

Acoustical and Thermodynamical Studies of L- Glycine in Buffer Solution of Different pH at 298.15 K

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Abstract

The volumetric, viscometric, and acoustical studies are used to investigate the exchange of L-Glycine in a buffer solvent system of different pH at 298.15K within a concentration range of 0.02 to 0.10 molal. L-glycine, being the simplest amino acid, serves as an important model compound for understanding molecular exchanges in biological systems. The effect of pH on the physicochemical properties of amino acid solutions is of considerable significance, as it governs the ionic form of the solute and thereby influences solute-solvent as well as solute-solute interactions, hydration behavior, and the structural organization of the solution. Acoustic impedance (Z), adiabatic compressibility (β), and hydration number (n_H) have been evaluated from density (ρ) & ultrasonic velocity (U) data. The limiting apparent molal volume (Φ_{0V}) and slope (S_V) are evaluated from the apparent molal volume (Φ_V). Similarly, the viscosity data (η) have been used to investigate relative viscosity (η_r), viscosity coefficients B & A . The variation of these parameters with molality and pH provides useful insight into the nature and strength of molecular exchanges. These findings contribute to a deeper understanding of amino acid behavior in buffered environments and provide useful information for pharmaceutical, biochemical, and industrial applications where pH-dependent molecular interactions are important. The results are discussed in terms of solute-solute and solute-solvent exchanges, as well as the structure-strengthening or structure-weakening effect of L-Glycine in the given solvent systems.

Keywords: L- glycine. apparent molal volume(Φ_V). density(ρ). ph. Viscosity B -coefficients. etc.

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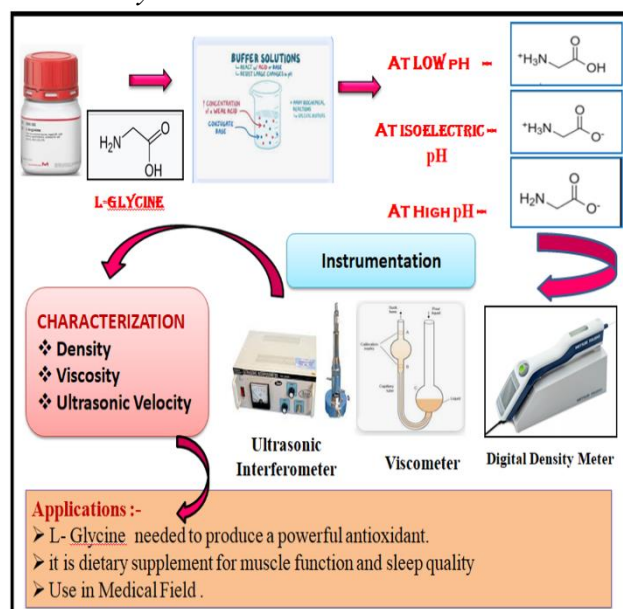
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INTRODUCTION

In various natural processes, volume changes & solvation of molecules take place. Its complete

understanding gives correct information about the state and nature of molecules in the medium [1]. To study the mechanism of molecular exchanges, the amino acids, which are the essential building blocks of proteins, are fairly suitable and helpful to investigate these molecular exchanges [2]. The acoustical & thermodynamical properties of amino acids in a solvent system at different pH levels can provide important data about the protein's stability [3]. Is this supportive in developing the progress of medicine & human science? Water, which is the most important component of bio system, is used for the preparation of buffer solutions of different pH because its presence leads to hydrophilic & hydrophobic forces [4].

In aqueous solutions, L-Glycine exists mostly as a zwitter-ions ($\text{H}_3\text{N}^+-\text{CH}_2-\text{COO}^-$), a polar molecule with +ve and -ve charge, making it greatly soluble in water [5]. L-Glycine is very important for protein and collagen synthesis, supports detoxification, acts as a neurotransmitter and antioxidant defense, and has wide applications in medicine, food, cosmetics, and biotechnology [6]. These studies are related to understanding the performance of L-Glycine in biological systems and in the production of new materials and drugs [7]. The pH of the surrounding environment plays an important role in finding the charge state and therefore the structure of L- Glycine as shown in figure 1 [8]. This ionization behavior is critical for understanding the role of amino acids in biological systems, including protein structure, enzyme function, and various metabolic processes [9].

During the last few years, ultrasonic velocity (U) measurements have gained much significance in assessing the nature of molecular exchanges and investigating the physicochemical behavior [10]. Acoustical and thermodynamic parameters calculated from these measurements are tremendously useful to learn the molecular exchanges in given liquid systems. In recent years, there has been substantial growth in the determination of thermodynamic and acoustic properties of liquid systems from viscosity (η) & density (ρ) measurements [11]. The measurement of viscosity (η), density (ρ), and sound velocity (U) is a useful tool to gain insight into solute-solute and solute-solvent interactions [12]. The investigation of these characteristics of amino acids in liquid medium has been a part of the curiosity of researchers. From the above observation, we have decided to organize the study of density (ρ), sound velocity (U), and viscosity (η) of L-Glycine of different compositions in buffer solution of different pH at 298.15 K.

EXPERIMENTAL

Chemicals

The L-Glycine of A.R. Grade was used from Merck. It is used without further purification. The purity of chemicals has been provided in Table 1. The stock solution of different pH has been prepared by using double distilled water which is used as solvent to make varied molal concentrations of given amino acids.

Methods

Densities (ρ) of L-Glycine solution of different molal composition, at 298.15K, at varied pH were measured concurrently and using a digital density meter [Mettler Toledo] with accuracy $\pm 0.001 \text{ g/cm}^3$. Viscosity (η) measurements were carried out by Ostwald's Viscometer. It was calibrated with double-distilled water. The speed of sound (U) was measured by an ultrasonic interferometer (Mittal Type: M-83 Model) operated at a frequency of 2 MHz with accuracy $\pm 0.1 \text{ m/s}$. The temperature of the system was maintained by using a thermostat. A buffer solution of expected pH was prepared by using CH_3COOH and CH_3COONa , and It is used as a stock solvent. Investigational solution from 0.02 m to 0.10 m for L-Glycine was prepared by using stock solvent. L-Glycine is a primary standard. 0.1065 $\approx$ 0.10 molal stock solution of L-Glycine was prepared by dissolving an appropriate amount in buffer solution of low pH i.e, below Ip. (pH=5.0). From this stock solution, lower concentrations such as 0.10, 0.08, 0.06, 0.04, and 0.02 molal were prepared by the dilution method. Similarly, stock solution of L-Glycine was prepared using buffer solution of Isoelectric point (pH $\approx$ 6.08) and buffer solution of high

pH i.e., above Ip. (pH $\approx$ 6.5) Further dilutions were carried out to obtain solutions of concentrations 0.10, 0.08, 0.06, 0.04 and 0.02 molal.

Amino Acid	a) At low pH	b) At isoelectric pH	c) At High pH
L- Glycine	$\text{H}_3\text{N}^+-\text{CH}_2-\text{COOH}$	$\text{H}_3\text{N}^+-\text{CH}_2-\text{COO}^-$	$\text{H}_2\text{N}-\text{CH}_2-\text{COO}^-$

Figure 1. Structure of L-glycine (a) at low pH (b) at isoelectric pH (c) at high pH.

Table 1. Chemicals used with Source, CAS No., M. wt./kg·mol⁻¹ and Mass fraction purity.

Chemical name	Source	CAS No	M·wt.(g/mol)	Mass fraction purity
L-Glycine (C ₂ H ₅ NO ₂)	Merck	56-40-6	75.07	0.990 or 99%

Theory and Calculation

Various physical parameters were calculated from the observed density (ρ), ultrasonic velocity (U) and viscosity (η) data.

Apparent Molal Volume (Φ_v):

$$\Phi_v = \frac{M}{\rho} + \frac{1000(\rho_0 - \rho)}{m \cdot \rho_0 \cdot \rho} \quad (1)$$

Where Φ_v , m , ρ , ρ_0 and M are the apparent molal volume, molality, density of the solution, density of solvent (i.e., Buffer) and molar mass of solute respectively.

If Graph is plotted among apparent molal volume & Sq. root of molality, then the following relation is derived

$$\Phi_v = \Phi_v^0 + S_v \sqrt{m} \quad (2)$$

Where, Φ_v^0 is the Partial molar volume and S_v is slope.

$$\text{Adiabatic compressibility, } (\beta) = 1/U^2 \rho \quad (3)$$

Where, U – Sound velocity, ρ – Density of solution

$$\text{Acoustic impedance } (z) = \rho \cdot U \quad (4)$$

$$\text{Relative Viscosity } (\eta_r) = \eta / \eta_0 \quad (5)$$

Viscosity data of L-Glycine solutions have been analyzed using the following equation.

$$\frac{\eta}{\eta_0} = 1 + A + B\sqrt{m} \quad (6)$$

Where, η_0 – viscosity of solvent, η - viscosity of solution, m is molality of solution.

RESULTS AND DISCUSSION

The density (ρ), viscosity (η), ultrasonic velocity (U), adiabatic compressibility (β_a), acoustic impedance (Z), and hydration number (n_H) of L-glycine solutions were determined at different molal concentrations under four pH conditions, namely aqueous medium, low pH (below the isoelectric point), isoelectric pH, and high pH (above the isoelectric point) at 298.15 K. The experimental values are presented in Table 2. The variations in these acoustical and thermodynamic parameters with concentration and pH provide valuable information regarding solute–solvent interactions, hydration behavior, and structural modifications occurring in the solution.

Ultrasonic Velocity

From Fig.2a, it is observed that the ultrasonic velocity is found to increase slowly with gain in molality of L-Glycine in all four systems in aqueous medium, at low pH (below Ip.), at isoelectric pH, and at high pH (above Ip.)). The rise in ultrasonic velocity in any solution suggests the greater stiffness of the medium [13]. At low pH i.e, pH=5.0 (acidic medium), L-Glycine exists mainly as a cationic form ($-\text{NH}_3^+-\text{CH}_2-\text{COOH}$). So strong electrostatic interactions occur between solute and solvent (Water / Buffer solution). This increases the solvent structure-weakening and enhancing solute-solvent structure. At isoelectric pH (pH = 6.08) L-Glycine exists in its zwitterionic form ($-\text{NH}_3^+-\text{CH}_2-\text{COO}^-$). Strong dipole–dipole and ion-solvent interactions occur [14]. The hydration shell becomes more structured,

lowering compressibility further. This leads to higher ultrasonic velocity. Both at low pH and at isoelectric pH, L-Glycine increases the ordering of surrounding water molecules and thus ultrasonic velocity increases [15]. At high pH i.e, pH = 6.5 (basic medium), L-Glycine exists mainly as the anionic form ($-\text{NH}_2\text{--CH}_2\text{--COO}^-$). In this state, the $-\text{COO}^-$ group dominates, producing strong ion-dipole interactions with water / solvent. These interactions bind water molecules tightly, leading to a greater structure-promoting effect of the solvent. Reduced adiabatic compressibility leads to a rise in velocity. At all three conditions (low, isoelectric, and high pH), L-Glycine interact strongly with water, reduce compressibility and hence ultrasonic velocity increases compared to pure solvent [16]. As pH increases, the L-Glycine molecule passes from cationic – zwitterionic anionic form. In all these states, ion–solvent and dipolar interactions strengthen water structure. This lowers compressibility, and therefore, ultrasonic velocity increases with pH.

Table 2. Density (ρ), viscosity (η), sound velocity (U), adiabatic compressibility (β_a), acoustic impedance (Z) and hydration number (n_H) values for L-Glycine solutions of different pH at 298.15K.

pH	M (Mol Kg ⁻¹)	Density, ρ ($\times 10^3$ kg m ⁻³)	η ($\times 10^{-3}$ N s m ⁻²)	U (m s ⁻¹) ¹	$\beta_a \times 10^{-7}$ Kg m ² S ¹	Z ($\times 10^6$ Pa s m ⁻³)	n_H
Aqueous pH	0.00	0.997	0.89	1496.91	4.48	1.492419	-
	0.02	0.9977	0.894111	1499.1	4.46	1.495652	10.0533
	0.04	0.9983	0.897847	1501.33	4.44	1.498778	9.9639
	0.06	0.99897	0.901878	1503.3	4.43	1.501722	9.64697
	0.08	0.9996	0.906149	1505.66	4.41	1.505013	9.812748
	0.10	1.0002	0.910043	1507.66	4.4	1.507962	9.646434
At low pH pH=5.00 (Below pI)	0.00	1.0021	0.909438	1509	4.38	1.5121689	-
	0.02	1.00295	0.915029	1512	4.36	1.5164604	13.35684
	0.04	1.0036	0.920445	1514	4.35	1.5194504	11.22067
	0.06	1.0041	0.925729	1515	4.34	1.5212115	9.149262
	0.08	1.0047	0.930964	1517	4.33	1.5241299	9.083297
	0.10	1.0053	0.936351	1519	4.31	1.5270507	9.035882
At Isoelectric pH pH=6.08	0.00	1.0011	1.017285	1567	4.07	1.5687237	-
	0.02	1.0015	1.023234	1567.6	4.06	1.5699514	3.343928
	0.04	1.002	1.02929	1568.2	4.06	1.5713364	3.484297
	0.06	1.0027	1.035557	1568.8	4.05	1.57303576	3.719269
	0.08	1.0033	1.041875	1569.4	4.05	1.57457902	3.763493
	0.10	1.004	1.048743	1570	4.04	1.576280	3.845422
At High pH pH=6.5 (Above pI)	0.00	1.0368	1.208019	1586	3.83	1.6443648	-
	0.02	1.03705	1.2142	1589.33	3.82	1.648214677	14.28652
	0.04	1.0374	1.220652	1590	3.81	1.649466	9.038721
	0.06	1.0379	1.227135	1591.5	3.8	1.65181785	8.556087
	0.08	1.0384	1.233926	1592.5	3.8	1.653652	7.8071
	0.10	1.0389	1.240572	1593.5	3.79	1.65548715	7.355126

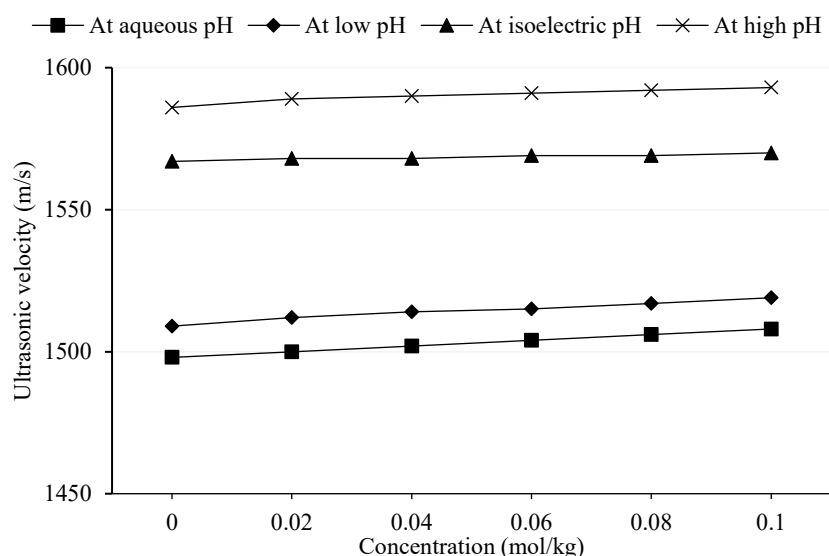


Figure 2a. Concentration vs ultrasonic velocity.

Adiabatic Compressibility

Adiabatic compressibility is an important acoustical parameter used to study the elastic properties and molecular interactions in liquid systems. From Fig.2b, it is observed that the adiabatic compressibility diminishes as the molality of L-Glycine in water or buffer systems of different pH increases. This indicates an enhancement in solute-solvent exchanges [17]. The gain in velocity and reduction in adiabatic compressibility with boost in molality indicates that the molecules are forming a more tightly bonded system. The close packing of molecules is caused by the H-bonds between the solute and solvent molecules [18]. The adiabatic compressibility (β_a) of L-Glycine reduces with gain in pH. At low pH i.e, in acidic medium, L-Glycine molecules are strongly hydrated due to electrostatic interactions, but the structure is less rigid, allowing higher compressibility [19]. At the isoelectric point ($pI \approx 6.0$), There is strong electrostatic interactions between $-NH_3^+$ and $-COO^-$ groups and surrounding water molecules increase hydration shells and reducing free space, hence compressibility diminishes. [20]. At high pH i.e, In alkaline medium, the strong hydration around the carboxylate ion decreases free volume further, which leads to lower compressibility [21]. Adiabatic compressibility of L-Glycine reduces as pH increases [acidic (pH=5.0) - neutral (pH=6.08) - basic (pH= 6.5)]. This decrease is due to increased electrostriction of water molecules and stronger solute-solvent exchanges, which make the solution less compressible.

Acoustic Impedance

Acoustic impedance is an important acoustical parameter used to study molecular interactions in liquid systems. It is defined as the product of density (ρ) and ultrasonic velocity (U) of the medium. In the case of amino acid solutions, acoustic impedance is strongly influenced by concentration, temperature, and pH. Acoustic impedance (Z) describes how much resistance a medium offers to the transmission of ultrasonic waves. It indicates the opposition that a medium provides to the passage of an acoustic wave [22]. Acoustic impedance $Z = \rho \times U$, where: ρ = Density; U = Ultrasonic velocity. From the above formula, it is clear that if density and ultrasonic velocity increase, acoustic impedance also increases. From the graph (Fig.2c), it is observed that when pH increases ionization state of amino acids changes. Ultrasonic velocity (U) usually increases with pH. This is because at higher pH, stronger ionic interactions and structured hydration shells reduce compressibility and increase sound speed [23]. Density (ρ) changes slightly, but velocity contributes more significantly. So, the net effect is an increase in acoustic impedance.

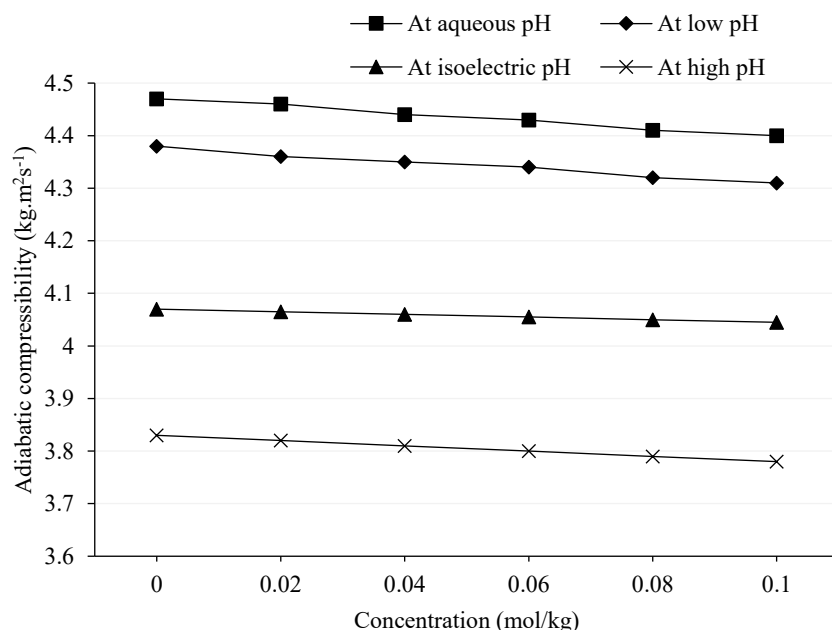


Figure 2b. Concentration vs adiabatic compressibility.

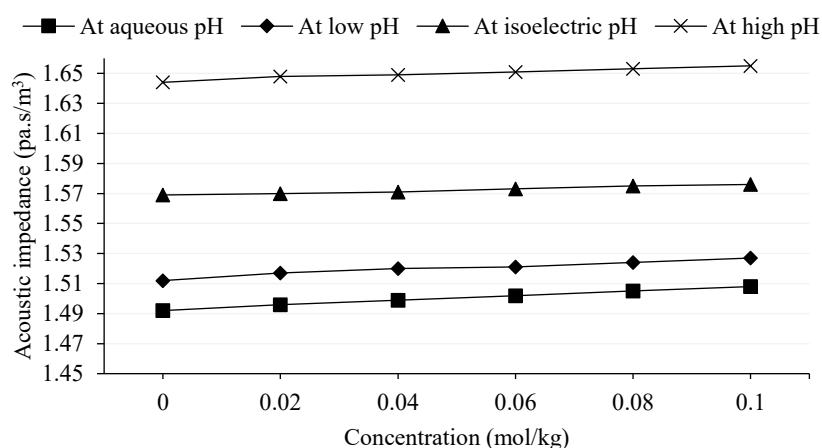


Figure 2c. Concentration vs acoustic impedance.

Hydration Number

Hydration number is an important physicochemical parameter that represents the number of solvent molecules, usually water, closely associated with a solute particle in solution. It provides valuable information about solute–solvent interactions, hydration behavior, and the structural arrangement of molecules in the liquid medium. The hydration number of L- Glycine in aqueous medium generally decreases with increasing molality of L- Glycine in fig 2 (d). At low concentration, L-Glycine molecules are well separated, and each ionized group interacts strongly with surrounding water molecules, hence a higher hydration number [24]. As molality increases, molecules come closer; ion-ion interactions reduce the free water molecules available for hydration. Overlapping hydration shells lead to a net decrease in hydration number [25]. At the isoelectric point (pI), L-Glycine exists primarily as a zwitterion. Both positive ($-\text{NH}_3^+$) and negative ($-\text{COO}^-$) groups are present. This dual charge enhances the electrostatic interaction with water molecules, leading to maximum hydration. Therefore, the hydration number is highest at pI [26]. At low pH (acidic), L- Glycine becomes fully protonated, and only a positive charge remains, reducing ionic interaction with water. Hence, the hydration number decreases. At high pH (basic), L-Glycine becomes fully deprotonated. Only a negative charge remains, again reducing hydration. Thus, the hydration number decreases.

Apparent Molal Volume

Apparent molal volume represents the effective contribution of one mole of solute to the total volume of the solution and provides valuable information about the structural changes occurring in the solvent system due to the presence of solute molecules. The apparent molal volume ((ΦV)) of L-Glycine in aqueous medium rises with molality. At low molality, L-Glycine molecules are well hydrated, and electrostriction (tight packing of water) reduces the effective volume [27]. As molality increases ion-solvent exchanges weaken because of overlapping hydration shells. Ion-ion interactions occur, reducing the degree of hydration [28]. This leads to a net increase in apparent molal volume as shown in table 3. At low pH (acidic), L-Glycine exists mainly in the cationic form. Strong hydration of the -NH_3^+ group reduces the electrostriction effect on water compared to the zwitterionic state. Due to less electrostriction, apparent molal volume increases [29]. At the isoelectric pH (pI) Glycine exists in zwitterionic form. Strong dipolar interactions and maximum hydration cause high electrostriction, and water molecules are more tightly bound and occupy less space. This results in a decline in apparent molal volume. At high pH (basic) L-Glycine exists in anionic form as in fig 2 (e). There is strong hydration around the -COO^- group, which also raises electrostriction, decreasing the apparent molal volume. When apparent molal volume (Φv) is plotted against molality, the slope S_v reflects solute-solute and solute-solvent exchanges. A positive S_v indicates structure - strengthening tendency which is common at low pH due to the strong positive charge on the L- Glycine molecule [30]. At high pH, L- Glycine becomes anionic ($\text{NH}_2\text{-CH}_2\text{-COO}^-$), and hydration diminishes, often giving negative or smaller S_v values due to weaker electrostriction [31].

Viscosity Coefficient constant B & A

The viscosity B -coefficient of L- Glycine is positive at low pH, high pH, and isoelectric pH, but its magnitude varies significantly with pH. $B > 0$ indicates that the solute behaves as a structure-strengthening in water i.e, it enhances the order of water molecules around it [32]. L- Glycine, being an amino acid, is highly polar in all ionic forms (cationic, anionic, zwitterionic), so it strongly interacts with water, creating a structured hydration layer [33]. At isoelectric pH, Strong dipolar nature suggests maximum hydration and structure-making effect. Therefore, B is highest at the isoelectric point. At low pH (cationic form $\text{NH}_3^+\text{-CH}_2\text{-COOH}$) positive charge dominates as shown in table 4. It forms strong hydration but less than zwitter-ion [34]. So, B is positive but lower than at the isoelectric pH. At high pH (anionic form $\text{NH}_2\text{-CH}_2\text{-COO}^-$) negative charge dominates. It forms hydration strong but similar reasoning to low pH. B is positive but lower than at the isoelectric pH [35].

The viscosity coefficient constant (A) represents long-range ionic exchanges. It is usually positive for strong electrolytes due to electrostatic attraction between oppositely charged ions [36]. By enhancing the pH of the L-Glycine solution. It goes towards anionic form ($\text{NH}_2\text{-CH}_2\text{-COO}^-$). There are like-charged species (COO^-) present, leading to repulsion instead of attraction. This reduces the predicted viscosity contribution from ion-ion interactions. In some cases, the interaction term becomes negative, giving $A < 0$. Negative A value indicates that the ionic atmosphere does not favor viscosity increase due to repulsive interactions among similarly charged species [37].

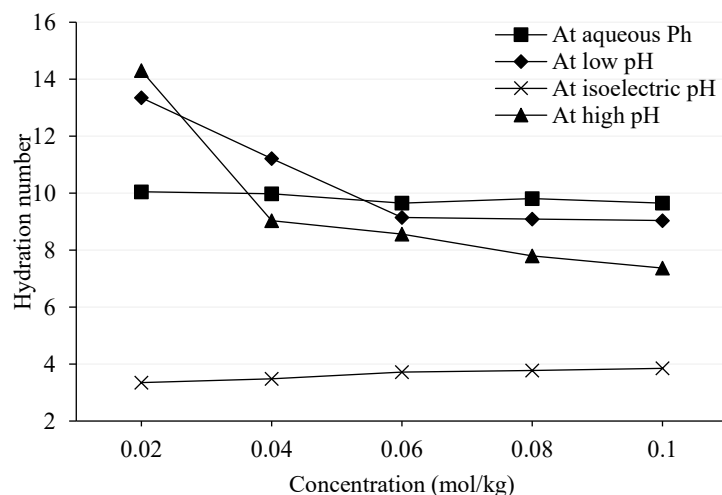


Figure 2d. Concentration vs hydration number.

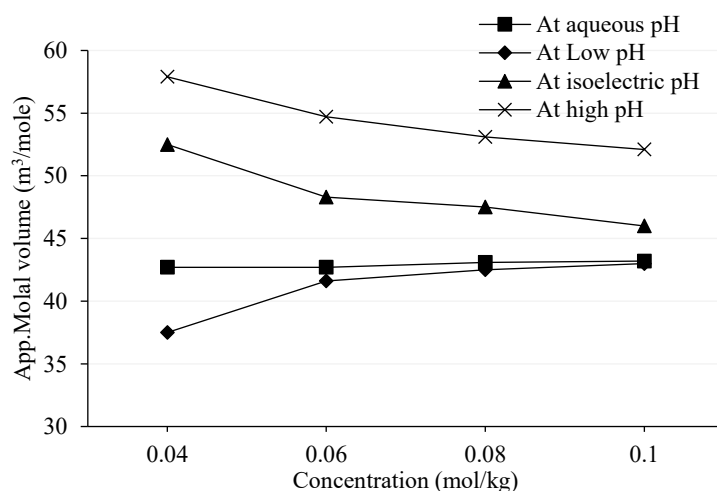


Figure 2e. Concentration vs apparent molal volume.

Table 3. Apparent molal volume values for L-Glycine solutions of different pH at 298.15K.

Concentration (mol/kg)	$\sqrt{m}$	Apparent molal volume Φ_v (m ³ mol ⁻¹)			
		Aqueous pH	Low pH (pH= 5.0)	Iso. pH (pH=6.08)	High pH (pH=6.5)
0.02	0.1414	40.19057	32.5	55.00949	60.34915
0.04	0.2	42.69809	37.49	52.51224	57.93789
0.06	0.2449	42.69809	41.65	48.35015	54.72287
0.08	0.2828	43.07422	42.48	47.51773	53.11535
0.10	0.3162	43.1996	42.98	46.01938	52.15085

Table 4. Values of limiting apparent molal volume (Φ_v^0), experimental slope (S_v), and Jones–Dole viscosity coefficients (A and B) for L-glycine solutions at different pH values at 298.15 K.

Parameter	Aqueous pH	Low pH (pH= 5.0)	Iso. pH (pH=6.08)	High pH (pH=6.5)
Φ_v^0 (m ³ mol ⁻¹)	38.65	37.67	62.57	67.35
S_v (m ³ Kg ^{1/2} mol ^{-3/2})	15.66	22.35	-53.52	-49.33
B (dm ³ mol ⁻¹)	0.228	0.285	0.321	0.281

$A \text{ dm}^{3/2} \text{ mol}^{-1/2}$	0	0.003	-0.004	-0.002
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CONCLUSION

In summary, acoustical and thermodynamical studies of L-glycine in buffer solutions of varying pH at 298.15 K demonstrate that thermo-acoustical investigations under these conditions provide valuable insights into solute–solvent and solute–solute interactions, hydration behavior, and structural modifications within the solution. As pH increases, the L-Glycine molecule passes from cationic - zwitterionic - anionic form. In all these states, ion-solvent and dipolar interactions strengthen water structure. This lowers compressibility, and therefore, ultrasonic velocity increases with pH. Adiabatic compressibility of L-Glycine decreases as pH increases [acidic (pH=5.0) – isoelectric (pH=6.08) – basic (pH= 6.5)]. This decrease is due to increased electrostriction of water molecules and stronger solute-solvent interactions, which make the solution less compressible. At low and high pH, the hydration number of glycine slightly decreases, whereas at neutral (aqueous) pH and at the isoelectric point (pI), it gradually increases. At acidic and basic pH, glycine exists in charged forms (cationic and anionic), leading to more dispersed and less compact hydration structures. In contrast, at neutral pH and especially at the isoelectric point, glycine exists as a zwitterion, which promotes stronger and more organized hydration through simultaneous interaction of $-\text{NH}_3^+$ and $-\text{COO}^-$ groups with water molecules. As a result, the hydration number shows a gradual increase under these conditions.

At low pH, increasing molality weakens hydration slightly, increasing apparent molal volume. At the isoelectric pH, strong structured hydration lowers it, while at high pH, enhanced electrostriction around $-\text{COO}^-$ reduces it further. At low pH, positive S_v indicates a structure-strengthening tendency due to positive charge. At high pH, L-glycine exists predominantly in its anionic form, and the comparatively weaker or less compact electrostriction around the solute may result in smaller or even negative S_v values. The viscosity B -coefficient of L-glycine remains positive over the entire pH range studied (acidic, basic, and isoelectric conditions), with its magnitude strongly dependent on pH. The positive B -values suggest that L-glycine behaves as a structure-promoting solute, facilitating increased organization of the solvent structure due to strong solute–solvent interactions.

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Author Contributions

Author 1: Conceptualization, Methodology, Investigation, Writing – Original Draft Preparation.

Author 2: Validation, Formal Analysis, Visualization, Writing – Review & Editing.

Author 3: Software, Resources, Supervision.

Author 4: Data Analysis, Writing – Review & Editing.

All authors have read and approved the final manuscript.

Data Availability

Data supporting the findings of this research can be accessed.

Declaration

I hereby declare that this research paper is my original work and has not been submitted elsewhere for publication. All sources used have been properly acknowledged.

Conflict of Interest

My research guide constantly helped in my research work as well as research paper examine well.

Ethical Approval

Data in this finding did not require ethical approval.

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