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Study of Interactions of L-Threonine with solvent as Urea (Aq)

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ABSTRACT

Urea is known to denature the peptide bonds. L-Threonine is the second important amino acid in biological processes. The measurements of density, viscosity and sound speed have been determined by experimental procedures using pre-calibrated bicapillary pyknometer, Ostwald's viscometer and Ultrasonic interferometer respectively. The experimental data related to density, viscosity, sound speed for the system L-Threonine with Urea (aq) are further utilized to derive parameters viz. isentropic compressibility (β), apparent molal volume (ϕ_v), apparent molal compressibility (ϕ_k), specific acoustic impedance(Z), Relative Association(R_A), Solvation Number(S_n) and Viscous Relaxation Time (τ). From these derived parameters we calculate limiting apparent molar volume (ϕ_v°), limiting apparent molar compressibility (ϕ_k°) and experimental slope (S_v* and S_k*) by using Masson's equation. The viscosity data are analysed using Jones-Dole equation. The concentration array selected for L-Threonine is 0.10m to 0.61m. The measurements are conducted at three temperatures viz. 298, 308, 318K. The results are interpreted on the basis of solute-solvent and solute-solute interactions, structure maker or breaker capacity of the solute, where the addition of solute and its concentration produces a major influence on the interactions. This shows the dependency of interactions on the concentration.

Keywords: apparent molal volume (ϕ_v), apparent molal compressibility (ϕ_k), solute-solute interactions, solute-solvent interactions, structure maker/breaker.

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INTRODUCTION

Animal tests on cereal protein have shown that L-Threonine is second required amino acid after L-Lysine for supplementation in the diet. The competency of L-Threonine in the body is considered to be limited by the fact that the hydrolysis of peptide linkage in which L-Threonine takes part is difficult. Moreover urea is recognized to denature proteins regardless of the composition of amino acids. Therefore changes in the solubility of amide back bone on introduction of urea to the solution are considered to be primary contributors to the denaturation process. L-amino acids are implicated in intracellular metabolism and maneuver unambiguous transport systems of the plasma membrane [1-3].

MATERIAL and METHODS

Analytical range (AR) grade L-Threonine with minimum assay of 99% purity obtained from Himedia were used as such without further purification. Distilled water was used to make the solutions. 0.01M aqueous solution of Urea was prepared the same day when it was to be used as solvent. The concentration range selected for L-Threonine is 0.10m to 0.61m. Weighing was done with a precision of $\pm 1 \times 10^{-4}$g on electronic digital balance. Density was measured using precalibrated bicapillary pyknometer having two limbs with equal divisions with an accuracy of ±0.06% [4, 5], viscosity by the use of precalibrated Ostwald's viscometer [6] up to an accuracy ±0.07%. Sound speed was measured using Ultrasonic interferometer (Mittal Enterprises, New Delhi, Model F-81) working at a fixed frequency of 2 MHz up to an accuracy of ±0.04% [7, 8]. All the experiments have been carried out at constant temperature in a refrigerated water bath maintaining temperature up to ±0.1°C and the data are concordant readings of the measurements taken. The solutions were hoard in airtight bottles so as to curtail the absorption of atmospheric moisture. The experimentation is carried out at three different temperatures viz. 298, 308, 318K so as to study the interactions precisely.

Formulae:

Apparent Molal Volume
$$\phi_v = \frac{1000(\rho_0 - \rho)}{m\rho_0\rho} + \frac{M}{\rho} \quad (1)$$

Apparent Molal Compressibility
$$\phi_k = \frac{1000(\rho_0\beta - \rho\beta_0)}{m\rho\rho_0} + \frac{\beta M}{\rho} \quad (2)$$

Specific Acoustic Impedance
$$Z = u\rho \quad (3)$$

Viscous relaxation time
$$\tau = \frac{4\eta}{3\rho u^2} \quad (4)$$

Relative Association
$$R_A = (\rho / \rho_0)(uo / u)^{1/3} \quad (5)$$

Solvation number $$Sn = (n_1 / n_2)(1 - \beta / \beta_0) \qquad (6)$$

where m is the concentration in moles kg^{-1}, ρ_o is the density of solvent (kg m^{-3}), ρ is the density of solution (kg m^{-3}), M is the Molecular weight of solute, η_o is the viscosity of solvent, η is the viscosity of solution, β_o is the adiabatic compressibility of solvent, β is that of solution, n_1 is moles of solvent, n_2 that of solution.

RESULTS AND DISCUSSIONS

The experimentally measured values of density (ρ) and sound speed (u) of the solutions of L-Threonine in Urea (aq) at 298, 308 and 318 K are recorded in Table 1.

Table 1: Density and Sound Speed of L-Threonine + Urea (aq) at 298, 308 and 318K

Conc. (mol/Kg)	Density(ρ) (Kg/m^3)				Sound Speed(u) m/s		
	298K	308K	318K		298K	308K	318K
0.0000	**996.9**	**994.3**	**991.3**		**1501.4**	**1509.2**	**1516.0**
0.1030	1000.3	997.3	993.8		1510.0	1521.4	1529.4
0.1545	1003.7	1000.3	996.6		1511.6	1525.4	1533.8
0.2060	1006.3	1002.8	998.7		1514.4	1529.8	1538.8
0.2575	1008.9	1005.4	1000.9		1517.4	1531.8	1542.8
0.3090	1010.1	1006.6	1002.7		1521.6	1536.2	1547.6
0.3604	1012.2	1007.9	1004.6		1525.2	1540.4	1551.8
0.4119	1014.3	1009.7	1006.4		1528.6	1544.4	1556.8
0.4634	1016.4	1011.3	1008.3		1532.2	1548.6	1562.2
0.5149	1017.9	1013.0	1009.5		1535.4	1552.8	1567.2
0.5664	1019.5	1014.7	1010.2		1539.4	1557.4	1570.4
0.6179	1021.4	1016.2	1011.1		1543.2	1562.2	1574.2

At 298K and 308K the values of ϕ_v increases with the increase of concentration of amino acid except at 0.10m. The result is similar for 318K with the exception at concentration of 0.20m. The increase with temperature may be ascribed to the fact that at a particular temperature the increase in solute concentration dwindles the size and number of intermolecular voids. With the increase of temperature the intermolecular distance between the solute and solvent molecules is affected due to an increase of accessible surface area of the amino acids resulting in the formation of cavity around the interacting molecules which escorts to positive changes in the volume. The apparent molal volume (ϕ_v) [Table 2] is positive demonstrating the presence of solute-solvent interactions [9].

Table 2: Apparent molal volume and Adiabatic Compressibility of L-Threonine+Urea (aq) at 298, 308 and 318K

Conc. (mol/Kg)	Apparent molal volume (ϕ_v x 10^{-3} m^3 /mol)				Adiabatic Compressibility (β x$10^{-10}$$m^2$ /N)		
	298K	308K	318K		298K	308K	318K
0.0000	-------	-------	--------		4.4501	4.4155	4.3894
0.1030	0.0858	0.0904	0.0954		4.3845	4.3320	4.3021
0.1545	0.0747	0.0805	0.0846		4.3605	4.2965	4.2652
0.2060	0.0729	0.0773	0.0826		4.3332	4.2609	4.2285
0.2575	0.0718	0.0754	0.0814		4.3050	4.2389	4.1976
0.3090	0.0754	0.0785	0.0814		4.2759	4.2095	4.1638
0.3604	0.0755	0.0807	0.0815		4.2469	4.1815	4.1337
0.4119	0.0756	0.0809	0.0815		4.2194	4.1524	4.0997
0.4634	0.0757	0.0813	0.0815		4.1910	4.1232	4.0640
0.5149	0.0768	0.0815	0.0827		4.1672	4.0939	4.0333
0.5664	0.0775	0.0818	0.0845		4.1390	4.0632	4.0139
0.6179	0.0776	0.0822	0.0858		4.1110	4.0323	3.9910

The values of β [Table 2] in amino acid system show a declining trend. It is assumed that the cavities contained in the framework of the solvent clusters are gradually filled up by the solutes (amino acid). This accompanies a decrease in the β and also in the dβ/dT in the solution [10]. The decrease in compressibility is accredited to the influence of electrostatic field of zwitterions formed from the amino acid in aqueous environment on the surrounding solvent molecules. The values of apparent molal compressibility (ϕ_k) are negative [Table 3] over the entire range of concentration which indicates occurrence of electrostriction and hydrophilic interactions in these systems, leading to solute- solvent interactions [11].

Table 3: Apparent molal Compressibility of L-Threonine+Urea (aq) at 298, 308 and 318K

Conc. (mol/ Kg)	Apparent molal compressibility (- ϕ_k x $10^{-10}$$m^2$ /N)		
	298K	308K	318K
0.1030	0.7846	0.9402	0.9604
0.1545	0.7734	0.9400	0.9597
0.2060	0.7661	0.9311	0.9426
0.2575	0.7644	0.8721	0.9089
0.3090	0.7469	0.8377	0.8915
0.3604	0.7441	0.8090	0.8687
0.4119	0.7379	0.7958	0.8603
0.4634	0.7344	0.7841	0.8569
0.5149	0.7184	0.7754	0.8396
0.5664	0.7133	0.7697	0.8024
0.6179	0.7106	0.7645	0.7778

The standard state (infinite dilution) partial molal volumes ϕ_v° were obtained from the relation

$$\phi_v = \phi_v^\circ + S_v^* \sqrt{m} \qquad (7)$$

where S_v^* is the experimental slope and is a measure of solute-solute interactions, while ϕ_v° provides information regarding solute-solvent interaction. The ϕ_v° and S_v^* values were obtained from the intercept and slope of ϕ_v vs. $m^{1/2}$ plots. The evaluated values of ϕ_v° and S_v^* are summarized in Table 4.

Table 4: Constant S_v^*, Limiting Apparent molal Volume, B-Coefficient, A-coefficient, Constant S_k^* and Limiting Apparent molal Compressibility for L-Threonine+Urea (aq) at 298, 308 and 318K

Temp in K	S_v^* (x10^{-3} m^3 $lt^{1/2}$ /$mol^{3/2}$)	ϕ_v° (x 10^{-3} m^3 /mol)	B(dm^3 /mol)	A ($dm^{3/2}$ /$m^{1/2}$)	S_k^* (x10^{-10} /Nm mol)	ϕ_k° (x10^{-10} m^2 /N)
298	-0.0018	0.0769	0.2498	0.1220	0.1452	-0.7973
308	-0.0016	0.0815	0.2762	0.0928	0.3963	-0.9810
318	-0.0084	0.0869	0.3607	0.0349	0.3507	-1.0054

Amino acids when dissolved in pure water exist as zwitterions. The hydration behaviour of amino acids can be explained in terms of the following interactions [12].

1. The terminal ionic groups NH_3^+ and COO^- of zwitterions of amino acids are hydrated in an electrostatic manner, whereas , hydration of intervening backbone depends on its nature which may be hydrophobic, hydrophilic or amphiphilic.
2. Electrostriction of NH_3^+ group is greater than COO^- by a factor of about 10.
3. The overlap of hydration cosphere of terminal NH_3^+ and COO^- groups and of adjacent groups' results in volume change.
4. Creation of cavity in the solvent large enough to accommodate the solute, followed by the placement of the solute into cavity.
5. The interaction between the solvent and the polar and charged groups of the protein molecules.

The observed values of ϕ_v° are due to the net combined effect of the aforeseen possible interaction processes in the mixtures of amino acids+Urea (aq) in system.

The volume behavior of a solute at infinite dilution is effectively represented by ϕ_v° which is independent of solute-solute interactions and reflects solute-solvent interactions. The large positive values of ϕ_v° suggest strong solute- solvent interactions and vice-versa. Thus for these systems the constructive values of ϕ_v° indicate solute-solvent interactions, which increase with rise in temperature. Negative values of S_v^* for the system illustrate weak ion-ion/solute-solute interactions. The negative values increase with rise in temperature which may be ascribed to the increase in solute-solute interaction with the increase of temperature.

The standard state (infinite dilution) ϕ_k° were obtained from the relation

$$\phi_k = \phi_k^\circ + S_k^* \sqrt{m} \qquad (8)$$

where S_k* is the experimental slope, while ϕ_k° provides information regarding solute-solvent interactions. The values of S_k* are positive [Table 4] suggesting the existence of solute-solute/ion-ion interactions [13]. The values of S_k* does not show linear pattern with temperature, but suggest an increase in solute-solute interactions with the increase in temperature.

A is the Falkenhagen coefficient [Table 4] that accounts for the solute-solute interactions and B, the Jones-Dole coefficient is a measure of the structural modification induced by solute-solvent interactions. The viscosity coefficients, A and B, were obtained from the intercepts and slopes of the plots $(\eta_r - 1)/m^{1/2}$ vs. $m^{1/2}$.The values of B-coefficient are large positive values as compared to A-coefficient values. The larger magnitude of B coefficient suggests the preeminence of solute-solvent interactions over solute- solute interactions [14]. The positive values of B-coefficient in system [Table 4] increases with the mounting of temperature, supporting ϕ_v°. Thus solute-solvent interaction increases with the ascending temperatures.

Table 5: Specific Acoustic Impedance and Viscous Relaxation Time for L-Threonine+Urea (aq) at 298, 308 and 318K

Conc. (mol/Kg)	Specific acoustic Impedance Z(x10^6 Kg /m^2s)				Viscous Relaxation Time τ (x10^{-13}s)		
	298K	308K	318K		298K	308K	318K
0.0000	**1.4967**	**1.5006**	**1.5028**		**5.2206**	**4.2097**	**3.5226**
0.1030	1.5104	1.5173	1.5199		5.4898	4.3862	3.6606
0.1545	1.5172	1.5258	1.5286		5.5734	4.4473	3.7080
0.2060	1.5239	1.5341	1.5369		5.6331	4.4654	3.7239
0.2575	1.5308	1.5401	1.5441		5.6805	4.4902	3.7415
0.3090	1.5370	1.5464	1.5518		5.7155	4.5210	3.7582
0.3604	1.5438	1.5525	1.5589		5.7550	4.5551	3.7871
0.4119	1.5505	1.5593	1.5668		5.7908	4.6252	3.8708
0.4634	1.5573	1.5661	1.5751		5.8986	4.6979	3.8827
0.5149	1.5629	1.5731	1.5820		5.9611	4.7283	3.9009
0.5664	1.5695	1.5803	1.5864		6.0147	4.7675	3.9547
0.6179	1.5763	1.5875	1.5917		6.0790	4.8335	4.0000

Z increase with the increase in concentration of solute as well as with temperature [Table 5]. The behavior can be explained on the basis of lyophobic interaction between solute and solvent molecules, which increase the intermolecular detachment and becomes liable for the promulgation of ultrasonic waves [15]. The Viscous relaxation time (τ) shows the existence of molecular interaction by the addition of solute at a given temperature [Table 5].With the increasing concentration the dissimilar molecules approach closer due to Hydrogen bonding [16].

R_A is influenced by two factors (i) The breaking up of solvent structure on addition of solute to it; and (ii) the solvation of solutes that are simultaneously present. Decrease in R_A is due to former and latter results in increase of R_A. The increase of R_A with concentration [Table 6] suggests that solvation of solutes prevail over the breaking up of solvent structure. With an increase of temperature the decreasing values of R_A in the system reveal the breaking of solvent molecules due to temperature [17].

Table 6: Relative Association and Solvation number of L-Threonine+Urea (aq) at 298, 308 and 318K

Conc.(mol/Kg)	Relative Association(R_A)				Solvation number $H_n/S_n(x10^{-3})$		
	298K	308K	318K		298K	308K	318K
0.1030	1.0015	1.0003	0.9996		1.4741	1.8901	1.9895
0.1545	1.0046	1.0024	1.0015		1.3426	1.7961	1.8864
0.2060	1.0065	1.0040	1.0025		1.3138	1.7509	1.8331
0.2575	1.0085	1.0062	1.0038		1.3046	1.6001	1.7478
0.3090	1.0088	1.0064	1.0046		1.3045	1.5551	1.7129
0.3604	1.0101	1.0067	1.0056		1.3044	1.5141	1.6645
0.4119	1.0114	1.0077	1.0064		1.2962	1.4894	1.6502
0.4634	1.0127	1.0084	1.0070		1.2938	1.471	1.6474
0.5149	1.0135	1.0092	1.0071		1.2713	1.4566	1.6225
0.5664	1.0142	1.0098	1.0072		1.2709	1.4505	1.5554
0.6179	1.0153	1.0103	1.0073		1.2701	1.4465	1.5127

S_n for system [Table 6] explain the intensification of the solvation of solute by Urea-H_2O structure with the increase of temperature and the solvated amino acid thus devastating the structure of water and thus bringing about an increase in the B-value as evident[18,19].

CONCLUSIONS

The values and magnitude of B coefficient, ϕ_v° and S_v^*exhibit, (B coefficient supports the results interpreted by ϕ_v° and S_v^*) existence of solute-solute as well as solute-solvent interactions in the system, but, solute-solvent interactions dominate over the solute-solute interactions. The incursion of urea molecule as such, having an affinity for both polar and non polar solvation, into protein interior interfaces can be easily appreciated. There is substantial polarity present at such interfaces, even though they are assigned a primarily hydrophobic origin. Intensifying the temperature produces thermal agitation thus abating the continuous hydrogen bond network and strengthens the bond between urea and water molecules. Apparently urea can reform the six hydrogen bonds that existed between the water dimer and the neighboring water molecules, so that no significant network rearrangements are needed to solvate urea. The decrease of free solvent molecules due to increase in association of urea-water aggregates causes a decrease in the β values. The values of B-coefficient increases with the increase of temperature dB/dT is thus positive, hence the amino acid behaves as structure breaker.

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