ION-SOLVENT AND MOLECULAR INTERACTION OF L-ARGININE AND L-THREONINE IN AQUEOUS UREA SOLUTIONS

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ABSTRACT

Densities and viscosities of L-Arginine and L-Threonine in aqueous urea (0.01M) solutions have been determined experimentally at 298 and 308 K. The results obtained from density and viscosity measurement have been used to calculate A and B coefficients, apparent molar volume, $\Phi_v$, the free energy of activation per mole of the solvent $\Delta \mu_1^{0\#}$ and free energy of activation per mole of the solute $\Delta \mu_2^{0\#}$, limiting apparent molar volume $\Phi_v^0$ at infinite dilution and experimental slope $S_v$. The results are discussed in terms of the dehydration effect of the urea upon the amino acids and weak solute-solute and strong solute-solvent interactions. The properties of these amino acids in water and water+ urea solution systems are discussed in terms of the charge, and hydrogen bonding effect.

KEYWORDS: L-Arginine And L-Threonine Aqueousurea, Apparentmolar Volume and Jones-Dole coefficients

INTRODUCTION

In continuation of our earlier work [1] on the study of interaction between amino acids and electrolytes medium. We present in this paper, the study of molecular interaction between aqueous urea and L-Arginine and L-Threonine at 298 and 308K. The study of interaction between amino acids and electrolytes in aqueous medium, there has been an increased interest in the physicochemical properties of amino acids in aqueous as well as aqueous electrolyte media to understand the role played by the biological molecules in living organism [2-6]. Amino acids have zwitter-ion and are the constituents of the most important class of biopolymers, i.e. Proteins. Disarrangement water and electrolyte balance in living systems cause a wide variety of health problems. In physiological media such as blood, membranes, cellulose fluids etc., the dipolar character of amino acids (in presence of ions such as Na$^+$, K$^+$, Mg$^{2+}$ and Cl$^-$ etc., dissolved in body water) has an important bearing on their biological functions. Amino acids are the chemical units or "building block" of the body that make up the proteins. All naturally
occurring amino acids are L-amino acids which can be metabolized by our enzymes. L-Threonine an essential amino acid helps to maintain the proper protein balance in the body, is important the formation of collagen, elastin and tooth enamels; aids liver and lipotropic function when combined with aspartic acid and methionine; prevents the building of fat in the liver; assists metabolism and assimilation [7]. L-Arginine a non-essential amino acid is considered "The Natural Viagra" by increasing blood flow to the penis; retards the growth of tumors and cancer by enhancing the immune system; increases the size and activity of the thymus gland, which manufacture the T cells, crucial components of the immune system, reduces the effect of chronic alcohol toxicity; used in treating sterility in men by increasing sperm count. The handling of urea by the kidneys is a vital part of human metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the counter current exchange system of the nephrons that allows for reabsorption of water and critical ions from the excreted urine [8]. In recent years, a number of workers have utilized density and viscosity data to deduce the thermodynamic properties (relative viscosity, Jones -Dole coefficient and free energy of activation of viscous flow) for a number of mixtures solutions [7-9]. Structural interactions of non-ionic solutes with ionic ones in different solvents are important in many fields of chemistry and bio-chemistry. Very recently, we have made systematic effort to investigate the ultrasonic and volumetric properties of amino acids in concentrated glucose and mannose solution [10-12]. It was found that NaCl CaCl₂ and MgCl₂ increase the apparent molar volume and decrease the adiabatic compressibility of electrolytes solution. This increase could be attributed to the interactions of the ions of the NaCl, KCl CaCl₂ and MgCl₂ electrolytes and aqueous glucose and mannose, causing the transfer of hydrated water molecule to the bulk state.

In the present paper, we report densities, $\rho$ and viscosities $\eta$ of L – Arginine and L-Threonine (0.1-0.5 M) in aqueous urea (0.01M) solutions have been determined experimentally at 298 and 308K. From these experimental data a number of thermodynamic parameters namely, apparent molar volume and limiting apparent molar volume; Jones- Dole equation to calculate $A$ and $B$ coefficients $\Delta \mu_1^{0\#}$ the free energy of activation per mole of the solvent and $\Delta \mu_2^{0\#}$ is the free energy of activation per mole of the solute respectively have been calculated. These parameters were utilized to study various interactions taking place in the solutions of in aqueous L – Arginine and L- Threonine in aqueous ureasolutions at 298 and 308K.
2. Experimental

2.1 Chemical and Preparation

L-Arginine and L-Threonine (99.8% purity) and urea were procured from Merck and Sd Fine Ltd. They were used as such without further purification, after drying over calcium chloride in desiccators for more than 48 hours. The viscosities and densities of the amino acids in aqueous electrolytes solution at various concentrations as well as in double distilled de-ionized water were measured experimentally. Aqueous solutions of urea (0.01M) were prepared and these were used as solvent to prepare L-Arginine and L-Threonine solutions on mass basis covering the whole composition range. All the solutions were prepared by mass in dry box and were stored in special air-tight bottles and kept in dark to avoid photo chemical degradation. The weighing was done on an Afcoset ER-120A electronic balance with an accuracy ± 0.1 mg.

2.2 Measurement of density and viscosity

The densities were measured with a single capillary pycnometer (made of Borosil glass) of bulb capacity of $8 \times 10^{-6}$ m$^3$. The marks of the stems were calibrated using double distilled water at 298 and 308K. The pycnometer was kept for about 30 minutes in a thermostatic water bath so that the thermal fluctuation in density was minimized. The viscosity measurements were carried out by Ubbelohde type suspended level viscometer which was first calibrated with doubled distilled water. The viscometer was allowed to stand in an electronically controlled thermostatic water bath for 30 minutes to minimize the thermal fluctuation. The time of fall was recorded with a stop-watch of least count 0.1 s. At least three time recorded were obtained, and the average value was used as the experimental flow time. Poiseuille's equation was employed to calculate the viscosity of the amino acid + electrolyte + urea solutions.

$$\eta = \pi \rho h g r^4 t = \rho \beta t$$  
$$81V$$  

Here $\rho$ is the density of the amino acids solutions, $h$ the height of the column in the viscometer, $g$ is the acceleration due to gravity, $r$ is the radius of the capillary, $l$ the length capillary and $t$ is the time of fall of the solution of volume $V$. The term $h$, $g$, $r$, $l$ and $V$ are constant for a given viscometer therefore these have been replaced by single term $\beta$. The temperature of the water bath was maintained at 298 and 308K. The viscosity and density data were found to be accurate within ± 0.1 % and ± 0.01% respectively.
3. Results and discussion

The densities and viscosities of L–Arginine and L-Threonine (0.1-0.5 M) in aqueous urea (0.01M) solutions determined at 298 and 308K and are presented in Table 1& 2. It is observed from Table 1&2 that densities $\rho$ and viscosities $\eta$ for all the ternary systems increase with increase in molarities of L–Arginine and L-Threonine decrease with rise in temperature. The values of $\rho$ and $\eta$ increase with increase in concentration of amino acids in all the ternary systems under investigation, which appear to be due to hydrophobic properties of solutes i.e. H-bond forming. This may be attributed to the formation of clusters by the amino acids and strong intermolecular forces in the solute. The changes in structure of solvent or solution as a result of H-bond formation lead to decrease in intermolecular free length [13]. Solute may occupy the interstitial spaces in solvent or get solvated forming new weaker bonds. It was suggested [14-16] that what is experimentally observed for any system, reflects the compromise between the tendency for the ion and the peptide to interact with each other and inclination of the solutes to associate with the solvent.

The viscosity data were used to calculate the relative viscosity using Jones-Dole equation [17]

$$\eta_{rel} = \eta / \eta_o = [ 1 + AC^{1/2} + BC ]$$

Where, $\eta$ and $\eta_o$ viscosities of the solutions and solvent respectively. B, is the Jones-Dole coefficient [17], an empirical constant, and is measure of ion-solvent interaction. Its values depend on the size and shape of the solute particles. The A is the Falkenhagen coefficient [18] which indicates ion-pair electrostatic interactions. They were obtained by a least square treatment as the intercepts and slopes of the linear plots of $\eta / \eta_o$ vs $1/C^{1/2}$ and their values are given in Table 3.

The viscosity, $\eta$ of dilute solution of non-electrolytes is represented by

$$\eta = \eta_o[ 1 + BC ]$$

For a dilute solution of unsolvated spherical colloidal suspension has derived by Einstein relation

$$\eta_{rel} = 1 + 2.5\phi$$
Where $\phi$ is the volume fraction of the solute [19]. If this equation is valid for the amino acids, Eq (3) becomes

$$\eta_{rel} = 1 + 0.0025V_hC$$

(5)

where $V_h$ is the hydrodynamic volume. Since $AC^{1/2}$ term from Equation (2) can be assumed to be negligible in a dilute solution, the following relation holds

$$B = 0.0025V_h$$

(6)

Hakin et al. [20] may be assumed that the partial molar volume at infinite dilution of the unsolvated solute particle in the continuum solvent. The greater $B$ values in the mixed solvent might mean a more hydrodynamic volume in the mixed solvent.

A perusal of Table 3 shows that the values of $A$ coefficients and $B$ coefficients decrease with increase the temperature which indicates ion-ion and on-solvent interactions are weak with rise the temperature. The $A$ coefficients are negative and very less positive those of $B$ coefficients are large positive for all the ternary systems under investigation, thereby suggesting the solute-solute interactions are weak and ion-solvent interactions are strong. The positive value of $B$ with urea concentration of water molecules as a result of shielding of polar terminal groups of $L$ – Arginine and $L$-Threoninemolecules is due to increased interaction between these polar ends and aqueous urea. Iqbal et al [21] have estimated that the contraction of water around the appositively charged group is caused by electrostatic ion- solvent interaction and is called as electrostriction. A mutual comparison of these amino acids shows that values of $B$ are larger in case of $L$ – Arginine than $L$ – Threonine. The $L$ – Arginine has an intense force field and hence a strong hydration co sphere around it. Therefore hydration of $L$ – Arginine (ion-solvent) will be much more than that of $L$ – Threonine. The greater $NH_3^+$ ion results in intense electric field and thus, the possibility of interactions with $L$ – Arginine is larger in case of $L$ – Arginine with aqueous urea ternary system. The studies suggest that ion- solvent interactions are stronger and solute -solute interaction are weak .The extent of interactions and structure making ability is greater in case of $L$ – Arginine . The hydration behaviour of amino acids considered the following interactions (a) Y.Akhtar[4] as the terminal groups of zwitter-ions of amino nature which may hydrophobic, hydrophilic or amphiphilic, acids, $-NH_3^+$ and $COO^-$ are hydrated in electrostatic manner whereas hydration of intervening backbone depends on its, (b) the overlap of hydration groups co-spheres of terminal ($-NH_3^+$ and $COO^-$)and the adjacent groups result in volume change. (c) Electrostriction of $NH_3^+$ group are greater than the $COO^-$by a factor of 10.
Table 2 shows that the values of B are positive which indicates strong solute-solvent interactions. From the experimental and computed data for solutions of amino acids in aqueous urea can be generalized that when urea dissolved in water fits into the water structure without significantly ordering or disordering it. The large attractive energy makes urea resemble both structurally and energetically water like structure. The structures of amino acids further influence the extent of interactions and size of molecules can be related to larger B-coefficient values. Two view points for protein denaturation are mentioned in the literature (i) urea act on a protein indirectly by mutating the H- bond structure of water and thus disturbing the water mediated hydrophobic interactions.(ii) Urea cooperates with water in solvation of amino acid. Due to this weakening of hydrophobic interactions occur because urea is more effective than water in solvating hydrophobic residues as reported by Parmar et al [22].

The apparent molar volume, $\phi_v$ were calculated from measured density data of amino acids in aqueous urea solution at different concentration and at 298 and 308 K using the following equation:

$$\phi_v = \left[1000 \left(\frac{\rho_0 - \rho}{C \rho \rho_0}\right)\right] + \frac{M}{\rho} \quad (7)$$

Where M is the molecular mass of the solute, $\rho_0$ and $\rho$ are densities of solvent and solution. The calculated values of $\phi_v$ of these ternary systems are given in Table 1& 2. In these cases where molarity dependence of $\phi_v$, having definite trend points and increase with increase the temperature which indicates ion-solvent interactions are strong with rise the temperature The $\phi_v$ values increase due to reduction in the electrostriction effect at terminals in the ternary systems whereas it decreases due to disruption of side group hydration by that of the charged end with increase the temperature.

The partial molar volume at infinite dilution $\phi^0_v$ was calculated by taking an average data points. The linear variation is obtained by least square fitting to the following equation.

$$\Phi_v = \phi^0_v + S_v C^{1/2} \quad (8)$$

The intercept which is the limiting apparent molar volume at infinite dilution $\phi^0_v$ is the experimental slope, $S_v$ which is considered to be volumetric pair wise coefficient. The derived values $\phi^0_v$ of along with $S_v$ are summarized in Table 3. The values of $\phi^0_v$ are positive of these ternary systems which indicate ion-solvent interactions are strong. The values of $\phi^0_v$ are less in presence of L–Threonine than the L – Arginine. A mutual comparison of these amino acids shows the values of $\phi^0_v$ are larger in case of L – Arginine, has an intense force field and hence a
strong hydration co-sphere around it. Therefore hydration of L-Arginine (ion-solvent) will be much more than that of L-Threonine. The greater NH$_3^+$ ion results in intense electric field and thus, the possibility of interactions with L-Arginine is larger in case of L-Arginine with aqueous urea ternary system. The $S_v$ values of amino acids in aqueous urea solution are negative and very less positive which indicates solute-solute interactions are weak. The studies suggest that ion-solvent interactions are stronger and solute-solute interaction are weak. The extent of interactions and structure making ability is greater in case of L-Arginine than that of L-Threonine.

According to the transition state theory of the relative viscosities of electrolytic solutions proposed by Feakins et al [23], the $B$-coefficient given as

$$B = \frac{(\bar{V}_1^0 - \bar{V}_2^0)}{1000 + \bar{V}_1^0\left[ (\Delta \mu_2^{0\#} - \Delta \mu_1^{0\#}) / RT \right]}$$

(9)

Where $\bar{V}_1^0$ and $\bar{V}_2^0$ are the partial molar volumes of the solvent and solute at infinite dilution, respectively, $\Delta \mu_1^{0\#}$ the free energy of activation per mole of the solvent and $\Delta \mu_2^{0\#}$ is the free energy of activation per mole of the solute. The $\Delta \mu_1^{0\#}$ and $\Delta \mu_2^{0\#}$ were calculated from the equation

$$\Delta \mu_1^{0\#} = RT \ln \left( \frac{\eta^0 \bar{V}_1^0}{hN_A} \right)$$

(10)

$$\Delta \mu_2^{0\#} = \Delta \mu_1^{0\#} + \frac{RT}{\bar{V}_1^0} \left[ 1000B - (\bar{V}_1^0 - \bar{V}_2^0)\right]$$

(11)

Where $R$, $h$ and $N$ are the gas constant, Planck’s constant and Avogadro’s number respectively and $T$ is the absolute temperature. The values of $\Delta \mu_1^{0\#}$ and $\Delta \mu_2^{0\#}$ of different compositions of L-Arginine and L-Threonine in aqueous urea are given in Table 3. The free energies of activation increases with increase the concentration of amino acids at all temperatures and decrease with temperature of L-Arginine + aqueous urea but increase with temperature for L-Threonine in aqueous urea solution. Increase in values with concentration might be due to closer approach of unlike molecules with formation of H-bonding also indicating the need for less time for rearrangement of molecules in solutions [24]. Table 3 shows that $\Delta \mu_2^{0\#}$ are larger than $\Delta \mu_1^{0\#}$ suggesting that the formation of transition state is accompanied by the breaking and distortion of the intermolecular bonds. Moreover, the greater values of $\Delta \mu_2^{0\#}$ than $\Delta \mu_1^{0\#}$ suggest that the aqueous urea under study, behave as structure makers/promoters in different concentration ranges of L-Arginine and L-Threonine. Greater values of $\Delta \mu_2^{0\#}$ have also been reported in mixtures of Ni, Cu, Co and Zn chlorides in aqueous glycine [24].
A comparison of $\Delta \mu_1^{0#}$ and $\Delta \mu_2^{0#}$ values of the two solutes result the structure making ability of L – Arginine is greater than L– Threonine which may be due to stronger solute-solvents interaction in L-Argenine solutions. Therefore the hydration of L-Argenine will is much more than that of L– Threonine. Thus the trends and magnitude of the various parameters obtained from viscosity measurement reported in this paper. The studies suggest that ion- solvent interactions are stronger and ion-ion interactions are weak. The extent of interactions and structure making ability is greater in case of L – Arginine. The dB/dT is a better criterion for determining the structure making/ breaking nature of any electrolyte rather than simply the B-coefficient.

**Conclusion**

In the present work the molecular and ion-solvent interaction of L-Argenine+ aqueous urea and L– Threonine + aqueous urea solution at 308K. We conclude from the values of B-coefficient and $\Phi_v$ that L-Argenine+ aqueous urea possess strong ion – solvent interaction than L– Threonine + aqueous urea and weak ion-ion interactions.

**Acknowledgement**

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**Table 1**

Densities ($\rho$) and viscosities ($\eta$) and apparent molar volume ($\Phi_v$) of L – Arginine and L-Threoninein aqueous urea solutions at 298K

<table>
<thead>
<tr>
<th>C (mol.l$^{-1}$)</th>
<th>$\rho$(kg m$^{-3}$)</th>
<th>$\eta$ ($\times 10^{-3}$Nm$^{-2}$s)</th>
<th>$\Phi_v$ ($\times 10^{-5}$m$^3$mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine + aqueous + (0.01M)urea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>996.9</td>
<td>0.8799</td>
<td>00.00</td>
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<td>0.10</td>
<td>1002.2</td>
<td>0.8906</td>
<td>11.73</td>
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<td>0.20</td>
<td>1006.4</td>
<td>0.9443</td>
<td>12.32</td>
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<tr>
<td>0.30</td>
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<td>1.0071</td>
<td>12.33</td>
</tr>
<tr>
<td>0.40</td>
<td>1015.0</td>
<td>1.0757</td>
<td>12.55</td>
</tr>
</tbody>
</table>

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### Table 2

Densities ($\rho$) and viscosities ($\eta$) and apparent molar volume ($\phi_v$) of L - Arginine and L-Threonine in aqueous urea solutions at 308K

<table>
<thead>
<tr>
<th>C (mol.l⁻¹)</th>
<th>$\rho$ (kg m⁻³)</th>
<th>$\eta$ (×10⁻³ Nm⁻²s)</th>
<th>$\phi_v$ (×10⁻⁵ m³mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine + aqueous + (0.01M) urea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>994.3</td>
<td>0.7150</td>
<td>00.00</td>
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<td>1013.0</td>
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</tr>
<tr>
<td>0.50</td>
<td>1017.5</td>
<td>0.9275</td>
<td>12.66</td>
</tr>
<tr>
<td>L-Threonine + aqueous + (0.01M) urea</td>
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<td></td>
</tr>
<tr>
<td>0.00</td>
<td>994.3</td>
<td>0.7150</td>
<td>00.00</td>
</tr>
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Table 3

Falkenhagen coefficient, (A), Jones Dole coefficient, (B), Limiting apparent molar volume \( \phi_{0v} \) and experimental slope \( S_v \), \( \Delta \mu_1^{0\#} \) and \( \Delta \mu_2^{0\#} \) of L – Arginine and L- Threonine in aqueous urea solutions at 298 and 308K

<table>
<thead>
<tr>
<th></th>
<th>A (dm(^{3/2})mol(^{-1/2}))</th>
<th>B (dm(^{3})mol(^{-1}))</th>
<th>( \phi_{0v} )</th>
<th>( S_v )</th>
<th>( \Delta \mu_1^{0#} ) (KJmol(^{-1}))</th>
<th>( \Delta \mu_2^{0#} ) (KJmol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
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<td>9.12</td>
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<tr>
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<td>128.22</td>
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<td>L-Threonine + aqueous + (0.01M)urea</td>
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</tr>
<tr>
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<td>-0.70</td>
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REFERENCES: