Thermophysical Properties and Hydration Behavior of L-Proline in the Presence of Mono-, Bis-, and Tris-(2-Hydroxyethyl) Ammonium Acetate Protic Ionic Liquids

MohammadAmin Morsali[1*]- Hemayat Shekaari[2]- masomeh mokhtarpor[3]

[1*] Department of Physical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran. ∗email: mohammadamin.morsali20@gmail.com

[2] Department of Physical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran. email: hemayatt@yahoo.com

[3] Department of Physical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran. email: masomeh64_m@yahoo.com

ABSTRACT

The hydration behavior of amino acids, which is vital for understanding the solvation of biological macromolecules, is significantly influenced by ammonium-based biomaterials. Protic ionic liquids (PILs) have garnered considerable attention in the food and pharmaceutical industries due to their non-toxic nature and tunable physicochemical properties. Consequently, investigating the hydration behavior of amino acids such as L-proline in the presence of PILs is essential for advancing our understanding of these interactions. In this study, the impact of PILs including mono-, bis-, and tris-(2-hydroxyethyl)ammonium acetate, which may occur naturally in the human body on the hydration behavior of L-proline was examined using COSMO-based calculations and thermophysical measurements. Experimental data, including density, speed of sound, viscosity, and refractive index, were collected for ternary solutions of L-proline, PILs, and water at various PIL concentrations across the temperature range of 298.15–318.15 K under atmospheric pressure. The results indicate that L-proline exhibits weaker interactions with water molecules compared to PILs ([2-HEA][Ac], [bis-2-HEA][Ac], and [tris-2-HEA][Ac]), primarily due to its compact structure and lower negative dielectric energy. In contrast, PILs form stronger hydrogen bonds with water molecules, leading to enhanced interactions. The hydration layer around L-proline is disrupted with increasing temperature, resulting in the release of more water molecules compared to PIL solutions. This effect is most pronounced for [tris-2-HEA][Ac], likely due to its larger molecular size and more intricate structure. While L-proline facilitates the formation of a more ordered water structure, PILs may interfere with this ordering by reorganizing water molecules and establishing their own hydrogen bonding networks.

Keywords: *Hydration behavior, L-proline, Protic ionic liquids, Volumetric properties, Viscosity B-coefficient, COSMO.*

1. *INTRODUCTION*

Water plays a critical role in stabilizing and regulating the behavior of biopolymers such as proteins and DNA through hydrogen bonding (H-bonding)[1].This interaction governs processes like solvation, protein folding, and DNA structure. Vibrational spectroscopy techniques, including infrared and Raman spectroscopy, are widely used to analyze H-bonding in simple and complex molecules[2,3]. L-proline, a model amino acid, has been extensively studied for its thermodynamic and solubility behavior in aqueous and electrolyte solutions. These investigations revealed that temperature and concentration significantly affect its solubility and interactions[4]. Dielectric permittivity measurements demonstrated stronger H-bonding and self-association in ethanol compared to water. Proline's cis/trans isomerization influences the phase behavior of elastin-like polypeptides without altering transition temperatures, highlighting its role in conformational stability[5,6]. Protic ionic liquids (PILs) have emerged as versatile solvents with unique properties derived *from their non-covalent interactions, particularly H-bonding. While the strength and directionality of H-bonds in PILs have been extensively explored, limited knowledge exists on their interactions with water[7]. This gap is significant as PIL-water systems are increasingly used in biomolecule stabilization and recrystallization. Carboxylate anions in PILs are particularly important due to their relevance in biomolecules and enzyme active sites[8,9]. Studying amino acids in PIL-water systems provides a simpler model to understand the interactions governing more complex biomolecular systems[10]. Thermophysical and thermodynamic studies of amino acids in PILs offer insights into solute-solvent and solute-solute interactions. These findings are essential for understanding the protective effects of PILs against amino acid denaturation and for designing biotechnological applications. Spectroscopic and computational tools remain indispensable in revealing the hydration properties and structural roles of H-bonds in these systems[11,12].*

In this work, the interactions between the L-proline and PILs (2-hydroxyethylammonium acetate, Bis-(2-hydroxyethylammonium acetate), Tris-(2-hydroxyethylammonium acetate) in aqueous media have been investigated. The density, speed of sound, viscosity and refractive index of aqueous L-proline in the presence of different concentration of the PILs were measured at (288.15 k to 318.15) K and atmospheric pressure. The measured data were used to compute partial molar volume ($V0\varphi$) partial molar isentropic compressibility ($\kappa 0\varphi$) viscosity Ɓ-coefficient and molar refraction RM. Also, the COSMO calculation has been carried out to understand the bonding and interactions between the L- proline and the ionic liquids in the term of σ-profiles and COSMO results.

1.1 Materials and methods

1.1.1.Materials:

All reagents including L-proline, ethanolamine, di-ethanolamine, triethanolamine, and acetic acid have been purchased from Merck, and used without further purification. Also, deionized ultrapure water with a specific conductance below $1 \mu S \cdot cm^{-1}$ was used to prepare the corresponding aqueous solutions of amino acid in the presence of ionic liquids.

1.1.2.Apparatus and procedure

the studied systems was conducted with a Shimadzu AW-220 analytical balance, featuring an uncertainty of $\pm 2 \times 10^{-4}$ g. Measurements of density and speed of sound were carried out using a digital vibrating U-shaped densitometer (Anton Paar DSA 5000) with resolutions of $\pm 1 \times 10^{-6}$ g cm^{-3} and 0.01 m s^{-1}, respectively. The uncertainties associated with these measurements were 4×10^{-5} g cm^{-3} for density and 0.7 m s^{-1} for speed of sound. Calibration of the instrument was performed using air and distilled water, and the speed of sound was measured at a frequency of 3 MHz.

Viscosity measurements were conducted using a digital rolling-ball viscometer (Lovis 2000 M), which was calibrated at room temperature (298.15 K) with doubly distilled water. Refractive index measurements were obtained using a digital Abbe refractometer (ATAGO), with an accuracy of ±0.0002 units. The refractometer was calibrated twice with doubly distilled water to ensure precision in refractive index determinations.

Density functional theory (DFT) calculations for the geometry optimization of the protic ionic liquids (PILs) were performed using $Dmol^3$ with the generalized gradient approximation (Vosko-Wilk-Nusair-BP functional). The BP functional was employed to replace the local correlation in the VWN functional. COSMO calculations were performed in two steps: geometry optimization and energy optimization, to obtain reliable solvation parameters.

1.2. Results and discussion

1.2.1..Sigma profile

The sigma-profile, a key concept in COSMO-based thermodynamics, represents the surface charge distribution of a molecule, serving as a "fingerprint" for predicting thermodynamic properties and molecular interactions. Sigma-profiles, obtained through computationally intensive density functional theory (DFT) calculations, are essential for COSMO models like COSMO-RS and COSMO-SAC. To enhance efficiency, software tools provide approximations for high-throughput applications[13]. In this study, COSMO results were derived using the $Dmol^3$ module of Materials Studio 2023, employing the GGA VWN-BP functional for geometry optimization and electronic property calculations. The VWN-BP functional integrates exchange and correlation effects, offering enhanced accuracy by considering electron density and its gradient, surpassing simpler local density approximation (LDA) methods. Water was chosen as the solvent for all analyses. Results *indicate that L-proline exhibits the smallest cavity volume and surface area, reflecting its compact structure, and has the least negative dielectric energy, indicating weaker solvent interactions. Conversely, the protic ionic liquids ([2-HEA][Ac], [bis-2-HEA][Ac], and [tris-2-HEA][Ac]) display progressively larger cavity volumes and surface areas, suggesting more extensive interactions with the solvent. These PILs also have more negative dielectric energies, signifying stronger solvation interactions. HOMO energies for all molecules are negative, while LUMO energies vary, underscoring differences in electronic properties among the compounds.*

1.2.2. Volumetric properties

The density of the ternary aqueous solution of the L-proline in the presence of [2-HEA][Ac], [bis-2-HEA][Ac], and [tris-2-HEA][Ac] has measured under atmospheric pressure (P = 0.086 MPa) and temperature range of (288.15− 318.15) K.

These data illustrate, that the density of the solutions increases with addition of the amino acid and PILs and decreases as temperature rising. apparent molar volumes of These solutions can be calculated by equation 1 [14]:

$$V_{\varphi} = \frac{M}{d} - \left[\frac{(d - d_0)}{mdd_0} \right] \qquad (1)$$

Where, M represents the molar mass of amino acids (kg·mol^{-1}), d and d0 are density of solution and pure solvent (kg·m^{-3}), respectively and m is molality of experimental solution mol·kg^{-1}.

The calculated apparent molar properties have been correlated using Redlich-Mayer model as given by Equation 2 [15]:

$$V_{\varphi} = V_{\varphi}^{0} + S_v m \qquad (2)$$

The structure making/breaking property of the solute (L-proline) in aqueous of (water + PILs) may be determined by helper s constant which is obtained by Equation 3[16]:

$$\left(\frac{\partial C_P}{\partial P} \right)_T = -T \left(\frac{\partial^2 V_{\varphi}^{0}}{\partial T^2} \right)_P = -2CT \qquad (3)$$

1.2.3. Ultrasonic and compressibility properties of the bulk

The Newton-Laplace formula has been used to calculate isentropic compressibility (Equation 4) [17]:

$$Ks = \frac{1}{du2} \qquad (4)$$

Where, the symbols, u, κs, κs0 represents the speed of sound, isentropic compressibility of solution, and isentropic compressibility of solvent, respectively.

1.2.4. Kinematic and dynamic viscosity

In order to use protic ionic liquids in industry viscosity information is required. Viscosity is an important dynamic property of a fluid that affect other properties such as conductivity and resistance of flow[18,19]. Accordingly, studying about this parameter is essential for fluid application in the industrial scale. Viscosity gives us useful information about ionic hydration and ion-solvent interaction by the changing of temperature or concentration of aqueous solutions. Dynamics viscosity and kinematic viscosity of (L-proline in (water + PIL) solution at (288.15 to 318.15) K[20,21].

Dynamic viscosity data has been fitted to the jones- dole equation (Equation 5) [22]:

$$\eta_r = \frac{\eta}{\eta_0} = 1 + A\sqrt{m} + Bm \qquad (5)$$

where, η is dynamic viscosity of solution and η0 is viscosity of solvent. The parameter A implies the long range columbic forces represents solute-solute interactions which is called is Falkenhagen coefficient and B is called B coefficient which shows solute-solvent interaction.

1.2.5. Refractive index and molar refraction

The refractive index of ternary solutions (L-proline + PILs + water) were measured in the temperature range of (288.15-318.15) K. The tendency of the temperature dependences of refractive indexes is closely *related to that of the density of these systems. The Lorentz–Lorenz was used to calculate the molar refraction (Equation 6) [23]:*

$$R_M = \frac{n_D^2 - 1}{n_D^2 + 2} \cdot \frac{x_1 \cdot M_1 + x_2 \cdot M_2}{\rho} \qquad (6)$$

Where, xi and M_1 and M_2 are the mole fraction and molecular weight of each component of the mixture and d is the density of the solution.

1.3. Conclusion

The findings indicate that L-proline exhibits weaker interactions with water molecules compared to the surrounding protic ionic liquids (PILs) [2-HEA][Ac], [bis-2-HEA][Ac], and [tris-2-HEA][Ac] due to its compact structure and less negative dielectric energy. In contrast, these PILs, characterized by their larger size and more intricate structures, demonstrate stronger interactions with water, primarily mediated through hydrogen bonding. With increasing temperature, the hydration layer surrounding L-proline is significantly altered, resulting in the release of more water molecules relative to the PIL solutions. This effect is most pronounced for [tris-2-HEA][Ac], likely due to its larger molecular size and more complex structure, which enable it to compete more effectively with L-proline for hydrogen bonding with water molecules. Furthermore, the positive helper constant observed suggests that L-proline promotes the formation of a more ordered structure in the surrounding water molecules. However, the PILs may disrupt the hydrogen bonding between L-proline and water by reorganizing the water molecules around L-proline and establishing their own hydrogen bonds with the water.

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