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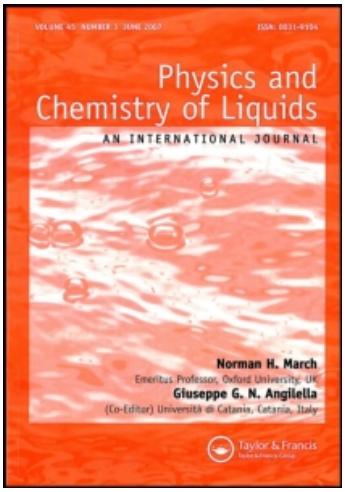

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Volumetric, viscometric and refractive index study of amino acids in mixed solvents at 308.15 K

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Densities, ρ, viscosities, η and refractive indices n_D for solutions of (0.10, 0.20, 0.30, 0.40 and 0.50 M) glycine (Gly), DL-alanine (Ala) and L-serine (Ser) in aqueous 1-propanol (10 and 30% v/v) at 308.15 K have been determined. These data have been used to calculate apparent molar volumes, ϕ_v, partial molar volumes, ϕ_v^0 and Falkenhagen and Jones–Dole coefficients, A and B, respectively. Free energies of activation of viscous flow, $\Delta\mu_1^{0\#}$ and $\Delta\mu_2^{0\#}$ per mole of solvent and solute, respectively, were obtained by using transition state theory to the B-coefficient data. The molar refractive indices, R_D were calculated from the experimental values of refractive indices for all the three amino acids + aqueous 1-propanol ternary mixtures. These parameters were used to discuss the solute–solute and solute–solvent interactions and also the effect of cosolvent (1-propanol) on these interactions.

Keywords: Partial molar volume; Viscosity B-coefficient; Activation energies; Amino acids; Mixed solvents

1. Introduction

Proteins are the biopolymers made up of the simpler monomers amino acids. Because of their complex nature, direct study of protein–water interaction is somewhat difficult. Therefore, a convenient approach for studying these interactions is to study the smaller amino acid molecule–water interactions, which participate in all the physiological processes of a living cell. This model compound approach has been widely used in the recent years [1–3].

A survey of the available literature reveals that a variety of thermodynamic properties, such as enthalpy of dissolution [4,5], partial molar heat capacity [6,7], partial molar volume [8–10], viscosity [11] and adiabatic compressibility [9,12] have been determined for aqueous solutions of amino acids. Recently, studies on amino acids in aqueous mixed solvents have drawn much attention [13–15]. This is because biological fluids are not pure water, but mixed solvents containing many organic and inorganic

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substances. Further, the mixed solvent (water + 1-propanol) chosen for the present study provides H-bonded environment (which plays a vital role in living systems) to amino acids for interaction. To the best of our knowledge, no study on interaction of amino acids (glycine, DL-alanine and L-serine) in aqueous 1-propanol has been reported from the point view of density, viscosity and refractive index measurements.

As a continuation of our work on volumetric, viscometric and refractive index investigations of amino acids in aqueous mixed solvent [15–17], the present work is aimed to get information regarding solute–solvent and solute–solute interactions, effect of the replacement of one H atom of Gly by $-CH_3$ group in DL-alanine and by $-CH_2OH$ group in L-serine molecule and, also, the effect of cosolvent (1-propanol) on the hydration behaviour of amino acids. These considerations led us to investigate the nature of interactions involved in amino acids + aqueous 1-propanol solutions.

The densities, ρ, viscosities, η and refractive indices n_D of (0.10, 0.20, 0.30, 0.40 and 0.50 M) glycine (Gly), DL-alanine (Ala) and L-serine (Ser) in aqueous 1-propanol (10 and 30% v/v) were measured at 308.15 K. From these experimental data, the derived parameters such as apparent molar volumes, ϕ_v, partial molar volumes, ϕ_v^0, Falkenhagen [18] and Jones–Dole [19] coefficients, A and B, respectively, free energies of activation of viscous flow, $\Delta\mu_1^{0*}$ and $\Delta\mu_2^{0*}$ per mole of solvent and solute respectively were obtained by using transition state theory, and the molar refractivities, R_D were derived. These simple thermodynamic parameters are found to be useful in the interpretation of solute–solvent/cosolvent and solute–solute interactions in the mixtures not easily obtained by other means.

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2. Experimental

High purity glycine (Merck, 99%), DL-alanine (Loba Chemicals, 99%) and L-serine (S.D. fine, 99%) were recrystallized twice from aqueous ethanol solutions and dried under vacuum at 373 K for 12 h. Then, they were stored over P_2O_5 in a desiccator before use. The 1-propanol (>99%) obtained from Merck was used without any further purification. Doubly distilled degassed water was used for preparation of the solutions. Aqueous 1-propanol (10 and 30% v/v) binary mixtures were used as a solvent to prepare solutions of 0.10, 0.20, 0.30, 0.40 and 0.50 M amino acids (Gly, Ala and Ser). All the solutions of ternary mixtures were prepared on molarity basis and were kept in airtight bottles. To prevent formation of air bubbles, all the solutions were preheated to 50°C above the measuring temperature before taking readings. The weighing was done on electronic balance Precisa XB–220A (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 single capillary pycnometer made of Borosil glass having a bulb capacity 8×10^{-3} dm^3 with graduated marks on the capillary. The marks on the capillary were calibrated by doubly distilled water. Viscosity measurements at 308.15 K were carried out with an Ubbelohde-type [20] suspended level viscometer with a flow time of 300 s for water. This viscometer was calibrated with pure water. The pycnometer and viscometer containing the test liquid were allowed to stand for about 30 min in thermostatic water bath so that thermal fluctuation was minimized. The accuracy in the density and viscosity measurements were found to be ± 0.01 kg m^{-3} and 3×10^{-6} N m^{-2} s, respectively. Refractive index was measured with the help of a thermostated Abbe refractometer to an accuracy of ± 0.0001. The calibration of the

refractometer was done by measuring the refractive indices of pure water and benzene at known temperatures [21]. All the measurements were repeated at least 3 times for each sample and were found to be reproducible within the precision quoted for the apparatuses. The temperature of the thermostated water bath (JULABO, Germany) was maintained within ±0.02 K.

3. Results and discussion

The experimentally measured values of ρ, η and n_D of the solutions of (0.10, 0.20, 0.30, 0.40 and 0.50 M) glycine, DL-alanine and L-serine in aqueous 1-propanol (10 and 30% v/v) at 308.15 K as a function of molarity of amino acids are reported in table 1. The densities were converted into apparent molar volumes, ϕ_v, of the amino acids in aqueous 1-propanol using the following equation:

$$\phi_v = \frac{M_2}{\rho_0} + \left[\frac{10^3(\rho_0 - \rho)}{C\rho_0}\right], \quad (1)$$

where C is the concentration of the solute (amino acid), ρ and ρ_o are the densities of the solution and solvent (aqueous 1-propanol), respectively, and M_2 is the molecular weight of the solute. The apparent molar volume can be considered to be a sum of the geometric volume of the solute molecule and changes in the solvent due to its interaction with the solute. The values of apparent molar volume at different molarities of amino acids are given in table 2. Since, for each amino acid studied, the ϕ_v was found to be a linear function of the molarity, C over the concentration range studied,

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Table 1. Densities, ρ, viscosities, η and refractive indices n_D of glycine, DL-alanine and L-serine in aqueous 1-propanol at 308.15 K.

C (mol L^{-1})	ρ (kg m^{-3})	η (10^{-3} N m^{-2} s)	n_D	ρ (kg m^{-3})	η (10^{-3} N m^{-2} s)	n_D
Gly + 10% 1-propanol				Gly + 30% 1-propanol		
0.0000	0.9809	0.9501	1.3391	0.9518	1.4226	1.3480
0.1000	0.9839	0.9615	1.3396	0.9552	1.4261	1.3485
0.2000	0.9870	0.9756	1.3415	0.9584	1.4420	1.3509
0.3000	0.9902	0.9906	1.3425	0.9615	1.4646	1.3531
0.4000	0.9935	1.0078	1.3440	0.9645	1.4875	1.3540
0.5000	0.9968	1.0310	1.3456	0.9673	1.5129	1.3558
DL-Ala + 10% 1-propanol				DL-Ala + 30% 1-propanol		
0.0000	0.9809	0.9501	1.3391	0.9518	1.4226	1.3480
0.1000	0.9839	0.9618	1.3399	0.9539	1.4479	1.3509
0.2000	0.9869	0.9824	1.3412	0.9566	1.4917	1.3522
0.3000	0.9898	1.0080	1.3423	0.9596	1.5336	1.3543
0.4000	0.9927	1.0287	1.3430	0.9627	1.5803	1.3553
0.5000	0.9956	1.0595	1.3440	0.9660	1.6155	1.3577
L-Ser + 10% 1-propanol				L-Ser + 30% 1-propanol		
0.0000	0.9809	0.9501	1.3391	0.9518	1.4226	1.3480
0.1000	0.9854	0.9663	1.3398	0.9597	1.4588	1.3520
0.2000	0.9899	0.9953	1.3414	0.9648	1.4954	1.3540
0.3000	0.9943	1.0205	1.3423	0.9683	1.5302	1.3559
0.4000	0.9986	1.0487	1.3438	0.9715	1.5647	1.3570
0.5000	1.0029	1.0717	1.3451	0.9745	1.6084	1.3591

Table 2. Apparent molar volume, ϕ_v, of glycine, DL-alanine and L-serine in aqueous 1-propanol at 308.15 K.

C (mol L^{-1})	ϕ_v (10^{-5} m^3 mol^{-1})	
	Gly + 10% 1-propanol	Gly + 30% 1-propanol
0.1000	4.6252	4.3150
0.2000	4.5589	4.4200
0.3000	4.5028	4.4901
0.4000	4.4493	4.5514
0.5000	4.4172	4.6302
	DL-Ala + 10% 1-propanol	DL-Ala + 30% 1-propanol
0.1000	6.0240	7.1527
0.2000	6.0444	6.8375
0.3000	6.0580	6.6274
0.4000	6.0750	6.4961
0.5000	6.0852	6.3752
	L-Ser + 10% 1-propanol	L-Ser + 30% 1-propanol
0.1000	6.0852	2.7411
0.2000	6.1260	4.2120
0.3000	6.1599	5.2626
0.4000	6.2024	5.8667
0.5000	6.2279	6.2712

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the standard-state (infinite dilution) partial molar volumes, ϕ_v^0 were obtained from the relation:

$$\phi_v = \phi_v^0 + S_v^* C^{1/2} \tag{2}$$

in which S_v^* is the experimental slope and is a measure of solute–solute interaction, while ϕ_v^0 provides information regarding solute–solvent interaction. The ϕ_v^0 and S_v^* values were obtained from the intercept and slope of ϕ_v *versus* $C^{1/2}$ plots. The evaluated values of ϕ_v^0 and S_v^* for all the three amino acids in aqueous 1-propanol are summarized in table 3.

It is well known that amino acids when dissolved in pure water exist predominantly as zwitterions. The hydration behaviour of amino acids can be explained by considering the following interactions [22–26]:

(i) The terminal ionic groups NH_3^+ and COO^- of zwitterions of amino acids are hydrated in an electrostatic manner, whereas, hydration of intervening backbone depends on its nature which may be hydrophobic, hydrophilic or amphiphilic.
(ii) Electrostriction of NH_3^+ group is greater than COO^- group by a factor of about 10.
(iii) The overlap of hydration cosphere of terminal NH_3^+ and COO^- groups and of adjacent groups results in volume change.

The observed values of ϕ_v^0 (table 3) are due to the net combined effect of the aforeseen possible interactions/processes in the ternary mixtures, amino acids + water +1-propanol. It is clear from the table that the values of ϕ_v^0 of all the three amino acids studied in 10% aqueous 1-propanol and those of Gly and Ala in 30% aqueous 1-propanol are large positive, suggesting stronger solute–solvent interactions. An increase in ϕ_v^0 from Gly to Ser in 10% aqueous 1-propanol may be due to the increase in hydrophobicity/nonpolar character of the side chain as the H atom of Gly is replaced by a hydrophobic group $-CH_3$ in Ala and by a relatively less hydrophobic

Table 3. Partial molar volume, ϕ_v^0, and its experimental slope, S_v^*, Falkenhagen coefficient, A, Jones–Dole coefficient, B and free energy of activation of viscous flow per mole of solvent, $\Delta\mu_1^{0\#}$ and solute $\Delta\mu_2^{0\#}$ for glycine, DL-alanine and L-serine in aqueous 1-propanol at 308.15 K.

	Gly + 10% 1-propanol	Gly + 30% 1-propanol
ϕ_v^0 (10^{-5} m^3 mol^{-1})	4.7986	4.0653
S_v^* (10^{-5} m^3 mol$^{-3/2}$ l$^{1/2}$)	−0.5431	0.7846
A (10^{-2} dm$^{3/2}$ mol$^{-1/2}$)	−3.0245	−6.1161
B (10^{-2} dm^3 mol^{-1})	20.4970	21.1250
$\Delta\mu_1^{0*}$ (kJ mol^{-1})	9.6865	10.8282
$\Delta\mu_2^{0\#}$ (kJ mol^{-1})	42.2243	41.7768
	DL-Ala + 10% 1-propanol	DL-Ala + 30% 1-propanol
ϕ_v^0 (10^{-5} m^3 mol^{-1})	5.9737	7.7491
S_v^* (10^{-5} m^3 mol$^{-3/2}$ l$^{1/2}$)	0.1577	−1.9831
A (10^{-2} dm$^{3/2}$ mol$^{-1/2}$)	−6.1140	−5.1560
B (10^{-2} dm^3 mol^{-1})	31.1470	35.1710
$\Delta\mu_1^{0*}$ (kJ mol^{-1})	9.6865	10.8282
$\Delta\mu_2^{0\#}$ (kJ mol^{-1})	58.6315	65.3617
	L-Ser + 10% 1-propanol	L-Ser + 30% 1-propanol
ϕ_v^0 (10^{-5} m^3 mol^{-1})	5.9634	0.0196
S_v^* (10^{-5} m^3 mol$^{-3/2}$ l$^{1/2}$)	0.3714	9.1505
A (10^{-2} dm$^{3/2}$ mol$^{-1/2}$)	−4.4724	−0.2498
B (10^{-2} dm^3 mol^{-1})	32.6050	25.9490
$\Delta\mu_1^{0*}$ (kJ mol^{-1})	9.6865	10.8282
$\Delta\mu_2^{0\#}$ (kJ mol^{-1})	60.6403	42.8122

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group –CH_2OH in Ser. As a result of (ii), the highly exposed N-terminal of Gly would cause the largest volume contraction followed by Ala in which N-terminal is shielded by –CH_3 group for electrostriction. The replacement of –CH_3 group of Ala by –CH_2OH group in Ser makes the latter a less hydrophobic than the former amino acids, resulting a slightly smaller ϕ_v^0 for Ser than for Ala. The shielding of N-terminal in Ala is further enhanced as the amount of 1-propanol (in other words, the amount of –C_3H_7 group) increases in 30% aqueous 1-propanol, resulting in a more pronounced increase in ϕ_v^0 from Gly to Ala. However, a sharp decrease in ϕ_v^0 for Ser suggests the weak solute–solvent interaction in 30% aqueous 1-propanol. Again, the small values of S_v^* for all the three amino acids in 10% aqueous 1-propanol and for Gly and Ala in 30% aqueous 1-propanol, while a large value of S_v^* for Ser in the latter solvent support our earlier view regarding ϕ_v^0. Similar conclusions were also reported by Banipal *et al.* [27]

The viscosity data are analyzed in the light of the Jones–Dole equation [19]

$$\eta_r = \eta/\eta_o = 1 + AC^{1/2} + BC \tag{3}$$

where η and η_o are the viscosities of solution and solvent (aqueous 1-propanol) respectively, η_r is the relative viscosity of the solution, A and B are the Falkenhagen [18] and the Jones–Dole [19] coefficients, respectively. A determines the solute–solute interactions and B measures the structural modification induced by the solute–solvent interactions [28,29]. The values of A and B are obtained from the intercepts and slopes of the plots of $(\eta_r - 1)/C^{1/2}$ *versus* $C^{1/2}$ and are summarized in table 3. Table 3 shows that the values of A coefficients for all the three amino acids are negative whereas those of B coefficients are larger positive, suggesting weak solute–solute and strong solute–solvent interactions in these ternary mixtures. Thus, the values of both A and B support the behaviour of ϕ_v^0 and S_v^* for the present amino acids mixtures.

Eyring *et al.* [30] calculated the free energy of activation of viscous flow, $\Delta\mu_1^{0*}$ per mole of solvent by using the relation:

$$\Delta\mu_1^{0*} = RT\ln(\eta_o\overline{V}_1^0/hN_A) \tag{4}$$

where $\overline{V}_1^0$, h and N_A are the partial molar volume of the solvent, Planck's constant and Avogadro's number, respectively. Feakins and coworkers [28,31] showed that if equations (3) and (4) are obeyed, then

$$B = [(\overline{V}_1^0 - \overline{V}_2^0) + \overline{V}_1^0(\Delta\mu_2^{0*} - \Delta\mu_1^{0*})/RT]/1000, \tag{5}$$

where $\overline{V}_2^0$ $(=\phi_v^0)$ is the partial molar volume of the solute (amino acid). After rearrangement, equation (5) yields:

$$\Delta\mu_2^{0*} = \Delta\mu_1^{0*} + \left(RT/\overline{V}_1^0\right)\left[1000\,B - \left(\overline{V}_1^0 - \overline{V}_2^0\right)\right] \tag{6}$$

The values of $\Delta\mu_1^{0*}$ and $\Delta\mu_2^{0*}$ are included in table 3. It is evident from table 3 that for all the three amino acids investigated, the values of $\Delta\mu_2^{0*}$ are positive and much larger than those of $\Delta\mu_1^{0*}$ in both the solvent mixtures. This suggests that the interactions between amino acids and solvent (aqueous 1-propanol) molecules in the ground state are stronger than in the transition state. Hence, in the transition state the solvation of the solute molecules is unfavourable in free energy terms. The values of $\Delta\mu_2^{0*}$, in general, are found to increase from Gly to Ser, indicating that the solvation of amino acids becomes unfavourable as the hydrophobic character of the side chain increases from Gly to Ser. Further, according to Feakins *et al.* [28], the solute (amino acids) in the aqueous 1-propanol solvent are found to be net structure makers because $\Delta\mu_2^{0*} > \Delta\mu_1^{0*}$.

The experimental refractive indices presented in table 4 show an increasing trend with increasing concentration of amino acids and 1-propanol in the mixtures. This indicates that the refractive index is directly related to the interactions in the solutions. For mixtures of interacting components the molar refractivity of each component is given by the equation:

$$R_D = 4\pi\alpha N \tag{7}$$

where N is the Avogadro's number and α is the molecular polarizability. The molar refractivity, R_D, of the mixtures can be calculated from the values of refractive indices, n_D, by using the Lorentz–Lorenz equation:

$$R_D = \left[\frac{n_D^2 - 1)}{(n_D^2 + 2)}\right]\left(\sum_{i=1}^{3} x_i M_i/\rho\right) \tag{8}$$

where x_i is the mole fraction and M_i is the molecular weight of the ith component of the mixture. The values of R_D at 308.15 K for the present systems are given in table 4 and its variation with concentration of the amino acids is shown in figure 1. As R_D is directly proportional to the molecular polarizability and is found to increase linearly with the increase in concentration of amino acids (figure 1) in both the solvent mixtures. This suggests that the overall polarizability of the ternary mixtures studied increases with the increasing amount of amino acids in the mixtures. Figure 1 reveals that this polarizability also increases with the increase in 1-propanol content of the mixtures.

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Table 4. Molar refractivity, R_D, of glycine, DL-alanine and L-serine in aqueous 1-propanol at 308.15 K.

C (mol L^{-1})	R_D (10^{-6}m^3 mol^{-1})	
	Gly + 10% 1-propanol	Gly + 30% 1-propanol
0.0000	3.8591	4.1208
0.1000	3.8750	4.1360
0.2000	3.9050	4.1726
0.3000	3.9254	4.2076
0.4000	3.9506	4.2292
0.5000	3.9771	4.2616
	DL-Ala + 10% 1-propanol	DL-Ala + 30% 1-propanol
0.0000	3.8592	4.1207
0.1000	3.8837	4.1735
0.2000	3.9137	4.2068
0.3000	3.9420	4.2478
0.4000	3.9664	4.2767
0.5000	3.9943	4.3201
	L-Ser + 10% 1-propanol	L-Ser + 30% 1-propanol
0.0000	3.8592	4.1207
0.1000	3.8829	4.1668
0.2000	3.9164	4.2039
0.3000	3.9432	4.2474
0.4000	3.9769	4.2841
0.5000	4.0088	4.3330

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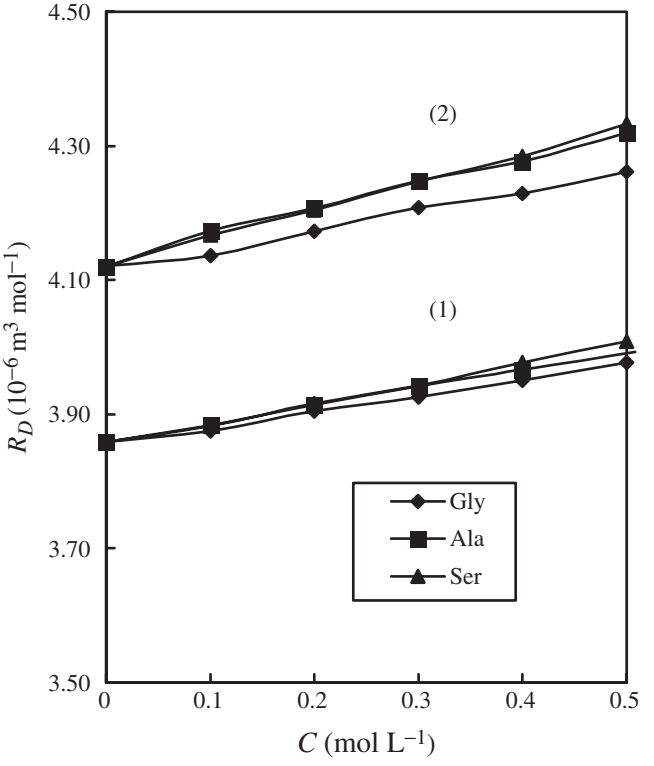

Figure 1. Variation of R_D, *vs.* C for ternary mixtures of amino acids + 10% 1-propanol + water (1) and amino acids + 30% 1-propanol + water (2) at 308.15 K.

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