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RESEARCH ARTICLE

VOLUMETRIC, VISCOMETRIC AND ULTRASONIC STUDY OF AMINO ACIDS IN MIXED SOLVENTS AT VARYING TEMPERATURE

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ABSTRACT

Densities (ρ), viscosities (η) and ultrasonic speeds (u), for solutions of (0.02, 0.04, 0.06, 0.08 and 0.10 M) L - Valine (Val), L - isoleucine (Ile) and L - Proline (Pro) in aqueous 1, 3 - dioxolane (10 % w/w) at 298.15, 303.15, 313.15 and 323.15K have been determined. These data have been used to calculate apparent molar volumes (ϕ_v), limiting apparent molar volume (ϕ_v°) and the slope (S_v), apparent molar compressibility (ϕ_k), limiting apparent molar compressibility (ϕ_k°) and the slope (S_k), Falkenhagen and Jones–Dole coefficients, A and B, respectively. These parameters were used to discuss the solute–solute and solute–solvent interactions and also the effect of cosolvent (1, 3 - dioxolone) on these interactions.

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INTRODUCTION

The specific interactions of water and other co-solvents with various functional groups of the protein, contribute to formation of the stable and folded structure (Hakin *et al.*, 1997, Pal and Kumar., 2002, Banipal *et al.*, 2002, Bag *et al.*, 2000). As the amino acid and peptide molecules contain functional groups similar to those existing more complex proteins, they are expected to mimic some common features of proteins (Hvidt and Westh, 1998). Recently, there has been an increased interest to understand the role of water, solved to soluble organics in the living cells (Millero *et al.*, 1978), owing to the fact that most biological macromolecules are physiologically active in aqueous solutions. The choice of water for preparing mixed solvent systems emerges from its important and unique role in determining the structure and stability of protein, since, its presence is known to give hydrophobic forces (Ronero *et al.*, 1999, Jolicoeur *et al.*, 1986, Wadi and Ramasami 1997; Belibagali and Ayranci, 1990, Wadi and goyal, 1992, Yan *et al.*, 1998; Kumar, 1999; Castronuovo *et al.*, 1999; Ren *et al.*, 1998, 1999 a,b, Li *et al.*, 1999, Lin *et al.*, 2000, Anwar Ali *et al.*, 2006), which are of prime importance in stabilizing the native globular structure of protein (Yan *et al.*, 2004, Lilley, 1993, 1994). The increasing use of cyclic diethers and their aqueous mixtures in many industrial processes, such as, pharmaceuticals and cosmetics has greatly stimulated the need for extensive information on their various properties. 1, 3 dioxolone is an important solvent for a number of separation processes, solution studies, Mannich reactions and as an electrolyte in batteries.

Thus, the knowledge of solute–solvent and solute–solute interactions of amino acids in aqueous 1, 3 - dioxolane medium has its biological significance.

In this study, we report the densities (ρ), ultrasonic speeds (U), and viscosities (η) of (0.02, 0.04, 0.06, 0.08 and 0.10) m L-valine (Vla) L-isoleucine (Ile) and L-proline (Pro) with aqueous 1, 3 - dioxolane (10% 1, 3 - dioxolane, w/w) at T = 298.15, 303.15, 313.15 and 323.15 K. The experimental results were used to calculate the apparent molar volume, ϕ_v , limiting apparent molar volume, ϕ_v° , and the slope, S_v , apparent molar compressibility, ϕ_k , limiting apparent molar compressibility, ϕ_k° , and the slope, S_k and Falkenhagen coefficient, A and Jones – Dole coefficient, B. These parameters were used to discuss the solute–solvent / cosolvent and solute–solute interactions in the aforementioned systems.

EXPERIMENTAL

High purity L - Valine, L-isoleucine (Merck, 99%), and L-serine (S.D. fine, 99%) were crystallized twice from aqueous ethanol solutions and dried under vacuum at 373K for 12 hrs. Then, they were stored over P₂O₅ in a desiccator before use. The 1, 3 - dioxolane (>99%) obtained from Sigma Aldrich was used without any further purification. Doubly distilled, degassed water was used for preparation of the solutions. Aqueous 1, 3 - dioxolone (10% v/v) binary mixtures were used as solvents to prepare solutions of (0.02, 0.04, 0.06, 0.08 and 0.10 M) amino acids (Vla, Ile and Pro). All the solutions of ternary mixtures were prepared on molarity basis and were kept in airtight bottles. To prevent formation of air

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bubbles, all the solutions were preheated to 50°C, above the measuring temperature, before taking readings. The weighing was done on an electronic digital balance, SHIMADZU AX200 (Swiss make) accurate up to 1.0×10^{-4} g.

The densities of mixed solvents and solutions of amino acids in these solvents were measured using a specific gravity bottle by relative measurement method with an accuracy of $\pm 0.01 \text{ kg m}^{-3}$. An Oswald's viscometer (10 ml capacity) was used for viscosity measurement and efflux time was determined using a digital chronometer to within ± 0.01 s. Sound speeds were determined with an uncertainty of 0.3%, using a single crystal variable path ultrasonic interferometer (Mittal Enterprises, New Delhi, India), operating at 3MHz, which was calibrated with water, methanol and benzene at 303.15K. During the measurements, the temperature of test solution was maintained to an accuracy of ± 0.01 K, in an electronically controlled thermostatic water bath (RAAGA INDUSTRIES, INDIA).

RESULTS

The experimental values of density (ρ), ultrasonic speed (U) and viscosity (η) of (0.02, 0.04, 0.06, 0.08 and 0.10m) L-Valine, L-isoleucine and L-Proline in aqueous 1, 3 - dioxolane (10% 1, 3 - dioxolane) as functions of amino acid concentration and temperature, which are listed in Table 1.

$$\phi_K = \frac{1000}{m\rho_0}(\beta\rho_0 - \rho\beta_0) + \left(\frac{\beta_0 M}{\rho_0}\right) \quad \dots(2)$$

where, β_0 and β are adiabatic compressibility of solvent and solution, respectively. The adiabatic compressibility is defined as,

$$\beta = \frac{1}{U^2 \rho} \quad \dots(3)$$

In the above equations, m is the molal concentration of the solute (amino acid), ρ and ρ_0 are the densities of solution and solvent respectively, M is the molecular mass of the solute (amino acid) and u , the sound velocity of the solution.

The calculated values of ϕ_v , as a function of amino acid concentration and temperature are given in Table 3. The values of ϕ_v are plotted against $C^{1/2}$ and the plots were found to be almost linear in the concentration range studied. The plots are well represented by the equation.

$$\phi_V = \phi_V^0 + S_V C^{1/2} \quad \dots (4)$$

$$\phi_K = \phi_K^0 + S_K m^{1/2} \quad \dots (5)$$

Table 1 Values of Density (ρ), viscosity (η) and velocity (U) for L-valine, L-isoleucine and L-proline + aqueous 10% 1,3-dioxolane (w/w) solutions at different temperatures

Molality m (mol.Kg ⁻¹)	DENSITY ρ (kg/m ³)				VISCOSITY η ($\times 10^{-3}$ NSm ⁻²) Temperature (K)				VELOCITY U/ms ⁻¹			
	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15
L-valine + aqueous 10% 1,3-dioxolane solution												
0.00	1013.40	1006.90	1001.20	997.70	0.9970	0.8134	0.6555	0.5673	1489.80	1508.60	1542.10	1579.40
0.02	1013.81	1007.31	1001.59	998.08	0.9984	0.8148	0.6589	0.5714	1491.41	1510.12	1543.51	1580.69
0.04	1014.20	1007.69	1001.92	998.45	1.0019	0.8177	0.6617	0.5741	1493.04	1511.64	1544.92	1581.98
0.06	1014.58	1008.06	1002.25	998.81	1.0091	0.8248	0.6689	0.5806	1494.63	1513.16	1546.33	1583.27
0.08	1014.96	1008.42	1002.57	999.17	1.0188	0.8346	0.6783	0.5890	1496.24	1514.68	1547.74	1584.56
0.10	1015.33	1008.79	1002.89	999.53	1.0272	0.8477	0.6894	0.5971	1497.86	1516.20	1549.15	1585.85
L-isoleucine + aqueous 10% 1,3-dioxolane solution												
0.00	1013.40	1006.90	1001.20	997.70	0.9970	0.8134	0.6555	0.5673	1489.80	1508.60	1542.10	1579.40
0.02	1014.25	1007.74	1002.03	998.51	0.9974	0.8139	0.6580	0.5718	1490.29	1508.81	1542.22	1579.51
0.04	1015.05	1008.49	1002.76	999.25	0.9982	0.8178	0.6629	0.5764	1490.71	1509.21	1542.56	1579.72
0.06	1015.86	1009.27	1003.50	999.95	1.0032	0.8242	0.6688	0.5819	1491.14	1509.62	1542.90	1579.93
0.08	1016.64	1010.04	1004.24	1000.68	1.0079	0.8300	0.6734	0.5861	1491.62	1510.03	1543.24	1580.14
0.10	1017.44	1010.68	1004.94	1001.41	1.0142	0.8354	0.6774	0.5923	1492.06	1510.42	1543.58	1581.55
L-proline + aqueous 10% 1,3-dioxolane solution												
0.00	1013.40	1006.90	1001.20	998.07	0.9970	0.8134	0.6555	0.5673	1489.80	1508.60	1542.10	1579.40
0.02	1013.87	1007.36	1001.65	998.15	1.0027	0.8188	0.6600	0.5709	1490.75	1509.48	1542.84	1580.12
0.04	1014.29	1007.78	1002.06	998.52	1.0132	0.8271	0.6684	0.5771	1491.62	1510.38	1543.64	1580.84
0.06	1014.68	1008.20	1002.47	998.90	1.0221	0.8361	0.6748	0.5839	1492.50	1511.26	1544.42	1581.56
0.08	1015.08	1008.59	1002.89	999.26	1.0341	0.8459	0.6821	0.5892	1493.38	1512.16	1545.07	1582.28
0.10	1015.47	1008.97	1003.30	999.64	1.0457	0.8552	0.6891	0.5963	1494.18	1513.07	1545.70	1583.03

The apparent molar volume (ϕ_v) and apparent molar compressibility (ϕ_K) of the amino acids in aqueous 1, 3 - dioxolane were computed from the density and sound velocity using the following equations.

$$\phi_v = \frac{1000(\rho_0 - \rho)}{m\rho\rho_0} \cdot \left[\frac{M}{\rho} \right] \quad \dots(1)$$

where, the intercepts, ϕ_v^0 or ϕ_K^0 by definition are free from solute-solute interactions and therefore, provide a measure of solute-solvent interactions, whereas, the experimental slope, S_v or S_k provides information regarding solute-solute interaction, and were obtained by using linear-regression of ϕ_v^0 and ϕ_K^0 vs m from equations (4) and (5) respectively.

The viscosity results were analyzed by using the Jones-Dole (1929) equation of the form

Table 2. Values of Adiabatic compressibility (β) in L-valine, L-isoleucine and L-proline + aqueous 10% 1,3-dioxolane (w/w) solutions at different temperatures

Molality m (mol.Kg ⁻¹)	$\beta/(\times 10^{-10} \text{ m}^2 \text{ N}^{-1})$											
	L-valine			L-isoleucine				L-proline				
	Temperature (K)											
	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15
0.00	4.4459	4.3638	4.2000	4.0181	4.4459	4.3638	4.2000	4.0181	4.4459	4.3638	4.2000	4.0181
0.02	4.4345	4.3533	4.1907	4.0100	4.4393	4.3590	4.1959	4.0142	4.4382	4.3567	4.1941	4.0126
0.04	4.4232	4.3429	4.1817	4.0020	4.4333	4.3534	4.1910	4.0102	4.4312	4.3497	4.1881	4.0074
0.06	4.4121	4.3326	4.1727	3.9940	4.4272	4.3477	4.1861	4.0063	4.4243	4.3429	4.1821	4.0023
0.08	4.4010	4.3223	4.1638	3.9861	4.4210	4.3420	4.1811	4.0023	4.4173	4.3360	4.1769	3.9972
0.10	4.3899	4.3121	4.1549	3.9781	4.4149	4.3370	4.1764	3.9923	4.4109	4.3292	4.1718	3.9919

Table 3. Values of apparent molal compressibility (ϕ_k) and apparent molal volume (ϕ_v) at different temperatures in L-valine, L-isoleucine and L-proline + aqueous 10% 1,3-dioxolane (w/w) solution

Molality m (mol.Kg ⁻¹)	$\phi_k(\times 10^{-8} \text{ m}^2 \text{ N}^{-1})$				$\phi_v(\times \text{m}^3 \text{ mol}^{-1})$			
	Temperature (K)							
	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15
L-valine + aqueous 10% 1,3-dioxolane solution								
0.02	-6.5882	-6.1598	-5.4643	-4.8019	9.5601	9.6088	9.7518	9.8295
0.04	-6.5640	-6.0862	-5.3317	-4.7763	9.6051	9.6791	9.8982	9.8509
0.06	-6.4953	-6.0416	-5.2831	-4.7507	9.6339	9.7166	9.9447	9.8725
0.08	-6.4709	-6.0043	-5.2450	-4.7352	9.6465	9.7460	9.9789	9.8815
0.10	-6.4495	-5.9872	-5.2195	-4.7238	9.6624	9.7522	9.9981	9.8854
L-isoleucine + aqueous 10% 1,3-dioxolane solution								
0.02	-5.1828	-5.4114	-4.8558	-4.3618	8.7988	8.8781	8.9548	9.0722
0.04	-4.9658	-5.2812	-4.7738	-4.2086	8.9134	9.0930	9.1973	9.2410
0.06	-4.9160	-5.2569	-4.7514	-4.1359	8.9306	9.1106	9.2569	9.3598
0.08	-4.8939	-5.2213	-4.7437	-4.1035	8.9723	9.1282	9.2832	9.3780
0.10	-4.8732	-5.1378	-4.7169	-4.0563	8.9749	9.2650	9.3364	9.3862
L-Proline + aqueous 10% 1,3-dioxolane solution								
0.02	-4.8867	-4.5296	-3.8949	-2.8102	9.0683	9.1613	9.2504	9.2750
0.04	-4.6540	-4.4680	-3.8898	-2.7848	9.1862	9.2561	9.3463	9.4723
0.06	-4.5401	-4.4259	-3.8681	-2.7552	9.2718	9.2850	9.3757	9.5189
0.08	-4.4922	-4.3849	-3.7784	-2.7447	9.3005	9.3348	9.3759	9.5656
0.10	-4.4061	-4.3560	-3.7046	-2.6558	9.3261	9.3731	9.3845	9.5720

Table 4. Values of limiting apparent molal compressibility (ϕ_k^0) and limiting apparent molal volume (ϕ_v^0) and their constants S_k and S_v of amino acids in aqueous 10% 1,3-dioxolane (w/w) solutions at different temperatures

Amino Acids	$\phi_k^0(\times 10^{-8} \text{ m}^2 \text{ N}^{-1})$																$S_k/(\times 10^{-8} \text{ N}^{-1} \text{ m}^{-1} \text{ mol}^{-1})$				$\phi_v^0(\times \text{m}^3 \text{ mol}^{-1})$				$S_v/(\text{N}^{-1} \text{ m}^{-1} \text{ mol}^{-1})$			
	Temperature (K)																											
	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15								
L-valine	6.7126	6.2888	5.6319	4.8645	8.3950	9.8312	13.6309	4.5129	94.796	94.972	95.846	97.796	5.9866	8.5734	13.9052	3.5575												
L-isoleucine	5.3623	5.5947	4.9404	4.5721	1.6696	14.0450	7.2560	1.6826	88.7270	90.0003	91.6355	90.8339	15.0943	11.8830	7.4674	16.7597												
L-proline	5.2203	4.6658	4.0804	2.9337	2.6343	9.8290	1.0684	0.7744	86.8026	86.4125	87.1838	88.4709	10.0272	19.1380	20.5549	18.5742												

Table 5. Values of Falkenhagen coefficient A, Jones-Dole coefficient B of amino acids in aqueous 10% 1,3-dioxolane (w/w) solutions at different temperatures

Amino acids	$A(\times \text{dm}^{-3/2} \text{ m}^{-1/2})$				$B(\times \text{dm}^3 \text{ mol}^{-1})$			
	Temperature (K)							
	298.15	303.15	313.15	323.15	298.15	303.15	313.15	323.15
L-valine	-0.0691	-0.0991	-0.0838	-0.0606	0.5086	0.6893	0.7366	0.6844
L-isoleucine	-0.0469	-0.0632	-0.0358	-0.0115	0.3055	0.4583	0.4619	0.4683
L-proline	-0.0501	-0.0479	-0.0361	-0.0464	0.6420	0.6647	0.6355	0.6586

$$\frac{\eta}{\eta_0} = 1 + Am^{1/2} + Bm \quad \dots (6)$$

where, η and η_0 are the viscosities of solution and the solvent (aqueous 1, 3 - dioxolane). A and B are the Falkenhagen (Falkenhagen and Dole, 1929, Falkenhagen and Vernon, 1932) and Jones-Dole (1929) coefficients, respectively, coefficient A accounts for the solute-solute interactions and B is a measure of structural modifications induced by the solute-solvent interactions (Feakins et al., 1974). The values of A and B were obtained as the intercept and slope from linear-regression of $[(\frac{\eta}{\eta_0} - 1)/c^{1/2}]$ vs. $c^{1/2}$ curves, which were found linear for these systems.

DISCUSSION

The trends observed in ϕ_v° values of amino acids in aqueous, 1, 3 - dioxolane may be due to their hydration behaviour, which comprises of following interactions in these systems (Kharakoz., 1991, Zhao *et al.*, 2005, Misra and Aluwalia, 1984, Aluwalia *et al.*, 1977, Kumar, 1997).

- The terminal groups of zwitterions of amino acids, NH_3^+ , and COO^- are hydrated in an electrostatic manner, whereas, hydration of the intervening backbone depends on its nature that may be hydrophobic, hydrophilic or amphiphilic.
- The overlap of hydration, co-spheres of terminal NH_3^+ and COO^- groups and of adjacent groups results in volume change. The ϕ_v° values increase due to reduction in the electrostriction at terminals, whereas, it decreases due to disruption of side group hydration by that of the charged end.

The values of ϕ_v° are positive and they increase with the rise in temperature for all three amino acids in aqueous, 1,3 - dioxolane solutions suggesting strong solute-solvent interactions. The observed positive ϕ_v° values indicate that the ion-hydrophilic and hydrophilic-hydrophilic group interactions exist in these systems.

The ϕ_v° values are in the sequence: Ile < pro < Val, which is also the order of the size of the side chain (hydrophobic group) of the three amino acids under study, i.e., the increases in ϕ_v° may be attributed to the increased hydrophobic/non-polar character of the side chain of these amino acids causing a reduction in electrostriction at the terminal charged groups. Ali *et al.* (2006) reported similar trends in ϕ_v° values with increasing size of the side chain of these amino acids and this may be rationalized by considering the structural changes occurring in the solution process. It has been pointed out by Franks *et al.* (1973) The ϕ_v° value (Table 4) increase with rise in temperature for all the three amino acids studied, which can be explained by considering the size of primary and secondary solvation layers around the dipolar ions. At higher temperatures, the solvent from the secondary solvation layer is released into the bulk of the solvent, resulting in the expansion of the solution (Ali *et al.*, 2007, Wadi and Ramasami, 1997). Furthermore, the S_v values,

Table 4, are positive for all three amino acids, suggesting strong solute-solute interactions.

The values of ϕ_k are negative (Table 3) for all the three amino acids in aqueous 1, 3 - dioxolane solutions, indicating that the water molecules around ionic charged groups of amino acids are less compressible than the water molecules in the bulk solution (Banerjee and Kishore, 2005). This further support the conclusion that the interaction of 1,3-dioxolane with amino acid zwitterions localized at the head groups decreases the electrostriction of water caused by the charged amino acids, resulting in an increase in volume, therefore, increasing the compressibility of the solution. The ϕ_k° values also increase with increase in 1, 3 - dioxolane concentrations due to increased 1, 3 - dioxolane - amino acid interactions. The ϕ_k° values also increase with increase in 1, 3 - dioxolane concentrations due to increased 1, 3 - dioxolane - amino acid interactions. The ϕ_k° values for these amino acids in the aqueous, 1, 3 - dioxolane solvents are in the sequence: Ile > pro > Val, which is in agreement with S_v values. The ϕ_k° values of are negative (table 4) and rise with an increase in temperature, indicating release of more water molecules from the secondary solvation layer into the bulk for all the three amino acids in aqueous 1, 3 - dioxolane solutions.

The values of A-coefficients are negative (Table 5), whereas those of B-coefficients are positive suggesting weak solute-solute and strong solute-solvent interactions in the amino acid solutions under study. Thus, the values of coefficients A and B support the behaviour of S_v , ϕ_k° and S_v , which suggest stronger solute-solvent interactions as compared to solute-solute interactions in these amino acid systems. Also, the increasing values of B-coefficients and decreasing values of A-coefficients with rise in temperature further support our earlier conclusion (drawn from the variations of S_v and ϕ_k° with temperature) that solute-solvent interactions increase with rise in temperature. The magnitude of B values is in the order: L-iso-leucine < L-proline < L-valine. This conclusion is in good agreement with that drawn from S_v and ϕ_k and S_k datas.

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