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Molecular interaction studies of aqueous L-proline using volumetric measurements at various temperatures

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Abstract

A detailed volumetric investigation of L-proline was undertaken in pure water and in aqueous media containing potassium chloride (electrolytic additive) and D-glucose (non-electrolytic additive). Density data were recorded within the molality interval 0.03-0.20 mol·kg⁻¹ over the temperature range 288.15-318.15 K at atmospheric pressure. Using the experimental density values, several thermodynamic quantities such as apparent molar volume (V_ϕ) limiting apparent molar volume (V_ϕ^0), limiting apparent molar transfer volume ($\Delta_{tr}V_\phi^0$) and Hepler's parameter were determined. These volumetric functions were employed to examine the nature of molecular interactions, particularly solute-solvent and solute-solute associations, and their temperature dependence. The obtained results demonstrate that both KCl and D-glucose markedly modify the hydration and solvation characteristics of L-proline. The study provides useful information regarding intermolecular behaviour in biologically significant aqueous systems and clarifies the effect of co-solutes on hydration phenomena.

Keywords: Density; L-proline; Potassium chloride; D-glucose; Hydration; Intermolecular interactions

1. Introduction

Research concerning the physicochemical behaviour of amino acids in aqueous and mixed-solvent environments continues to attract considerable scientific interest because of its importance in biochemical and pharmaceutical systems. Amino acids are the primary structural units of proteins, and their solution properties offer significant information about protein stability, folding mechanisms, and molecular recognition processes. Since direct investigations on proteins are often hindered by their complicated structures and high molecular masses, amino acids and small peptides are frequently employed as simplified model compounds for studying intermolecular interactions at the microscopic level [1].

Protein conformational stability is highly dependent upon the surrounding solvent medium. Variations in temperature, solvent composition, or the presence of supplementary solutes may disturb the equilibrium of intermolecular forces, thereby promoting stabilization or denaturation of biomolecular structures. Co-solutes can either interact directly with biomolecules or alter the structural organization of water indirectly, influencing hydration shells and solvent dynamics. Consequently, understanding these effects requires systematic examination of simpler aqueous systems.

Potassium chloride (KCl), a strong electrolyte, undergoes complete ionization in water to produce potassium and chloride ions. It is biologically important for maintaining osmotic and ionic equilibrium and participates in physiological activities including muscular contraction and nerve signal propagation. Besides its physiological role, KCl also finds widespread applications in agriculture and chemical industries. Numerous studies have addressed its influence on ion-solvent and solvent-solvent interactions [2, 3].

D-glucose is an important nonelectrolyte that serves as a major metabolic fuel in living organisms. It is involved in essential biochemical pathways such as glycolysis and cellular respiration. In aqueous solutions, glucose establishes extensive hydrogen-bonding interactions with water molecules, thereby influencing the arrangement and structural

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ordering of the solvent network. Owing to these characteristics, D-glucose represents an appropriate model compound for examining nonelectrolyte effects on solvation and hydration behaviour^[4, 5].

In the present investigation, density measurements of L-proline were carried out in aqueous KCl and aqueous D-glucose media over a broad temperature range. From these measurements, various volumetric and thermodynamic parameters were evaluated. The central aim of this work is to explore the influence of temperature and co-solutes on molecular interactions, particularly solute-solvent and solute-solute interactions, in binary and ternary liquid systems.

2. Materials and Methods

2.1 Materials

L-proline, potassium chloride and D-glucose used in this study were procured from Sigma-Aldrich (India). All reagents were of analytical-reagent purity and employed without additional purification. Prior to use, the compounds were dried in a vacuum oven at 343.15 K and subsequently stored in a vacuum desiccator containing fused anhydrous calcium chloride for more than 48 h.

2.2 Instrumentation and Experimental Procedure

Deionized distilled water with conductivity lower than $0.1 \mu\text{S}\cdot\text{cm}^{-1}$ was supplied by an Evoqua purification unit. Solutions were prepared gravimetrically at ambient temperature within the concentration range $0.03\text{--}0.20 \text{ mol}\cdot\text{kg}^{-1}$ and utilized immediately after preparation. Weighing was performed using a Sartorius Quintix

analytical balance (Model 125D-101N) having an accuracy of $\pm 0.01 \text{ mg}$.

Density and ultrasonic velocity measurements were obtained using an Anton Paar DSA 5000M digital densimeter-sound analyzer (Austria). The instrument operates on the Pulsed Excitation Method, providing density accuracy up to $0.007 \text{ kg}\cdot\text{m}^{-3}$. The corresponding sensitivities for density and sound velocity measurements are 1×10^{-3} and $1\times 10^{-2} \text{ m}\cdot\text{s}^{-1}$, respectively. Estimated standard uncertainties were $0.5 \text{ kg}\cdot\text{m}^{-3}$ for density and $1.0 \text{ m}\cdot\text{s}^{-1}$ for sound velocity. Temperature regulation was maintained by an integrated PT100 thermostat with stability of $\pm 0.01 \text{ K}$. Prior to each measurement sequence, calibration was verified using deionized water throughout the investigated temperature range. The liquid sample was positioned between two piezoelectric transducers; one generated ultrasonic waves of nearly 3 MHz frequency, while the other detected the transmitted signal.

3. Results and Discussion

3.1 Density Behaviour

Densities of aqueous L-proline solutions and ternary systems containing $0.01 \text{ mol}\cdot\text{kg}^{-1}$ KCl or $0.1 \text{ mol}\cdot\text{kg}^{-1}$ D-glucose were measured at temperatures from 288.15 K to 318.15 K over different solute concentrations. The experimental observations indicate that density increases almost linearly with increasing concentration of L-proline in all systems studied. Conversely, at constant concentration, density decreases slightly with rise in temperature due to thermal expansion of the solvent medium. These findings are graphically represented in Figure 1.

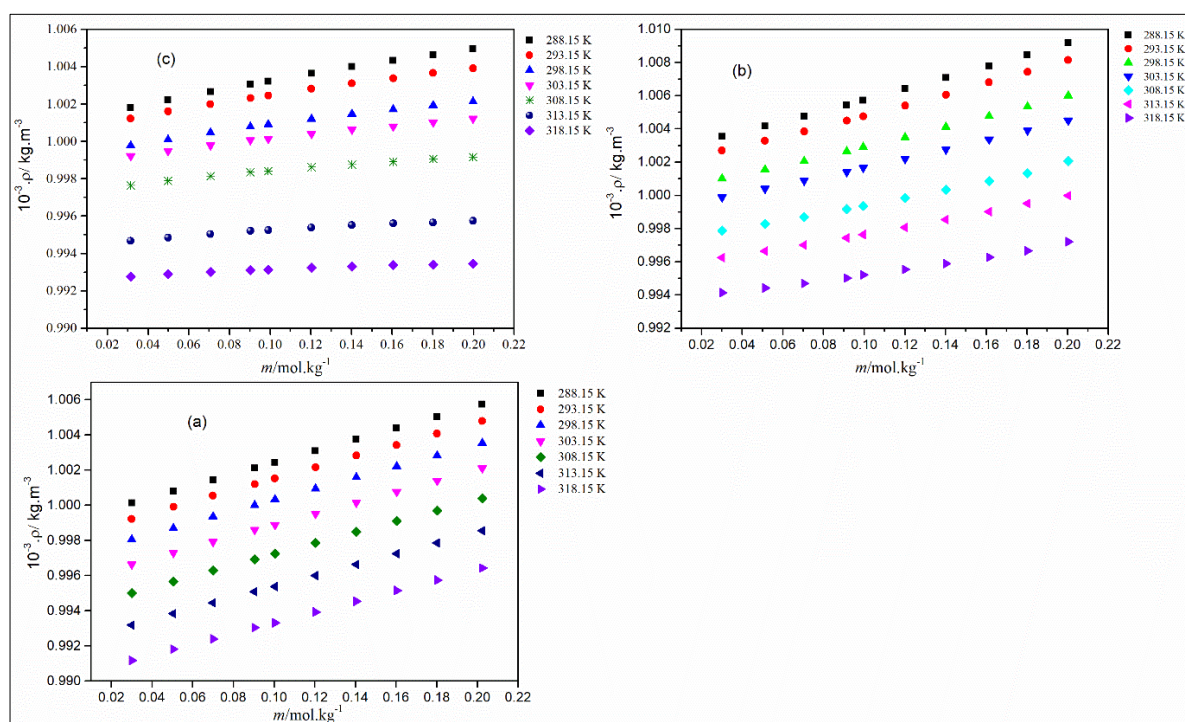


Fig 1: Plot of variation of density (ρ) against molality (m) for aqueous solution of L-proline (a) in water (b) in aqueous $0.1 \text{ mol}\cdot\text{kg}^{-1}$ KCl and (c) in aqueous $0.1 \text{ mol}\cdot\text{kg}^{-1}$ D-glucose at $T = (288.15\text{--}318.15) \text{ K}$.

3.1.1 Apparent Molar Volume(V_ϕ)

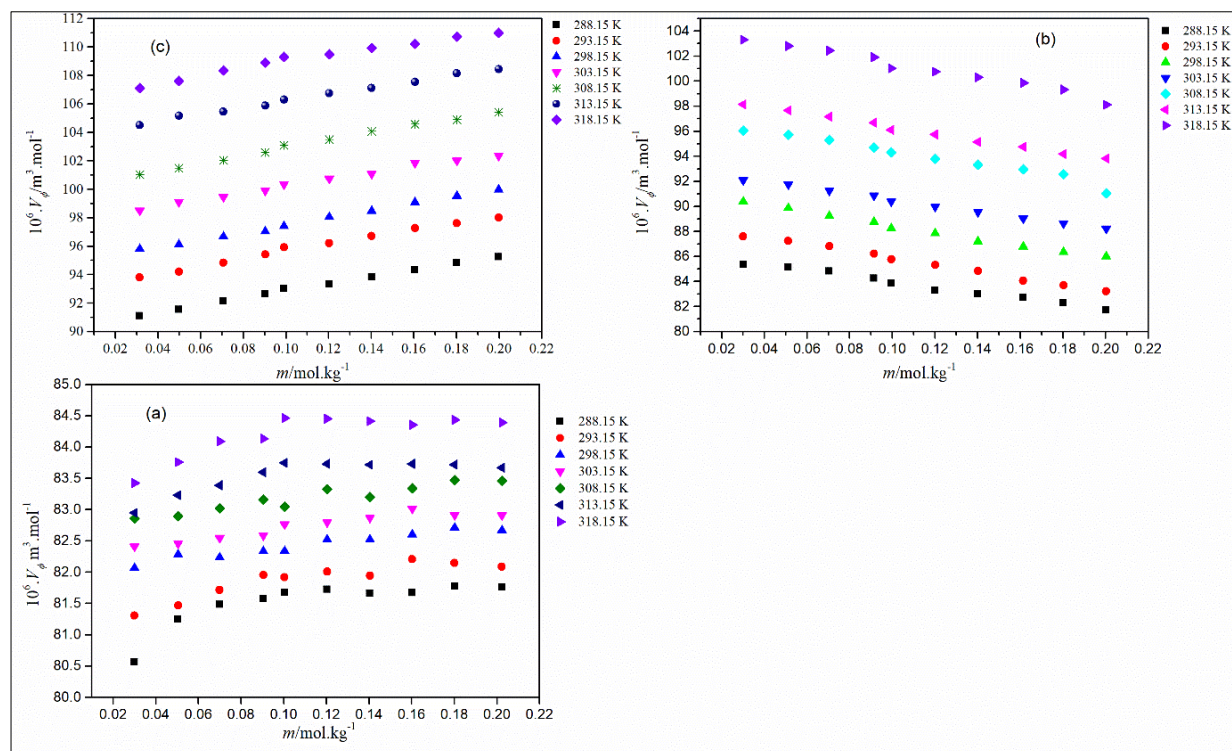
The apparent molar volume (V_ϕ) was evaluated from density data using the expression:

$$V_\phi = \frac{M}{\rho} - \frac{(\rho - \rho_0)}{m\rho\rho_0} \quad (1)$$

where M denotes molar mass of the solute, m is molality, and ρ and ρ_0 represent densities of solution and solvent, respectively. The calculated values are reported in Table 1 and graphically shown in Figure 2^[6, 7].

Table 1: Values of apparent molar volumes (V_ϕ) of L-proline in water, and aqueous 0.1 mol. kg⁻¹ KCl and aqueous 0.1 mol.kg⁻¹ D-glucose at $T=(288.15-318.15)$ K and experimental pressure at $1.013\cdot10^5$ Pa.

$m/$ (mol.kg ⁻¹)	$10^6 \cdot V_\phi / (\text{m}^3 \cdot \text{mol}^{-1})$						
	$T=288.15$ K	$T=293.15$ K	$T=298.15$ K	$T=303.15$ K	$T=308.15$ K	$T=313.15$ K	$T=318.15$ K
L-proline + water							
0.0300	80.56	81.31	82.07	82.41	82.86	82.95	83.42
0.0505	81.25	81.47	82.29	82.45	82.90	83.23	83.76
0.0700	81.48	81.71	82.23	82.55	83.02	83.39	84.09
0.0905	81.58	81.95	82.33	82.58	83.16	83.60	84.13
0.1004	81.67	81.92	82.34	82.76	83.04	83.75	84.46
0.1204	81.73	82.01	82.52	82.79	83.32	83.73	84.45
0.1404	81.66	81.94	82.52	82.86	83.20	83.72	84.41
0.1603	81.67	82.21	82.60	83.01	83.34	83.73	84.35
0.1801	81.78	82.15	82.71	82.91	83.47	83.72	84.43
0.2023	81.76	82.09	82.67	82.91	83.46	83.67	84.39
L-proline + 0.1 mol.kg⁻¹ KCl							
0.0301	85.34	87.60	90.36	92.10	96.05	98.15	103.30
0.0512	85.17	87.24	89.86	91.76	95.71	97.67	102.81
0.0705	84.84	86.81	89.24	91.25	95.29	97.16	102.44
0.0914	84.26	86.21	88.76	90.85	94.70	96.69	101.91
0.0995	83.86	85.76	88.24	90.37	94.30	96.10	101.02
0.1201	83.28	85.33	87.85	89.94	93.81	95.76	100.75
0.1402	83.04	84.83	87.20	89.54	93.33	95.14	100.31
0.1614	82.73	84.05	86.77	89.03	92.95	94.75	99.87
0.1803	82.27	83.71	86.36	88.63	92.57	94.18	99.33
0.2003	81.71	83.21	86.00	88.21	91.04	93.82	98.11
L-proline + 0.1 mol.kg⁻¹ D-glucose							
0.0314	91.11	93.82	95.81	98.50	101.03	104.51	107.12
0.0498	91.54	94.21	96.13	99.10	101.49	105.17	107.60
0.0706	92.12	94.84	96.67	99.45	102.03	105.46	108.34
0.0903	92.64	95.43	97.03	99.90	102.60	105.87	108.91
0.0991	93.00	95.92	97.40	100.34	103.08	106.29	109.29
0.1203	93.31	96.21	98.05	100.74	103.48	106.75	109.47
0.1402	93.81	96.71	98.48	101.09	104.06	107.11	109.95
0.1605	94.33	97.27	99.08	101.85	104.57	107.53	110.23
0.1801	94.83	97.61	99.52	102.02	104.89	108.16	110.73
0.1998	95.24	98.01	99.98	102.36	105.40	108.45	110.98

**Fig 2:** Plot of V_ϕ against molality (m) for L-proline in (a) in water (b) in aqueous 0.1 mol.kg⁻¹ KCl and (c) in aqueous 0.1 mol.kg⁻¹ D-glucose at $T = (288.15-318.15)$ K.

Positive values of V_ϕ were obtained for all concentrations and temperatures, suggesting strong interactions between solute and solvent molecules. Increasing temperature resulted in higher V_ϕ values, indicating enhanced solvation effects. In aqueous D-glucose medium, V_ϕ increased noticeably, reflecting stronger solute-solvent association, whereas a decreasing trend was observed in KCl solutions [14-15].

3.1.2 Limiting Apparent Molar Volume (V_ϕ^0)

The limiting apparent molar volume (V_ϕ^0) was determined by extrapolating plots of V_ϕ versus molality to zero concentration using Masson's relation:

$$V_\phi = V_\phi^0 + A_V \sqrt{m} + S_V m \quad (2)$$

where S_V denotes the experimental slope associated with solute-solute interactions. An increase in V_ϕ^0 with temperature may be attributed to the release of solvent molecules from secondary solvation shells into the bulk medium. Elevated temperatures reduce electrostrictive effects around zwitterionic centres, thereby expanding the solution volume. Positive V_ϕ^0 values indicate dominant solute-solvent interactions.

The V_ϕ^0 values in aqueous D-glucose solutions were found to exceed those in KCl systems, demonstrating stronger hydration interactions in the former medium. Positive S_V

values observed in some systems imply stronger solute-solute interactions, while negative values suggest comparatively weaker association effects [8, 9].

3.1.3 Limiting Apparent Molar Volume of Transfer ($\Delta_{tr}V_\phi^0$)

The limiting apparent molar transfer volume was evaluated from:

$$\Delta_{tr}V_\phi^0 = V_\phi^0(KCl/D-glucose) - V_\phi^0(water) \quad (3)$$

The values of $\Delta_{tr}V_\phi^0$ for all the studied systems are reported in Table 2. Depending upon the co-sphere overlap model regarding the values of $\Delta_{tr}V_\phi^0$ there is negligible contribution from (solute + solute) interactions and hence they provide information regarding (solute + solvent) interactions. The various interactions that occur between amino acid and solvent molecules are graphically represented and can be summarised as follows,

1. (hydrophilic + hydrophilic) interactions (between hydrophilic groups of L-proline and hydrophilic groups of KCl/D-glucose).
2. (ion/hydrophilic + hydrophobic) interactions (between ions of KCl/hydrophilic groups of D-glucose and hydrophobic centres of L-proline).
3. (ion-ion) interactions between zwitterionic centres of L-proline and ions of KCl.

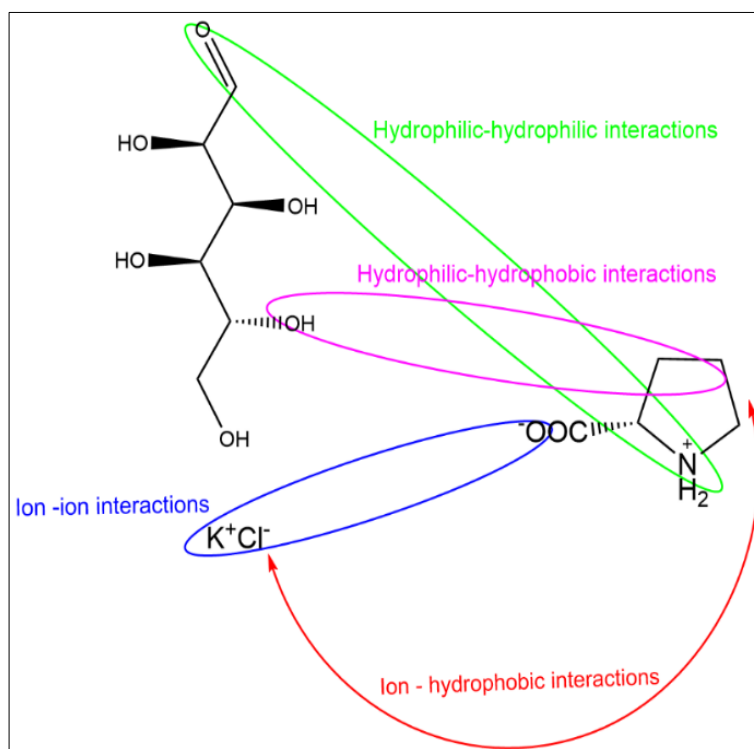


Fig 3: Plausible interactions between L-proline and KCl/D-glucose

Positive transfer volume values were obtained for both co-solutes, indicating predominance of hydrophilic-hydrophilic interactions according to the co-sphere overlap model proposed by Gurney, Frank, and Evans [10-17]. These interactions arise mainly between polar groups of amino

acids and the hydrophilic moieties of KCl or D-glucose. Table 2 reports the V_ϕ^0 , S_V and $\Delta_{tr}V_\phi^0$ values for all the systems under study.

Table 2: Limiting apparent molar properties (V_{ϕ}^0), experimental slope (S_V), V_{ϕ}^0 (Calculated) and $\Delta_{tr}V_{\phi}^0$ of L-proline in water, 0.1 mol.kg⁻¹ aqueous KCl and 0.1 mol.kg⁻¹ aqueous D-glucose at different temperatures i.e. $T = (288.15 - 318.15)$ K.

T/K	$10^6 \cdot V_{\phi}^0 / \text{m}^3 \cdot \text{mol}^{-1}$	$10^6 \cdot S_V / \text{m}^3 \cdot \text{mol}^{-2} \cdot \text{kg}$	$10^6 \cdot \Delta_{tr} V_{\phi}^0 / \text{m}^3 \cdot \text{mol}^{-1}$	$10^6 \cdot V_{\phi}^0 / \text{m}^3 \cdot \text{mol}^{-1}$ (calculated)
L-proline + water				
288.15	80.94	5.07		80.94
293.15	80.87	3.07		80.87
298.15	81.69	2.26		81.62
303.15	81.98	2.26		81.98
308.15	82.4	2.36		82.48
313.15	82.69	2.63		82.69
318.15	83.07	3.41		83.07
L-proline + 0.1 mol.kg⁻¹ KCl				
288.15	88.19	-13.84	7.25	88.19
293.15	91.02	-16.92	10.15	91.02
298.15	93.56	-16.77	11.87	93.45
303.15	95.04	-14.86	13.06	95.04
308.15	99.54	-16.98	17.14	95.39
313.15	101.36	-16.54	18.67	101.36
318.15	106.94	-18.17	23.87	106.94
L-proline + 0.1 mol.kg⁻¹ D-glucose				
288.15	88.09	15.59	7.15	88.09
293.15	90.7	16.18	9.83	90.70
298.15	92.53	16.15	10.84	92.12
303.15	95.7	14.76	13.72	95.70
308.15	97.78	16.74	15.38	98.70
313.15	101.72	14.68	19.03	101.72
318.15	104.49	14.57	21.42	104.49

3.1.4 Temperature Dependence and Structure-Making/Breaking Effects

The variation of limiting apparent molar volume with temperature is described by:

$$V_{\phi}^0 = A + B(T - T_{ref}) + C(T - T_{ref})^2 \quad (4)$$

where $T_{ref} = 298.15$ K and A, B, and C are empirical constants.

From this relation, limiting apparent molar expansibility

(E_{ϕ}^0) and Hepler's constant, $\left(\frac{\partial E_{\phi}^0}{\partial T}\right)_P$ were derived:

$$E_{\phi}^0 = B + 2C(T - T_{ref}) \quad (5)$$

According to Hepler's criterion,

$$(\partial E_{\phi}^0 / \partial T)_P = (\partial^2 V_{\phi} / \partial T^2)_P = 2C \quad (6)$$

Table 3. Empirical parameters of Eq. (4), limiting apparent molar expansivity (E_{ϕ}^0) of solute, coefficient of thermal expansion (α^*) and Hepler's constant ($\partial E_{\phi}^0 / \partial T$) for L-proline in aqueous and in aqueous solutions of 0.1 mol.kg⁻¹ KCl and 0.1 mol.kg⁻¹ D-glucose at $T = (288.15 - 318.15)$ K and $P = 1.013 \cdot 10^5$ Pa.

T/K	$A \cdot 10^6 / \text{m}^3 \cdot \text{mol}^{-1}$	$B \cdot 10^6 / \text{m}^3 \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$	$C \cdot 10^6 / \text{m}^3 \cdot \text{mol}^{-1} \cdot \text{K}^{-2}$	$10^6 \cdot E_{\phi}^0 / \text{m}^3 \cdot \text{mol} \cdot \text{K}^{-1}$	α^* / K^{-1}	$10^6 \cdot \frac{\partial E_{\phi}^0}{\partial T} / \text{m}^3 \cdot \text{mol} \cdot \text{K}^{-2}$
L-proline + water						
288.15	81.8	0.099	0.001550	0.068		
293.15	81.2	0.084	0.001550	0.068	0.00093	
298.15	81.6	0.066	0.001550	0.066	0.00136	-0.0004
303.15	81.5	0.099	0.000015	0.099	0.00087	-0.00005
308.15	81.5	0.091	0.000007	0.091	0.00086	-0.00001
313.15	81.0	0.111	0.000007	0.111	0.00081	
318.15	80.9	0.111	0.000007	0.111		
L-proline + 0.1 mol.kg⁻¹ aqueous KCl						
288.15	88.9	0.071	-0.000200	0.075		
293.15	91.4	0.073	-0.000200	0.075	0.00590	
298.15	93.4	0.111	-0.000200	0.111	0.00430	0.0061
303.15	94.7	0.071	-0.000025	0.071	0.00629	0.00021
308.15	98.8	0.071	-0.000003	0.071	0.00635	0.00010
313.15	100.4	0.067	-0.000003	0.067	0.00730	
318.15	105.6	0.067	-0.000003	0.067		
L-proline + 0.1 mol.kg⁻¹ aqueous D-glucose						
288.15	93.8	0.598	0.003050	0.537	0.00490	
293.15	93.5	0.568	0.003050	0.537	0.00540	0.0081
298.15	92.1	0.402	0.003050	0.402	0.00549	0.00008
303.15	92.7	0.597	0.000103	0.598	0.00616	0.00011
308.15	91.5	0.631	0.000050	0.632	0.00660	

313.15	90.6	0.738	0.000050	0.74		
318.15	89.7	0.738	0.000050	0.74		

The sign of this parameter provides information regarding structure-making or structure-breaking tendencies of solutes. Positive values correspond to structure-promoting behaviour, whereas negative values indicate disruption of solvent structure [18-20]. The present systems predominantly exhibited positive values, suggesting that both co-solutes act as structure makers in aqueous L-proline solutions.

The thermal expansion coefficient was calculated using:

$$\alpha^* = \frac{1}{V_\phi^0} \left(\frac{\partial V_\phi^0}{\partial T} \right) \quad (5)$$

The obtained α^* values indicate comparatively stronger solute-solvent interactions in aqueous KCl and D-glucose systems than in pure aqueous L-proline solutions [21-25].

4. Conclusion

The present investigation offers a comprehensive volumetric examination of L-proline in aqueous as well as mixed-solvent environments containing potassium chloride and D-glucose. The evaluated thermodynamic parameters reveal that solute-solvent interactions become progressively stronger with increasing temperature, as evidenced by the rise in apparent molar volume and limiting apparent molar volume values.

Among the investigated co-solutes, D-glucose exerts a more pronounced influence on the interaction behaviour of L-proline compared with KCl. This difference may be attributed to the extensive hydrogen-bonding capability and structural characteristics of glucose molecules in aqueous media. In contrast, the electrolyte KCl primarily affects the system through ion-solvent interactions and electrostatic effects.

The calculated Hepler parameters indicate that L-proline predominantly behaves as a structure breaker in most of the studied solutions. However, in aqueous D-glucose medium, the amino acid exhibits structure-promoting characteristics, suggesting enhanced ordering of the surrounding solvent network. Such behaviour highlights the important role of co-solutes in modifying hydration structure and intermolecular association patterns.

Overall, the present findings improve the understanding of amino acid-solvent and amino acid-co-solute interactions in biologically relevant systems. These observations are valuable for interpreting the influence of ions and nonelectrolytes on amino acid solubility, conformational stability, and hydration behaviour. Furthermore, the study provides useful insight into the role of co-solutes in protein denaturation and renaturation phenomena, where amino acids are often employed as simplified model compounds. Knowledge of these thermophysical properties also contributes to a better understanding of protein folding mechanisms, molecular stability, and solvent-mediated biochemical processes.

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Conflict of interests

The author declares that there is no conflict of interest.

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