueous methanol at different temperatures (K).

Mole fraction of MeOH	288.15 K	293.15 K	298.15 K	303.15 K	308.15 K
0	1.1312	1.2468	1.3698	1.5402	1.7397
	1.13[44]	1.24[44]	1.228[43]		1.74[44]
			1.37[44]		
0.2038	1.0492	1.1440	1.2734	1.4326	1.6207
0.4050	0.9670	1.0556	1.1786	1.3318	1.4974
0.6045	0.8840	0.9682	1.0867	1.2291	1.3896
0.8092	0.8032	0.8789	0.9904	1.1232	1.2685
1	0.7204	0.7912	0.9015	1.0225	1.1468
	0.72[44]		0.90[44]		1.15[44]

Note: Standard uncertainties (u), $u(T) = 0.10$ K; relative uncertainties in solubility, $u_r(S) = 0.017 \times 10^{-3}$.

Fig. 1. Mole fraction solubility of L-tryptophan at various temperatures in pure solvents: water (black) and methanol (red).

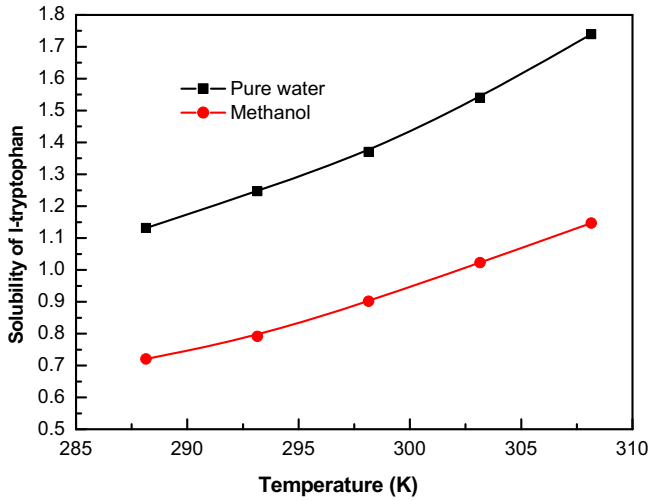
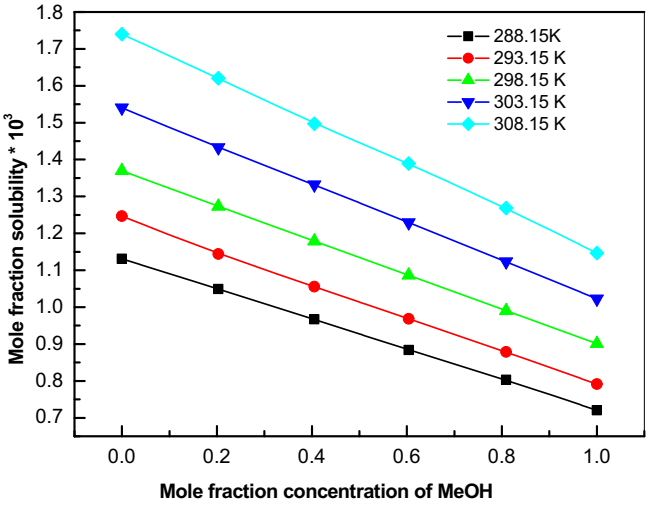


Fig. 2. Mole fraction solubility of L-tryptophan with mole fraction concentration of methanol in water–methanol mixed solvent systems at various temperatures: (■) 288.15 K; (●) 293.15 K; (▲) 298.15 K; (▼) 303.15 K; and (♦) 308.15 K.



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

Here, γ is the molal activity coefficient and "S" is the saturated solubility of the solute L-tryptophan in mole fraction scale (Table 3). Amino acids basically exist as zwitterions in solutions; this leads to expectation of existing high dipole–dipole interaction among them. Therefore, the contribution of activity coefficient factor $-RT\ln\gamma$ might be prime to $\Delta G_s^0(i)$. This is only true in the case of high concentration of solute in solution. For the present system, it is found that the mole fractions of L-tryptophan present in different compositions

Table 4. Standard Gibbs energies of solutions (ΔG_s^0) on molal scale in their respective solubilities of L-tryptophan in aqueous methanol at different temperatures (K).

288.15 K		293.15 K		298.15 K		303.15 K		308.15 K	
S (mole fraction)	ΔG_s^0 kJ/mol	S (mole fraction)	ΔG_s^0 kJ/mol	S (mole fraction)	ΔG_s^0 kJ/mol	S (mole fraction)	ΔG_s^0 kJ/mol	S (mole fraction)	ΔG_s^0 kJ/mol
0.0011312	16.2534	0.0012468	16.2983	0.0013698	16.3431	0.0015402	16.3216	0.0017397	16.2788
0.0010492	16.4337	0.0011440	16.5080	0.0012734	16.5239	0.0014326	16.5042	0.0016207	16.4603
0.0009670	16.6292	0.0010556	16.7040	0.0011786	16.7157	0.0013318	16.6881	0.0014974	16.6631
0.0008840	16.8442	0.0009682	16.9147	0.0010867	16.9169	0.0012291	16.8903	0.0013896	16.8545
0.0008032	17.0738	0.0008789	17.1505	0.0009904	17.1469	0.0011232	17.1174	0.0012685	17.0881
0.0007204	17.3344	0.0007912	17.4067	0.0009015	17.3801	0.0010225	17.3542	0.0011468	17.3465

Table 5. The values of coefficients *a*, *b*, and *c*, and total transfer Gibbs energies $\Delta G_t^0(i)$ and entropies $T\Delta S_t^0(i)$ of L-tryptophan in mole fraction scale from H_2O to H_2O–MeOH mixture at 298.15 K.

Mole fraction of MeOH	*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	−106.88	2.7595	−0.41179	0	0
0.2038	−121.94	3.1047	−0.46341	0.1725	0.1580
0.4050	−104.95	2.7271	−0.40702	0.3729	0.1349
0.6045	−105.68	2.7545	−0.41128	0.5756	0.4723
0.8092	−103.67	2.7145	−0.40531	0.8011	0.4769
1	−63.31	1.8172	−0.27145	0.9393	0.8033

Note: Uncertainty $u(T) = 0.10$ K; estimated uncertainties in $\Delta G_t^0(i)$ and $\Delta T\Delta S_t^0(i)$ are about 0.0006 and 0.0010 kJ/mol, respectively.

of water and water–methanol mixtures given in Table 3 are quite small, and for simplicity the molal activity coefficients are considered as unity. However, there are a number of different theoretical aspects and assumptions based on which activity coefficient is resolved to unity throughout the calculation of Gibbs energy of solution of many amino acids in solution, in this study as well as in many previous studies.[8,11,13,19,24,27–30]

Furthermore, our motto in the present investigation was to search the total transfer free energy ($\Delta G_t^0(i)$) in said solvent system and so it becomes easy to consider activity coefficient as unity. Since $\Delta G_t^0(i)$ is related as $\Delta G_t^o(i) = \Delta G_s^0(i) - \Delta G_R^0(i)$, (where $\Delta G_s^0(i)$ and $\Delta G_R^0(i)$ are the related free energy of the L-tryptophan in mixed solvent system and water, respectively), so, $\Delta G_t^0(i) = -\mathrm{RTln}m_s\gamma_s + \mathrm{RTln}m_R\gamma_R = -\mathrm{RTln}m_s\gamma_s/m_R\gamma_R = -\mathrm{RTln}m_s/m_R - \mathrm{RTln}\gamma_s/\gamma_R$ ("s" and "R" are used as subscripts with the parameters to categorize the input in mixed binary water–methanol solvent and water, respectively). In this case, the ratio of activity coefficient factor, $-\mathrm{RTln}\gamma_s/\gamma_R$ ("s" for water–methanol mixed solvent and "R" for H_2O), is near to be zero or is negligibly small. The factor holding the ratio of activity coefficient, $-\mathrm{RTln}\gamma_s/\gamma_R$, in computing transfer free energies ($\Delta G_t^o(i) = \Delta G_s^0(i) - \Delta G_R^0(i)$), which is our ultimate goal, was supposed to be negligibly small.

We employ least square method for evaluating *a*, *b*, and *c* required for the calculation of the resultant free energies of solution ΔG_s^0 at different temperatures.

$$\Delta G_s^0 = a + bT + cT\ln T \quad (4)$$

T is the temperature in absolute scale. The ΔG_s^0 values are shown in Table 4 and the values of *a*, *b*, and *c* are presented in Table 5. Transfer Gibbs free energies $\Delta G_t^0(i)$ and entropies ΔS_t^0 of the L-tryptophan from water to aqueous methanol solutions were estimated at 298.15 K by using eqs. 5 and 6.[11,13,17]

$$\Delta G_t^0(i) = {}_s\Delta G_s^0(i) - {}_R\Delta G_R^0(i)$$

i.e.,

$$\Delta G_t^0(i) = (a_s - a_R) + (b_s - b_R)T + (c_s - c_R)T\ln T \quad (5)$$

and

$$\Delta S_t^0(i) = (b_R - b_s) + (c_R - c_S)(1 + \ln T) \quad (6)$$

"R" and "s" carry the same meaning as previously stated.

M_R and M_s are the molar mass of water and aqueous methanol, respectively. The values of $\Delta G_t^0(i)$ and $T\Delta S_t^0(i)$ are shown in Tables 5 and 6.

3.3. Calculation of transfer energetics owing to cavity formation, dipole–dipole interactions, enthalpy, and entropy

The total transfer energetics in terms of Gibbs free energetics and entropies (P = G or S) may be attributed as the total of the following terms (assuming dipole-induced dipole term to be negligibly small).[13,23]

$$\Delta P_t^0(i) = \Delta P_{t,cav}^0(i) + \Delta P_{t,d-d}^0(i) + \Delta P_{t,ch}^0(i) \quad (7)$$

Here, $\Delta P_{t,cav}^0(i)$ denotes the transfer energy part due to the cavity-forming effect in aqueous methanol solutions for the L-tryptophan, and $\Delta P_{t,d-d}^0(i)$ is the transfer energy due to the

Table 6. Transfer Gibbs energies $\Delta G_t^0(i)$, $\Delta G_{t,cav}^0(i)$, $\Delta G_{t,dd}^0(i)$, $\Delta G_{t,ch}^0(i)$ and enthalpy of transfer $\Delta H_{t,cav}^0(i)$ and transfer entropies $T\Delta S_t^0(i)$, $T\Delta S_{t,cav}^0(i)$, $T\Delta S_{t,dd}^0(i)$, and $T\Delta S_{t,ch}^0(i)$ of L-tryptophan from H_2O to H_2O–MeOH at 298.15 K in kJ/mol.

Mole fraction of MeOH	$\Delta G_t^0(i)$ kJ/mol	$\Delta G_{t,cav}^0(i)$ kJ/mol	$\Delta G_{t,dd}^0(i)$ 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	$T\Delta S_{t,dd}^0(i)$ kJ/mol	$T\Delta S_{t,ch}^0(i)$ kJ/mol
0	0	0	0	0	0	0	0	0	0
0.2038	0.1725	−1.61	0.039	1.744	0.1580	0.85	2.463	0.035	−2.340
0.4050	0.3729	−2.83	0.113	3.089	0.1349	1.77	4.600	0.104	−4.569
0.6045	0.5756	−3.77	0.188	4.158	0.4723	3.17	6.940	0.176	−6.644
0.8092	0.8011	−4.52	0.254	5.067	0.4769	5.48	10.000	0.241	−9.764
1	0.9393	−5.13	0.306	5.763	0.8033	8.52	13.650	0.293	−13.139

Note: Uncertainty $u(T) = 0.10$ K; estimated uncertainties in $\Delta G_t^0(i)$ and $T\Delta S_t^0(i)$ are about 0.0006 and 0.0010 kJ/mol, respectively.

dipole–dipole interaction effect between zwitterionic amino acid L-tryptophan and solvated methanol molecules.

Various short range chemical interactions like acid–base interactions, hard−soft, short-range dispersive interactions, hydrophilic and hydrophobic hydration, hydrogen bonding and structural effect influence $\Delta P_{t,ch}^0(i)$, the chemical transfer Gibbs energetics and entropies. Structural effects here mainly arise due to solute–solute, solute–solvent, and solvent–solvent interactions.

Scale particle theory is applied to estimate $\Delta P_{t,cav}^0(i)$,[31–33] where the solute and solvent molecules are considered as hard sphere models that are dictated by their respective parameters (Table 2).

Again

$$(8)\qquad \Delta G_{t,d-d}^0(i) = ({}_s\Delta G_{d-d}^0(i) - {}_R\Delta G_{d-d}^0(i)$$

and $\Delta S_{t,d-d}^0(i) = ({}_s\Delta S_{d-d}^0(i) - {}_R\Delta S_{d-d}^0(i))$ have been calculated by using Keesom orientation expression,[34] for ${}_s\Delta G_{d-d}^0(i)$ in a solvent S, as given below:

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

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

$$(10)\qquad {}_s\Delta S_{d-d}^0(i) = -\left\{\delta_s\Delta G_{d-d}^0(i)/\delta T\right\}_p$$

i.e., ${}_sT\Delta S_{d-d}^0(i) = {}_s\Delta G_{d-d}^0(i)[1 + T\alpha]$, where N stands for Avogadro's number, and μ_s and μ_x are the dipole moment of aqueous methanol mixtures and amino acid, respectively (Table 2).

σ_{s-x} is equal to $1/2(\sigma_s + \sigma_x)$ where σ_s and σ_x are the hard sphere diameters of co-solvents and amino acid molecule, respectively (Table 2), and α is the isobaric thermal expansibility constant of the solvents and measured by eq. 11.[18,19]

$$(11)\qquad \alpha = (\delta \ln V_s/\delta T)_P = -(\delta \ln d_s/\delta T)_P$$

According to Marcus[32] and Kim et al.,[34] to get actual mole fraction part due to dipole–dipole interaction $\Delta P_{t,d-d}^0(i)$ value on mole fraction scale, the amount was multiplied by χ_{s1}.

$$(12)\qquad \mathrm{X}_{s1} = \mathrm{X}_s(\mu_s/\sigma_s^3)/(\mu_R/\sigma_R^3)$$

After subtraction of $\Delta P_{t,cav}^0(i)$ and $\Delta P_{t,d-d}^0(i)$ from the total $\Delta P_t^0(i)$, we get the value of $\Delta P_{t,ch}^0(i)$, which is presented in Table 6.

4. Discussion

4.1. Analysis of solubility

The investigation on solubilities of L-tryptophan in water and water–methanol solvent systems was done in the temperature range 288.15–308.15 K. A linear rise of solubility with increasing temperature is shown according to Fig. 1 in pure water and methanol. Experimental result also denotes that the solubilities of L-tryptophan decrease with increasing methanol concentration at particular temperature (Fig. 2; Table 3). From Fig. 2, it can also be concluded that L-tryptophan is more soluble in pure aqueous system than in water–methanol system for a particular temperature. The last one can be explained on the basis of structural ground of L-tryptophan. Nitrogen atom present within L-tryptophan has a capability of forming H-bonding with polar protic solvent like water through its proton acquiring due to zwitterionic form with electronegative oxygen present within water.[35] On the other hand, the carboxyl group (COO^-) can also make H-bond with H_2O through its neutral oxygen and thus in aqueous solution zwitterionic form of L-tryptophan produces H-bonding with water. In comparison with methanol, water has a smaller molecular structure and also greater polarity with higher dielectric constant (32.63 for methanol and 78.54 for water at 298.15 K).[38] These all lead to produce better H-bonding as well as easy accommodation in interspaced directing to greater solvation than methanol. So, we reach the conclusion that the solubility of L-tryptophan in pure water is higher than methanol solution at any temperature. Side-by-side methanol cannot overcome electrostatic interaction due to low dielectric constant value and consequently fails to create solvated ions. Hydrocarbon part of methanol also plays a crucial role. Polarity of methanol is low compared with water due to the presence of hydrocarbon part carrying more covalent character decreasing effectiveness of H-bonding. However, in the mixture, the -OH group of methanol can produce H-bonding with water more effortlessly than that with L-tryptophan. Hence, higher concentration of methanol in solution reduces the water molecules available for L-tryptophan, leading to lower solubility of L-tryptophan. Therefore, water was proved to be better solvent for L-tryptophan than methanol.[36,39] Thus, it is clear that methanol shows antisolvent effect for L-tryptophan. Figure 1 supports the behavior of methanol as successful antisolvent

Fig. 3. Mole fraction solubility (solubility (S) $\times$ 10^4) of L-tryptophan in water–methanol solvent mixtures with variations of temperatures in different mole fraction concentrations of methanol.

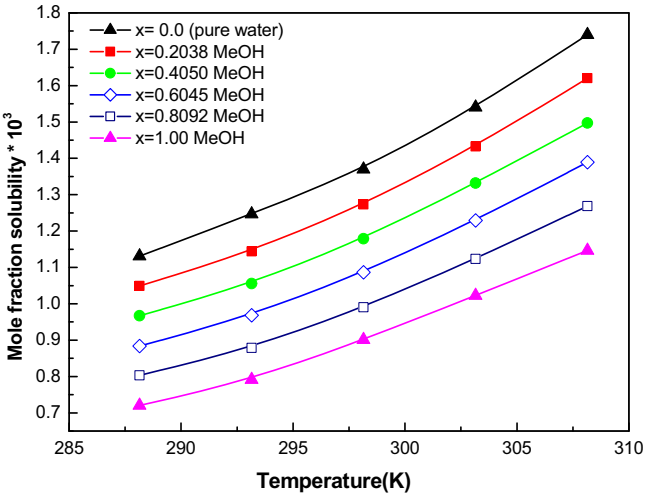
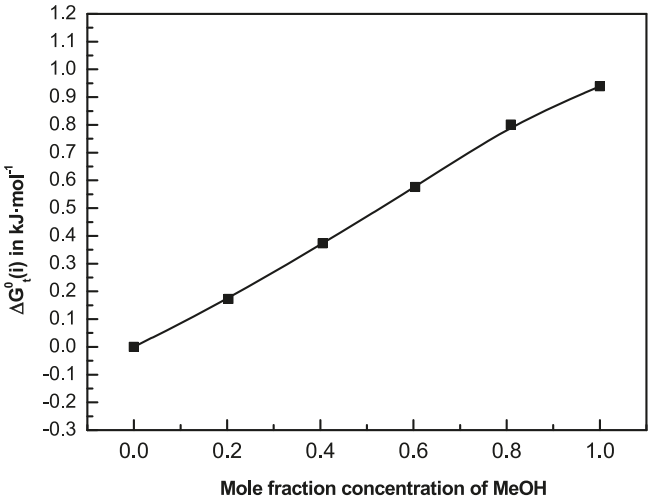


Fig. 4. Variation of transfer Gibbs free energy ($\Delta G_t^0(i)$) of L-tryptophan with mole fraction concentration of methanol solvent systems in kJ·mol^{-1} at 298.15 K.



for L-tryptophan. Now, Fig. 3 shows that the solubilities of L-tryptophan in both water and water–methanol system increase with enhancement of temperature for particular concentration. This conclusion might be owing to the fact that the kinetic energy of the solvent molecule increases with the rising of temperature. This helps the solvent molecule to distort the association of solute molecule held by strong intermolecular force. Therefore, L-tryptophan can now effectively make interaction with solvent molecules and consequently solubility increases.

4.2. Transfer Gibbs free energies and probable stability of L-tryptophan in aqueous methanol media

For better understanding of the solvation process as well as the probable stability of L-tryptophan, we become eager to calculate Gibbs free energies arising due to cavity-forming interaction, dipole–dipole interaction, and chemical interaction. Figure 4 shows the variation of $\Delta G_t^0(i)$ with mole fraction of methanol in aqueous methanol media. From this figure, it is clear that $\Delta G_t^0(i)$ becomes more and more positive with increasing concentration of methanol and it leads to destabilization. This also suggests that L-tryptophan becomes more stable in aqueous media than in methanol. This stability may be explained by the gradual change in cavity interaction, dipole–dipole interaction, dispersion interaction, acidity basicity, and other associated factors between L-tryptophan and mixed solvent system.

In this study, dipole-induced dipole interaction becomes negligible.[31,33] It is found that $\Delta G_{t,cav}^0(i)$ becomes more and more negative gradually with the increasing mole fraction of methanol in binary solvent mixture. The experimental result also leads to the conclusion that L-tryptophan gains higher stability owing to cavity interaction with enhancing mole fraction of methanol. As methanol (3.6A)[37] is larger than water (2.74A),[32] the involved energy change due to creation of cavity for L-tryptophan in methanol–water mixture will be more favorable.

Again, $\Delta G_{t,(d-d)}^0(i)$ values (Table 6) for L-tryptophan become gradually positive indicating less stabilization with increment of methanol concentration. This stabilization/destabilization can be explained with their corresponding dipole moment value. The dipole moment values for L-tryptophan, water, and methanol are 1.37, 1.85, and 1.70 D, respectively. So, these data predicted that dipole–dipole interaction becomes better between water and L-tryptophan compared with L-tryptophan and methanol. Consequently, with increasing methanol concentration, dipole–dipole interaction becomes weak leading to destabilization in this medium, which in turn results in the positive increment of $\Delta G_{t,(d-d)}^0(i)$ values.

$\Delta G_{t,ch}^0(i)$ of L-tryptophan is computed after subtraction of $\Delta G_{t,cav}^0(i)$ and $\Delta G_{t,d-d}^0(i)$ from $\Delta G_t^0(i)$. The $\Delta G_{t,ch}^0(i)$ value involves the chemical interactions such as H-bonding, acid–base, hard–soft, dispersion, hydrophilic hydration, and hydrophobic hydration between L-tryptophan and solvent molecules in water–methanol mixtures.

The variation of $\Delta G_{t,ch}^0(i)$ of L-tryptophan with mole fraction of methanol is shown in Fig. 5. This value rises up to 100 mole fraction of methanol in aqueous organic solvent system. This leads to destabilization of L-tryptophan. This can be explained on the basis of solvent and solvent–solute interaction. L-tryptophan has ability to H-bond with water but when methanol is added to the solution, then the mentioned H-bonding present between solvent and solute breaks down with the enhancement of concentration of methanol. H_2O molecules produce H-bonding with methanol in a more preferable way rather than with tryptophan due to increasing hydrocarbon part in L-tryptophan; thus, solvent–solvent interaction becomes more favorable rather than solute–solute and solvent–solute interactions. Consequently, $\Delta G_{t,ch}^0(i)$ val-

Fig. 5. Variation of transfer Gibbs free energy due to chemical interactions ($\Delta G^0_{t,ch}(i)$) of L-tryptophan with mole fraction concentration of methanol solvent systems in kJ·mol^{-1} at 298.15 K.

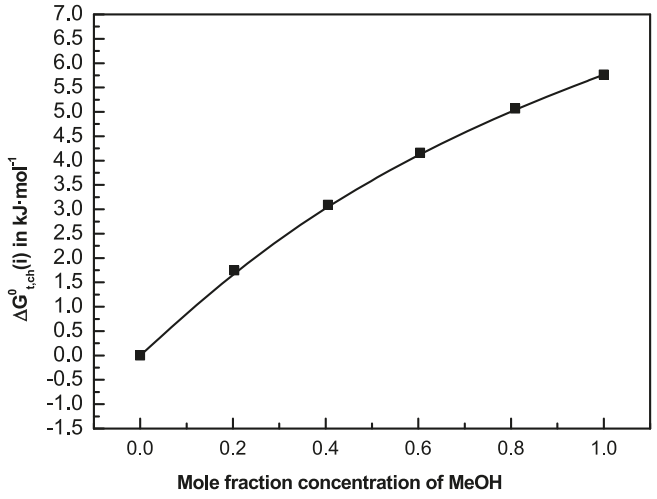
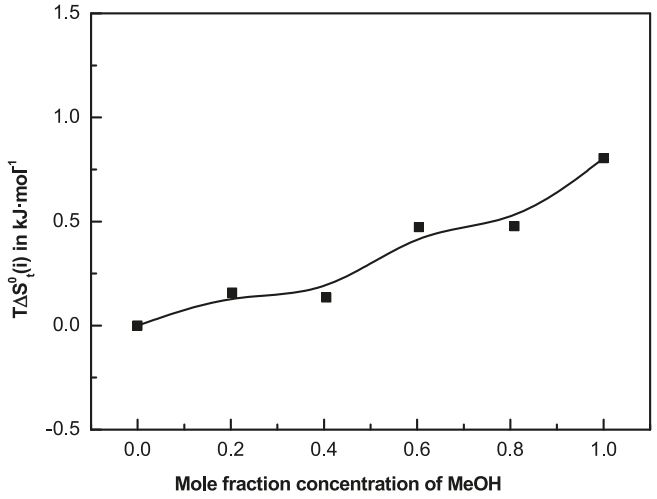


Fig. 6. Variation of total transfer entropy ($T\Delta S^0_t(i)$) of L-tryptophan with mole fraction concentration of methanol in kJ·mol^{-1} at 298.15 K.

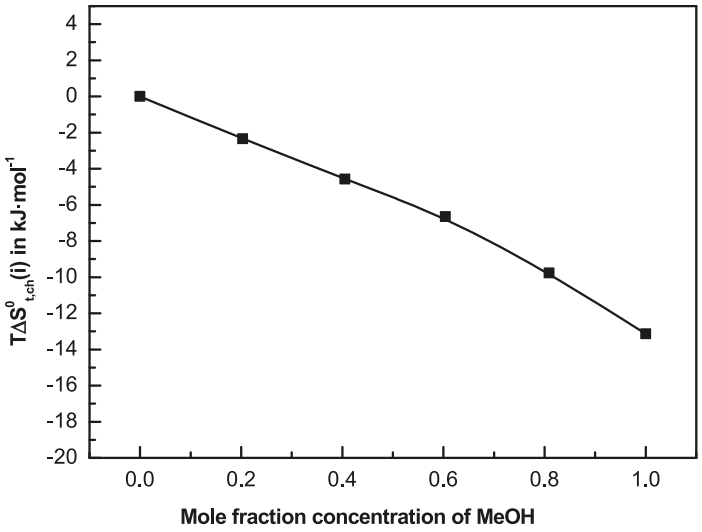


ues of L-tryptophan become more positive with increasing concentration of methanol in water. Due to lower dielectric constant value of methanol, it shows lower stability toward L-tryptophan compared with water. The stability order of L-tryptophan is water–methanol < water. The result also supports the solubility data of L-tryptophan in experimental solvent system.

4.3. Amino acids induced solvent–solvent interactions in terms of transfer entropy

Figure 6 reflects the change of $T\Delta S^0_t(i)$ of L-tryptophan in water–methanol solvent systems with mole fraction of methanol. These thermodynamic terms also comprised several interactions like cavity, dipole–dipole, and various other

Fig. 7. Variation of total transfer entropy ($T\Delta S^0_{t,ch}(i)$) of L-tryptophan with mole fraction concentration of methanol in kJ·mol^{-1} at 298.15 K.



chemical interactions:

$$T\Delta S^0_t(i) = T\Delta S^0_{t,cav}(i) + T\Delta S^0_{t,d-d}(i) + T\Delta S^0_{t,ch}(i) \quad (13)$$

where the first two terms stand for the difference in the entropy change involved in creating appropriate cavities for accommodating the L-tryptophan and in interaction of dipolar L-tryptophan with the solvent dipole, respectively. These two effects in conjunction, being superimposed on other effects, might become a key factor for an initial increase followed by a maximum in the $T\Delta S^0_t(i)$ versus mole fraction of methanol profile for the L-tryptophan.

Dipole-induced dipole interactions are ignored for sake of simplicity. Complex nature of variation is mainly found due to different types of chemical interactions between solvent molecules induced by solute molecules. Such several types of interactions were also investigated in many past studies for different amino acids in divergent solvent mixtures.[13,23,31,33,40]

$T\Delta S^0_t(i)$ and $T\Delta S^0_{t,ch}(i)$ values are presented in Table 6. $T\Delta S^0_{t,ch}(i)$ values for L-tryptophan are computed after deduction of $T\Delta S^0_{t,cav}(i)$ and $T\Delta S^0_{t,d-d}(i)$ from $T\Delta S^0_t(i)$. Figure 7 reflects the change of $T\Delta S^0_{t,ch}(i)$ values for L-tryptophan in water–methanol solvent systems. Those experimental data reveal that $T\Delta S^0_{t,ch}(i)$ decreases with increased co-solvent mixture for the amino acid L-tryptophan. Since the transfer of L-tryptophan from water to aqueous methanol consists of four successive steps,[41] the variation is complex in nature and needs to be explained in terms of the following steps.

The very first step is the cleavage of solute–water bonds (H-bond, acid–base etc.) followed by the removal of a molecule from the cavity to vacuum leading to positive entropy change $\Delta S^0{}_1$. The second step is the formation of normal water structure to fill up the cavity, associating negative entropy change $-\Delta S^0{}_2$. The third step is the formation of the suitable cavity in the mixed solvent by breaking of solvent–solvent bond associating positive entropy change $\Delta S^0{}_3$. The last step is the

introduction of the molecule from vacuum into the solvent cavity and the formation of the ion solvent bond leading to again negative entropy change $-\Delta S^0{}_4$. Thus

(14) $\Delta S_{t,ch} = \Delta S_1^0 - \Delta S_2^0 + \Delta S_3^0 + -\Delta S_4^0$

These composite types of entropy changes might be the reasons for the variations or deviations of $T\Delta S_{t,ch}^0(i)$ values as shown in Fig. 7.

Initially in aqueous solution of L-tryptophan H-bonding, acid–base interaction and hydrophilic interaction play a crucial role for the stability of L-tryptophan in aqueous media. But when methanol was introducing the 3D structure of water breakdown and due to high polar nature of methanol, solvent–solvent interaction will be more favorable. The hydrophilic character of methanol is also responsible for this fact. This is why gradual negative increment of entropy is observed. Intermolecular H-bonding is taking place between solvent–solvent molecules leading to lowering of the solubility as well as stability of L-tryptophan in water–methanol solvent system.

5. Conclusion

The solubility of L-tryptophan in mixed solvent was investigated at five equidistant temperatures (288.15–308.15 K). The investigated data reveal that the solubility of L-tryptophan varies inversely with concentration at a particular temperature. The experimental results also indicate that L-tryptophan becomes less soluble in methanol compared with pure water, leading to more effective crystallization process. Additionally, some important thermodynamic parameters for the solvation process such as transfer Gibbs free energy, enthalpy, and entropy were calculated and the values were explained.

The present study showed that both of the values of standard molar enthalpy change due to cavity-forming interaction and standard transfer Gibbs free energy change of solution were positive, which specify that the solvation process of L-tryptophan was endothermic and nonspontaneous. The standard transfer Gibbs free energy change due to chemical interactions of solution suggested that L-tryptophan is more stable in pure water rather than in mixed water–methanol solvent systems. The negative tendency of chemical entropy transfer establishes the entropy-driving force to be the most important aspect in the overall solvation process of the experimental molecule in water–methanol solvent mixtures.

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Data availability

Data generated or analyzed during this study are provided in full within the published article.

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Formal analysis: AS, KM
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Methodology: KM
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Competing interests

The authors declare no conflict of interest.

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