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Thermodynamic study of solvation of some amino acids, diglycine and lysozyme in aqueous and mixed aqueous solutions

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Abstract

Apparent molar adiabatic compressibilities and viscosities of glycine, DL-α-alanine, DL-α-amino-*n*-butyric acid, L-valine, L-leucine and diglycine have been determined in aqueous and mixed aqueous solutions of $m_B = 1.0$, 2.0, 3.0, 4.0 and 5.0 aqueous *n*-propanol solutions at 298.15 K. From these data the partial molar adiabatic compressibilities and viscosity *B*-coefficients have been evaluated to calculate the corresponding transfer functions. The partial molar adiabatic compressibilities of transfer at infinite dilution $\Delta_{tr}K^{\circ}_{2,S}$ for all the studied model compounds are positive and increase with the concentration of *n*-propanol. Positive and negative *B*-coefficients of transfer $\Delta_{tr}B$ have been observed for the studied amino acids in lower and in higher concentration of *n*-propanol, respectively. The activation free energy for viscous flow in aqueous and mixed aqueous *n*-propanol solutions has been calculated from *B*-coefficient and partial molar volume data. Hydration numbers and interaction coefficients have also been calculated from these data. These parameters have been discussed in terms of solute–cosolvent interactions. Thermal denaturation of lysozyme has also been studied using UV-visible spectrophotometer in aqueous and in mixed aqueous solutions of *n*-propanol, 1,2-propandiol and glycerol. The thermodynamic parameters accompanying the thermal denaturation have been evaluated. The results have been explained on the basis of competing patterns of interactions of the cosolvents with the native ↔ denatured reaction. The preferential interaction parameters have been calculated from these thermodynamic data and by correlating the surface tension data of *n*-propanol and 1,2-propandiol to the surface area of the protein. Some parallelism in the patterns of interactions has been observed for the studied model compounds and protein in the aqueous solutions of these solvents.

Keywords: Adiabatic compressibility; Viscosity; Amino acids; Diglycine; Thermal denaturation; Lysozyme

1. Introduction

Proteins play a vital role in nearly all-chemical and biological processes. The detailed three-dimensional structure of proteins and nucleic acids provides critical information about the molecules but they provide no information about the stability of a molecule or the energetics of its interactions [1]. The interactions of water with the various functional groups of proteins are important factors in determining the conformational stability of proteins [2–4]. The study of native and denatured states is necessary to understand the role of hydration in protein folding/unfolding processes [5]. Thus in the recent years there has been considerable interest in the determination of various thermodynamic, transport, surface properties etc., of proteins and model compounds which mimic some aspects of protein and thus provide insight into protein hydration [5-14].

Simple low molecular weight compounds are extensively used as models for studies of such systems [5,6], which have at least two main advantages [6c]. The first is the relative ease of the microscopic interpretation of experimental data derived from the studies on low molecular weight model compounds, secondly one can systemically alter the structure, so that the contribution of a chosen atomic group can be addressed e.g. the side chain groups of the amino acid residues which constitute a protein represent a wide range of properties and are therefore capable of being involved in a range of interactions [14].

Different views have been reported about the factors responsible for the protein stability. It has generally been accepted [15a] that proteins are being stabilized because of hydrophobic effect, which arises from the peculiar features

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of the water structure. However, Murphy has reported [15b] that this simple view of protein stability is inadequate to account for experimental data. Makhatadze and Privalov have reported [16] that the simple binding model describes well all experimentally observed thermodynamic properties of proteins in the presence of urea and guanidine hydrochloride. Recently Franks has reported [17] about the protein stability in the article "Protein Stability: The value of old literature". He has strongly emphasized about the involvement of the hydrophobic rather than binding phenomenon for protein stability, which has been discussed in terms of alteration of water structure. He has also argued [17] that structure-breaking effect of urea results in weakening of hydrophobic interactions rather than binding which is responsible for solublizing and denaturing effects.

The effect of alcohols on proteins and model compounds is useful for considering how protein specific structures are stabilized in an aqueous environment. It is well known that typical monohydric alcohols such as methanol, ethanol, *n*-propanol generally destabilize [18,19] the native structure and stabilize [20–23] the α-helical conformation in unfolded proteins and peptides, however at lower concentration some alcohols have been found to slightly stabilize the native state of protein. In spite of large number of studies the mechanism through which alcohols affect the stability and solubility of protein remains unresolved. Further various studies have been reported about the peculiar behavior of alcohols and attempts have been made to rationalize their behavior in aqueous solutions. Koga and coworkers have reported [24] different mixing schemes for alcohols in aqueous solutions. They have reported that 1- and 2-propanol in aqueous solutions behave as typical "hydrophobic" or "structure making" in most water rich region (mixing scheme I) i.e. solute molecule enhance the hydrogen bonding net work of water in their immediate vicinity with a concomitant reduction in the hydrogen bond probability in the bulk away from the solute molecule. As the solute composition increases, the hydrogen bond probability in the bulk water is reduced to the bond percolation threshold and thus the mixture now consists of two kinds of clusters, each rich in each component (mixing scheme II). Mixing scheme III is operative in the most solute rich region where solute molecules form clusters of their own kind like in pure state. Similarly in case of glycerol, they have reported [24] that in water rich region the characteristics of water are reduced gradually as glycerol concentration increases but the essential features of liquid water are retained in region I. However the detailed picture is not yet emerged.

Thus in light of above picture of the aqueous solutions of alcohols it will be worthwhile to study systematically the amino acids, peptides and proteins in alcohols having different number of hydroxyl groups. These studies may shed some light on the mechanism that how the alcohols effect the stability of proteins. Therefore in continuation of our studies [9,10,25] on amino acids and peptides we report sound velocities and viscosities of model compounds (glycine, DL-α-alanine, DL-α-amino-*n*-butyric acid, L-valine, L-leucine and diglycine) in water and in $m_B = 1.0$, 2.0, 3.0, 4.0 and 5.0 aqueous solutions of *n*-propanol at 298.15 K. Partial molar adiabatic compressibilities ($K^{\circ}_{2,S}$), viscosity *B*-coefficients, their corresponding transfer functions, hydration numbers, side chain contributions of amino acids and interaction coefficients have been calculated. The activation free energy of viscous flow has also been calculated. Further we have investigated thermal denaturation of lysozyme in various mixed aqueous solutions of *n*-propanol, 1,2-propandiol and glycerol using UV-visible spectrophotometer. From this denaturation temperature T_m, enthalpy ΔH_m, free energy ΔG° and entropy ΔS_m have been evaluated. Preferential interaction parameters have also been calculated from these thermodynamic data and by correlating the surface tension data of aqueous solutions of *n*-propanol and 1,2-propandiol at 298.15 K to the surface area of the lysozyme. These parameters have been discussed in terms of various interactions.

2. Experimental

All the model compounds glycine (G-7126), DL-α-alanine (A-7502), DL-α-amino-*n*-butyric acid (A-1754), L-valine (V-0500), L-leucine (L-8000) and diglycine (G-7278) were procured from Sigma Chemical Co. and used after drying for 24 h in vacuum oven. The glycerol (AR 072929), from SRL and 1,2-propandiol (AR, 133378), from Thomas Baker were used as such and *n*-propanol (AR 1629104) from SRL was purified [25c] before use. Doubly distilled degassed water with specific conductance less than $10^{-6}\,\Omega^{-1}\,cm^{-1}$ was used for preparation of the solutions. All the solutions were prepared by mass on Mettler Balance within accuracy of ±0.01 mg. Hen egg white Lysozyme (L-6876) was also procured from Sigma Chemical Co. and the stock solution was prepared by extensive dialysis of the protein at 4 °C in double distilled deionized water at pH 2.0 with at least four changes. The pH of the solutions was measured using pH meter (Elico LI 127) at room temperature.

The Multifrequency Ultrasonic Interferometer (Model M-82, Mittal Enterprises) was employed for measurement of sound velocities [9,11c], with maximum uncertainty in velocity of $0.5\,m\,s^{-1}$. The interferometer was calibrated against the speeds of sound of aqueous sodium chloride at 298.15 K. The sound velocities were precise to be within $0.1\,m\,s^{-1}$. The average of 10 readings was treated as a final value of sound velocity. The temperature was maintained within 0.01 K by circulating water from constant temperature circulator bath (Model: MV F 25 Julabo/Germany). The values of adiabatic compressibilities, K_S are accurate to 0.06%.

Viscosity measurements were carried out using Ubellohde type capillary viscometer, which was calibrated with doubly distilled deionized water at four temperatures [10]. The efflux time was measured with electronic watch with

the resolution of 0.01 s. The average of at least five readings reproducible within 0.1 s was used as final efflux time. The temperature around viscometer was maintained within ±0.01 K. The measured viscosities were accurate to within ±0.001 mPa s.

Surface tension measurements were carried out by drop weight method. The rate of flow through the capillary was controlled to five to seven drops per minute. The temperature around the stalagmometer was maintained at 298.15 ± 0.1 K by circulating the water through the glass jacket and was stopped during the collection of drops to avoid the vibrations [13]. In each experiment the weight of 30 drops was measured on balance immediately after the collection.

The thermal denaturation experiments on lysozyme in water and in various mixed aqueous solutions of *n*-propanol, 1,2-propandiol and glycerol at pH = 2.0 were carried out using a Shimadzu 160-A UV-visible double beam spectrophotometer to which TCC-240-A temperature controller was attached. The temperature stability of the solutions in cuvettes was ±0.1 K. The concentration of lysozyme in all the experiments was ≈0.4 mg/ml. The reference was double distilled water or cosolvent solution when the measurements were made in water or in the presence of cosolvent, respectively. Thermal unfolding of protein was examined by observing the absorbance at around ≈280 nm as a function of temperature. Heating of the solutions in the cuvettes was started at a temperature below the thermal transition. The heating rate in these measurements was 0.4 K min^{-1}. To check the reversibility of thermal denaturation, the sample in first run was heated a little over complete denaturation temperature, cooled immediately and then reheated. The thermal transitions were reversible in all the cases and hence amenable to equilibrium thermodynamic analysis.

3. Results and discussion

3.1. Compressibilities and viscosities of amino acids and diglycine

The apparent molar adiabatic compressibilities $K_{2,S\phi}$ for amino acids and diglycine in aqueous and mixed aqueous solutions at 298.15 K (Table 1) were determined using the following equation:

$$K_{2,S\phi} = \frac{K_S^\circ M}{d} - \frac{K_S^\circ d - K_S d^\circ}{m_A d d^\circ} \quad (1)$$

where M is the molecular mass of the solute; d and d°, K_S and K_S° the densities [24] and adiabatic compressibilities of solution and solvent, respectively; and m_A molality of the solution. The adiabatic compressibilities of solvent/solutions were calculated from the corresponding sound velocities (U) and densities as

$$K_S = \frac{1}{U^2 d} \quad (2)$$

At infinite dilution the apparent molar adiabatic compressibility becomes equal to partial molar adiabatic compressibility $K_{2,S}^\circ$. The $K_{2,S}^\circ$ values were calculated from $K_{2,S\phi}$ data using the following equation:

$$K_{2,S\phi} = K_{2,S}^\circ + S_K m_A \quad (3)$$

where S_K is the slope. In most of the cases the $K_{2,S}^\circ$ were determined by taking average of all the data points. The $K_{2,S}^\circ$ values are given in Table 2, along with literature values [9,26,27]. The $K_{2,S}^\circ$ values increase with the increase in concentration of *n*-propanol.

The viscosities (η) of the solutions were determined from the following equation:

$$\frac{\eta}{d} = at - \frac{b}{t} \quad (4)$$

where t is the efflux time, a and b the constants for viscometer. The relative viscosities, η_r ($\eta_r = \eta/\eta_0$, where η and η_0 are viscosities of the solution and solvent, respectively) of the studied compounds in water and in various mixed aqueous *n*-propanol solutions are summarized in Table 3 . The *B*-coefficients were calculated by using the following Jones–Dole equation by least squares method:

$$\eta_r = \frac{\eta}{\eta_0} = 1 + Bc \quad (5)$$

where c is the molarity (calculated from molality) of the solution. The values of *B*-coefficients in water are given in Table 4 which agree well with literature values except in case of L-leucine, where the present value is higher which may be due to different form of leucine [28]. Positive values of *B*-coefficients for the studied amino acids increase with increase in size of the side chain of amino acids both in water and in aqueous *n*-propanol solutions.

The $K_{2,S}^\circ$ and viscosity *B*-coefficient data in aqueous and in mixed aqueous solutions have been used to calculate corresponding transfer functions at infinite dilution ($\Delta_{tr}K_{2,S}^\circ$ and $\Delta_{tr}B$, respectively) as follows:

$$\Delta_{tr}K_{2,S}^\circ/\Delta_{tr}B = K_{2,S}^\circ/B\text{-coefficient (in aqueous } n\text{-propanol solution)} - K_{2,S}^\circ/B\text{-coefficient (in water)} \quad (6)$$

The $\Delta_{tr}K_{2,S}^\circ$ and $\Delta_{tr}B$ values are summarized in Tables 5 and 6 and illustrated in Figs. 1 and 2, respectively. Fig. 1 shows that $\Delta_{tr}K_{2,S}^\circ$ values are positive for the amino acids and diglycine which increase with the increase in concentration of *n*-propanol. In case of glycine $\Delta_{tr}K_{2,S}^\circ$ values increase almost linearly in lower concentration range while tend to level off at higher concentration. $\Delta_{tr}K_{2,S}^\circ$ values increase non-linearly in case of DL-α-alanine, DL-α-amino-*n*-butyric acid, L-valine and diglycine with the increase in concentration of *n*-propanol and have little tendency (more in case of diglycine) to level off at higher concentration. $\Delta_{tr}K_{2,S}^\circ$ values show linear behavior with concentration of *n*-propanol in case of L-leucine. In the lower

Table 1
Apparent molar adiabatic compressibilities $K_{2,S\phi}$ of some amino acids and diglycine in water and in aqueous solutions of *n*-propanol at 298.15 K

m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})	m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})
Glycine in water					
0.09803	1504.30	−26.19	0.13721	1506.33	−25.60
0.17739	1508.45	−25.38	0.20348	1509.78	−25.14
0.22549	1510.87	−24.90			
Glycine in aqueous *n*-propanol solutions					
$m_B = 1.0$ ($U_0 = 1544.87$)					
0.22232	1555.07	−20.43	0.30167	1559.07	−20.91
0.30776	1559.27	−20.74	0.41683	1564.67	−20.90
0.46627	1566.67	−20.39	0.54250	1570.67	−20.66
$m_B = 2.0$ ($U_0 = 1579.35$)					
0.17819	1585.55	−13.65	0.29105	1589.35	−13.18
0.32389	1590.75	−13.54	0.38009	1592.55	−13.17
0.43760	1595.75	−14.45			
$m_B = 3.0$ ($U_0 = 1593.75$)					
0.24211	1600.07	−8.67	0.27910	1601.07	−8.72
0.31904	1602.07	−8.65	0.36518	1604.03	−9.71
0.46762	1606.83	−9.64			
$m_B = 4.0$ ($U_0 = 1580.55$)					
0.17443	1583.27	−2.67	0.20895	1583.85	−2.74
0.26900	1584.99	−3.07	0.40040	1587.13	−2.94
0.49979	1588.95	−3.07			
$m_B = 5.0$ ($U_0 = 1557.23$)					
0.19641	1559.03	0.82	0.25007	1559.63	0.66
0.32461	1560.33	0.80	0.33680	1560.53	0.67
0.39298	1561.03	0.82			
DL-α-Alanine in water					
0.08231	1504.15	−25.30	0.13261	1507.45	−24.96
0.18631	1510.93	−24.76	0.25324	1515.23	−24.13
0.32264	1519.70	−23.95			
DL-α-Alanine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.10071	1551.07	−21.07	0.16033	1554.87	−21.48
0.23525	1559.67	−21.67	0.26319	1561.27	−21.29
0.28808	1563.07	−21.72			
$m_B = 2.0$					
0.14394	1587.75	−17.95	0.21204	1591.53	−17.61
0.25749	1594.33	−17.97	0.33249	1598.35	−17.34
0.36407	1600.33	−17.53			
$m_B = 3.0$					
0.12576	1599.15	−10.16	0.16215	1600.75	−10.21
0.18952	1602.15	−10.77	0.22240	1603.55	−10.59
0.31322	1607.35	−10.17			
$m_B = 4.0$					
0.14509	1585.37	−5.33	0.18627	1586.67	−5.15
0.21495	1587.57	−4.94	0.25635	1588.97	−4.98
0.26739	1589.33	−4.96			
$m_B = 5.0$					
0.09950	1559.44	1.00	0.16722	1560.84	1.18
0.20236	1561.64	1.08	0.23415	1562.24	1.34
0.29415	1563.44	1.62			
DL-α-Amino-*n*-butyric acid in water					
0.11688	1507.84	−25.16	0.19445	1514.04	−25.30
0.20062	1514.64	−25.62	0.22534	1516.64	−25.67
0.25542	1519.04	−25.64	0.30306	1522.83	−25.58

Table 1 (*Continued*)

m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})	m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})
DL-α-Amino-*n*-butyric acid in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.06724	1550.03	−23.50	0.11236	1553.47	−23.28
0.13807	1555.44	−23.19	0.16156	1557.27	−23.28
0.19634	1560.07	−23.57			
$m_B = 2.0$					
0.07735	1584.75	−18.94	0.11377	1587.35	−19.10
0.12785	1588.35	−19.10	0.16141	1590.75	−19.12
0.17993	1592.03	−18.98			
$m_B = 3.0$					
0.09807	1598.55	−7.76	0.14240	1600.55	−7.05
0.16825	1601.95	−7.50	0.20291	1603.55	−7.20
0.22812	1604.75	−7.10			
$m_B = 4.0$					
0.08887	1584.23	−4.01	0.12344	1585.73	−4.21
0.15890	1587.23	−4.15	0.17721	1588.03	−4.19
0.23244	1590.35	−4.00			
$m_B = 5.0$					
0.11542	1560.72	2.13	0.13310	1561.32	1.91
0.14095	1561.52	2.10	0.15017	1561.84	1.97
0.16597	1562.32	2.03			
L-Valine in water					
0.03271	1502.94	−30.05	0.04359	1503.04	−30.39
0.04824	1503.44	−29.61	0.05547	1504.24	−30.57
0.06271	1504.84	−29.42	0.08321	1506.82	−29.95
L-Valine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.04038	1548.67	−26.57	0.04567	1549.07	−26.36
0.06573	1551.07	−26.60	0.07585	1551.93	−25.88
0.09093	1553.33	−25.82			
$m_B = 2.0$					
0.05851	1584.18	−19.62	0.07248	1585.33	−19.54
0.07738	1585.73	−19.49	0.09492	1587.23	−19.70
0.10361	1587.93	−19.55			
$m_B = 3.0$					
0.04274	1596.75	−13.37	0.05512	1597.63	−13.35
0.08083	1599.43	−13.06	0.09951	1600.75	−12.94
0.12198	1602.43	−13.18			
$m_B = 4.0$					
0.03407	1582.00	0.74	0.05974	1583.20	0.47
0.08127	1584.23	0.29	0.09709	1584.95	0.45
0.10853	1585.45	0.66			
$m_B = 5.0$					
0.03933	1558.53	6.31	0.04970	1558.88	6.50
0.07846	1559.93	6.20	0.09026	1560.38	6.02
0.11886	1561.38	6.20			
L-Leucine in water					
0.04238	1503.34	−31.38	0.04658	1503.84	−31.50
0.06236	1505.64	−31.22	0.08782	1508.64	−31.30
0.10527	1510.64	−31.09			
L-Leucine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.01801	1546.67	−22.97	0.02501	1547.43	−22.45
0.04354	1549.27	−22.72	0.06152	1551.07	−22.40
0.07066	1552.07	−22.96			
$m_B = 2.0$					
0.01421	1580.57	−13.83	0.02872	1581.83	−14.09

Table 1 (*Continued*)

m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})	m_A (mol kg^{-1})	U (m s^{-1})	$10^{15} \times K_{2,S\phi}$ (m^3 mol^{-1} Pa^{-1})
0.04022	1582.83	−14.12	0.05876	1584.43	−13.95
0.09223	1587.33	−13.73			
$m_B = 3.0$					
0.01617	1594.83	−3.77	0.02345	1595.33	−3.93
0.03125	1595.83	−3.38	0.04660	1596.88	−3.48
0.05494	1597.48	−3.75			
$m_B = 4.0$					
0.00720	1580.93	4.05	0.01608	1581.40	4.00
0.02101	1581.66	4.06	0.03122	1582.20	4.18
0.04581	1582.60	4.72			
$m_B = 5.0$					
0.00980	1557.58	14.65	0.01723	1557.83	15.23
0.02627	1558.17	14.76	0.03132	1558.33	15.18
0.04275	1558.73	15.30			
Diglycine in water					
0.07242	1504.44	−40.10	0.09379	1506.24	−39.71
0.12138	1508.64	−39.77	0.16838	1512.64	−39.76
0.20998	1516.24	−39.52	0.21813	1516.84	−39.24
Diglycine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.09405	1552.27	−35.27	0.11533	1554.07	−35.72
0.15656	1557.43	−35.71	0.19121	1560.27	−35.67
0.21125	1561.87	−35.51			
$m_B = 2.0$					
0.10805	1587.23	−30.65	0.12706	1588.53	−30.11
0.15149	1590.37	−30.17	0.17793	1592.63	−30.96
0.21158	1595.23	−30.96			
$m_B = 3.0$					
0.09324	1598.35	−17.98	0.10668	1598.95	−17.61
0.12931	1600.15	−17.90	0.15748	1601.75	−18.44
0.17913	1602.75	−18.05			
$m_B = 4.0$					
0.08207	1583.33	−9.91	0.11090	1584.43	−10.42
0.14772	1585.75	−10.39	0.16053	1586.33	−10.77
0.17848	1586.95	−10.61			
$m_B = 5.0$					
0.08277	1559.23	−5.20	0.10697	1559.93	−5.54
0.12148	1560.23	−5.10	0.14015	1560.83	−5.53
0.16694	1561.43	−5.07			

Table 2
Partial molar adiabatic compressibilities $K^{\circ}_{2,S}$ of some amino acids and diglycine in water and in aqueous solutions of *n*-propanol at 298.15 K[a]

Compound	$10^{15} \times K^{\circ}_{2,S}$ (m^3 mol^{-1} Pa^{-1})					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	−27.04 ± 0.09 (9.49), −27.0[b], −27.09[c]	−20.67 ± 0.22	−13.60 ± 0.47	−9.08 ± 0.49	−2.90 ± 0.17	0.75 ± 0.09
DL-α-Alanine	−25.69 ± 0.13 (5.75), −25.03[b],−26.28[c]	−21.45 ± 0.24	−17.68 ± 0.24	−10.38 ± 0.25	−5.07 ± 0.15	1.24 ± 0.22
DL-α-Amino-*n*-butyric acid	−25.56 ± 0.20	−23.36 ± 0.14	−19.05 ± 0.07	−7.32 ± 0.28	−4.11 ± 0.09	2.03 ± 0.08
L-Valine	−30.0 ± 0.48, −30.62[b], −29.82[d]	−26.05 ± 0.39	−19.58 ± 0.07	−13.18 ± 0.16	0.52 ± 0.16	6.25 ± 0.16
L-Leucine	−31.30 ± 0.16, −31.78[b], −31.59[d]	−22.70 ± 0.24	−13.94 ± 0.15	−3.66 ± 0.20	4.20 ± 0.26	15.02 ± 0.26
Diglycine	−39.68 ± 0.26, 39.92[c]	−35.58 ± 0.17	−30.57 ± 0.37	−18.00 ± 0.27	−10.41 ± 0.29	−5.29 ± 0.21

[a] Slopes are in parenthesis.
[b] Ref. [26].
[c] Ref. [9].
[d] Ref. [27].

Table 3
Relative viscosities η_r of some amino acids and diglycine in water and in aqueous solutions of *n*-propanol at 298.15 K

c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r
Glycine in water					
0.03811	1.0019	0.06468	1.0089	0.13163	1.0193
0.20500	1.0309	0.26758	1.0380	0.36481	1.0521
Glycine in aqueous *n*-propanol solutions					
$m_B = 1.0$ ($\eta_0 = 1.1408$)					
0.21758	1.0360	0.30010	1.0459	0.40455	1.0613
0.45158	1.0688	0.52369	1.0810		
$m_B = 2.0$ ($\eta_0 = 1.4059$)					
0.17349	1.0195	0.28201	1.0359	0.31338	1.0393
0.36686	1.0483	0.42132	1.0556		
$m_B = 3.0$ ($\eta_0 = 1.6514$)					
0.23352	1.0243	0.26877	1.0333	0.30671	1.0388
0.35039	1.0444	0.44675	1.0542		
$m_B = 4.0$ ($\eta_0 = 1.8568$)					
0.16754	1.0225	0.20039	1.0256	0.25730	1.0319
0.38078	1.0407	0.47325	1.0500		
$m_B = 5.0$ ($\eta_0 = 2.0197$)					
0.18706	1.0194	0.23760	1.0242	0.30741	1.0349
0.31879	1.0364	0.37104	1.0404		
DL-α-Alanine in water					
0.06650	1.0151	0.12286	1.0311	0.34544	1.0736
0.39707	1.1041	0.49568	1.1214	0.55811	1.1479
0.72002	1.2057				
DL-α-Alanine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.09891	1.0260	0.15692	1.0435	0.22923	1.0627
0.25603	1.0702	0.27983	1.0807		
$m_B = 2.0$					
0.14002	1.0388	0.20544	1.0530	0.24882	1.0631
0.31988	1.0825	0.34 962	1.0904		
$m_B = 3.0$					
0.12166	1.0275	0.15653	1.0366	0.18266	1.0432
0.21394	1.0514	0.29971	1.0727		
$m_B = 4.0$					
0.13923	1.0297	0.17832	1.0370	0.20543	1.0450
0.24439	1.0612	0.25475	1.0636		
$m_B = 5.0$					
0.09502	1.0209	0.15906	1.0328	0.19208	1.0401
0.22184	1.0482	0.27770	1.0625		
DL-α-Amino-*n*-butyric acid in water					
0.03852	1.0087	0.07040	1.0193	0.10580	1.0332
0.12496	1.0392	0.16286	1.0521	0.17981	1.0583
0.22655	1.0778				
DL-α-Amino-*n*-butyric acid in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.06611	1.0244	0.11011	1.0346	0.13505	1.0471
0.15775	1.0560	0.19123	1.0669		
$m_B = 2.0$					
0.07546	1.0253	0.11070	1.0372	0.12427	1.0422
0.15651	1.0519	0.17424	1.0600		
$m_B = 3.0$					
0.09490	1.0324	0.13736	1.0433	0.16199	1.0554
0.19487	1.0605	0.21868	1.0707		
$m_B = 4.0$					
0.08546	1.0244	0.11841	1.0365	0.15203	1.0475
0.16932	1.0542	0.22120	1.0705		

Table 3 (*Continued*)

c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r
$m_B = 5.0$					
0.10995	1.0314	0.12663	1.0362	0.13402	1.0396
0.14269	1.0450	0.15752	1.0469		
L-Valine in water					
0.03378	1.0109	0.04678	1.0181	0.05430	1.0218
0.05846	1.0235	0.07333	1.0296	0.08770	1.0360
0.08816	1.0370				
L-Valine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.03976	1.0173	0.04494	1.0193	0.06457	1.0260
0.07445	1.0337	0.08913	1.0404		
$m_B = 2.0$					
0.05711	1.0239	0.07066	1.0298	0.07541	1.0328
0.09236	1.0383	0.10074	1.0445		
$m_B = 3.0$					
0.04150	1.0188	0.05347	1.0238	0.07823	1.0330
0.09615	1.0397	0.11763	1.0478		
$m_B = 4.0$					
0.03288	1.0108	0.05752	1.0205	0.07810	1.0294
0.09318	1.0353	0.10405	1.0430		
$m_B = 5.0$					
0.03766	1.0109	0.04754	1.0162	0.07486	1.0267
0.08603	1.0316	0.11301	1.0418		
L-Leucine in water					
0.01966	1.0098	0.02849	1.0165	0.03675	1.0211
0.04485	1.0260	0.05692	1.0343	0.06748	1.0392
0.08143	1.0469				
L-Leucine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.01776	1.0155	0.02465	1.0167	0.04283	1.0280
0.06040	1.0368	0.06930	1.0418		
$m_B = 2.0$					
0.01392	1.0126	0.02809	1.0201	0.03930	1.0262
0.05730	1.0338	0.08963	1.0488		
$m_B = 3.0$					
0.01573	1.0104	0.02280	1.0134	0.03036	1.0156
0.04520	1.0238	0.05324	1.0274		
$m_B = 4.0$					
0.00696	1.0042	0.01554	1.0077	0.02029	1.0107
0.03012	1.0139	0.03797	1.0181		
$m_B = 5.0$					
0.00472	1.0019	0.00941	1.0042	0.01652	1.0083
0.02517	1.0108	0.02999	1.0120		
Diglycine in water					
0.07182	1.0268	0.09286	1.0338	0.11992	1.0419
0.16574	1.0585	0.20603	1.0717	0.21390	1.0751
Diglycine in aqueous *n*-propanol solutions					
$m_B = 1.0$					
0.09227	1.0314	0.11297	1.0376	0.15288	1.0439
0.18622	1.0615	0.20544	1.0679		
$m_B = 2.0$					
0.10516	1.0287	0.12348	1.0372	0.14695	1.0419
0.17226	1.0525	0.20432	1.0644		
$m_B = 3.0$					
0.09024	1.0262	0.10315	1.0318	0.12482	1.0380
0.15169	1.0460	0.17226	1.0503		

Table 3 (*Continued*)

c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r	c (mol dm^{-3})	η_r
$m_B = 4.0$					
0.07894	1.0227	0.10644	1.0305	0.14140	1.0435
0.15350	1.0462	0.17043	1.0521		
$m_B = 5.0$					
0.07902	1.0207	0.10194	1.0313	0.11564	1.0349
0.13322	1.0392	0.15836	1.0483		

Table 4
Viscosity *B*-coefficients of some amino acids in water and in aqueous solutions of *n*-propanol at 298.15 K

Compound	*B*-coefficient (dm^3 mol^{-1})					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	0.143 ± 0.004, 0.143[a], 0.146[b]	0.154 ± 0.005	0.129 ± 0.008	0.122 ± 0.008	0.112 ± 0.006	0.110 ± 0.005
DL-α-Alanine	0.251 ± 0.004, 0.249[a], 0.258[b]	0.279 ± 0.009	0.258 ± 0.009	0.239 ± 0.006	0.235 ± 0.006	0.218 ± 0.007
DL-α-Amino-*n*-butyric acid	0.325 ± 0.005, 0.352[b]	0.347 ± 0.007	0.338 ± 0.004	0.323 ± 0.008	0.315 ± 0.005	0.298 ± 0.008
L-Valine	0.423 ± 0.005, 0.447[b,c], 0.405[d]	0.440 ± 0.008	0.428 ± 0.007	0.417 ± 0.007	0.386 ± 0.021	0.361 ± 0.008
L-Leucine	0.576 ± 0.008, 0.487[b,e]	0.625 ± 0.019	0.584 ± 0.039	0.529 ± 0.023	0.483 ± 0.027	0.427 ± 0.019
Diglycine	0.352 ± 0.006, 0.315[c]	0.323 ± 0.006	0.302 ± 0.016	0.299 ± 0.007	0.301 ± 0.009	0.298 ± 0.006

[a] Ref. [28(a)].
[b] Ref. [28(b)].
[c] Value for DL-valine.
[d] Ref. [28(c)].
[e] Value for DL-leucine.

Table 5
Partial molar adiabatic compressibilities of transfer $\Delta_{tr}K^{\circ}_{2,S}$ of some amino acids and diglycine from water to aqueous *n*-propanol solutions at 298.15 K

Compound	$10^{15} \times \Delta_{tr}K^{\circ}_{2,S}$ (m^3 mol^{-1} Pa^{-1})				
	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	6.37 ± 0.24	13.44 ± 0.48	17.96 ± 0.50	24.14 ± 0.19	27.79 ± 0.13
DL-α-Alanine	4.24 ± 0.27	8.01 ± 0.27	15.31 ± 0.27	20.62 ± 0.20	26.93 ± 0.25
DL-α-Amino-*n*-butyric acid	2.20 ± 0.24	6.51 ± 0.21	18.24 ± 0.34	21.45 ± 0.22	27.59 ± 0.21
L-Valine	3.95 ± 0.62	10.42 ± 0.49	16.82 ± 0.50	30.52 ± 0.50	36.25 ± 0.50
L-Leucine	8.60 ± 0.29	17.36 ± 0.22	27.64 ± 0.26	35.50 ± 0.30	46.32 ± 0.30
Diglycine	4.10 ± 0.31	9.11 ± 0.45	21.68 ± 0.37	29.27 ± 0.40	34.39 ± 0.33

concentration range of *n*-propanol $\Delta_{tr}K^{\circ}_{2,S}$ values decrease from glycine to DL-α-alanine to DL-α-amino-*n*-butyric acid and starts increasing from L-valine (although less than in glycine) to L-leucine. $\Delta_{tr}K^{\circ}_{2,S}$ for DL-α-alanine, DL-α-amino-*n*-butyric acid, L-valine and diglycine show a slight dip in the plots of $\Delta_{tr}K^{\circ}_{2,S}$ vs m_B. Dip has also been observed [25c] earlier in case of partial molar volume data in the lower concentration range. At higher concentration $\Delta_{tr}K^{\circ}_{2,S}$ values are almost same for glycine, DL-α-alanine and DL-α-amino-*n*-butyric acid whereas values are more

Table 6
Viscosity *B*-coefficients of transfer $\Delta_{tr}B$ of some amino acids and diglycine from water to aqueous *n*-propanol solutions at 298.15 K

Compound	$\Delta_{tr}B$ (dm^3 mol^{-1})				
	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	0.011 ± 0.006	−0.014 ± 0.009	−0.021 ± 0.009	−0.031 ± 0.007	−0.033 ± 0.006
DL-α-Alanine	0.028 ± 0.010	0.007 ± 0.010	−0.012 ± 0.007	−0.016 ± 0.007	−0.033 ± 0.008
DL-α-Amino-*n*-butyric acid	0.025 ± 0.009	0.013 ± 0.006	−0.002 ± 0.009	−0.010 ± 0.007	−0.027 ± 0.009
L-Valine	0.017 ± 0.009	0.005 ± 0.008	−0.006 ± 0.008	−0.037 ± 0.021	−0.062 ± 0.008
L-Leucine	0.049 ± 0.020	0.008 ± 0.039	−0.047 ± 0.024	−0.093 ± 0.028	−0.149 ± 0.020
Diglycine	−0.029 ± 0.008	−0.050 ± 0.017	−0.053 ± 0.009	−0.051 ± 0.010	−0.054 ± 0.008

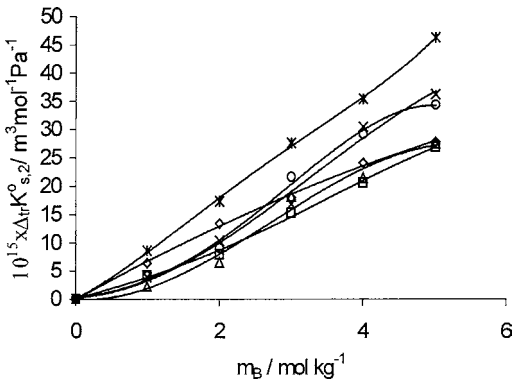

Fig. 1. Partial molar adiabatic compressibilities of transfer of some amino acids and diglycine vs concentration of *n*-propanol: (◇) glycine; (□) DL-α-alanine; (△) DL-α-amino-*n*-butyric acid; (×) L-valine; (✕) L-leucine; (○) diglycine.

in case of L-valine and L-leucine. Both positive and negative $\Delta_{tr}B$ values have been observed for the amino acids studied (at lower and higher concentration, respectively). $\Delta_{tr}B$ values are negative for diglycine and decrease almost linearly up to $m_B \approx 2.0$ and then get leveled off at higher concentration of *n*-propanol (Fig. 2). Further $\Delta_{tr}B$ values show maxima around $m_B \approx 1.0$ *n*-propanol for the amino acids. After passing through maxima the $\Delta_{tr}B$ values decrease with increase in concentration of *n*-propanol and the decrease is sharp for L-valine and very sharp for L-leucine. Similarly sharp changes in partial molar volumes of transfer ($\Delta_{tr}V^{\circ}_2$) and partial molar adiabatic compressibilities of transfer ($\Delta_{tr}K^{\circ}_{2,S}$) for L-leucine have been observed at higher concentration of *n*-propanol.

The $\Delta_{tr}V^{\circ}_2$ values [25c] are positive for glycine, DL-α-alanine and diglycine throughout the studied range of *n*-propanol. However for DL-α-amino-*n*-butyric acid and L-leucine both positive and negative $\Delta_{tr}V^{\circ}_2$ values have been observed, where as $\Delta_{tr}V^{\circ}_2$ is negative for L-valine throughout the concentration range of *n*-propanol. The minima in $\Delta_{tr}V^{\circ}_2$ have been observed for latter three amino acids, which lie around $m_B \approx 2.5$ *n*-propanol and the magnitude of $\Delta_{tr}V^{\circ}_2$ at minima increased with increase in

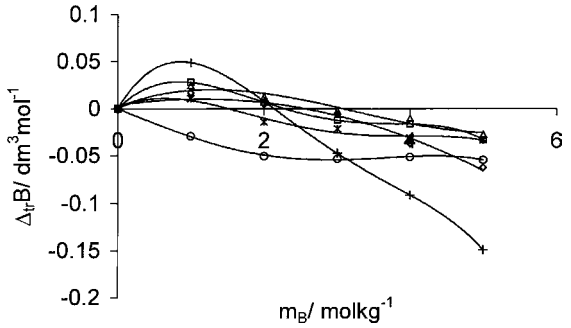

Fig. 2. Viscosity *B*-coefficients of transfer of some amino acids and diglycine vs concentration of *n*-propanol: (✕) glycine; (□) DL-α-alanine; (△) DL-α-amino-*n*-butyric acid; (◇) L-valine; (+) L-leucine; (○) diglycine.

side chain of amino acids. In case of glycine, DL-α-alanine and diglycine, ion/hydrophilic–hydrophilic interactions are dominating throughout the concentration of *n*-propanol. For other amino acids the hydrophobic–hydrophobic or hydrophilic–hydrophobic interactions are dominating at lower concentration, and at higher concentration, there is a tendency of cooperative aggregation of side chains of amino acids and that of hydrophobic part of *n*-propanol. The surface properties of aqueous *n*-propanol solutions have shown [29] that *n*-propanol forms clathrate like structures in water. Presently the increase in viscosity with the increase in concentration of *n*-propanol in aqueous solutions also indicates the increase in structure.

Positive $\Delta_{tr}K^{\circ}_{2,S}$ values for glycine indicate the dominance of ion/hydrophilic–hydrophilic interactions, where the overlap of cospheres of *n*-propanol and glycine results in release of water to the bulk, which is more compressible than the water in electrostricted region. At the lower concentration range, a small dip in $\Delta_{tr}K^{\circ}_{2,S}$ values in case of DL-α-alanine and DL-α-amino-*n*-butyric acid (Fig. 1), suggests that increase in hydrophobic/non-polar side chain results in disruption of hydration sphere of charged centers of amino acids and thus the positive contribution to $\Delta_{tr}K^{\circ}_{2,S}$ gets reduced. Further increase in side-chain, as in case of L-valine, $\Delta_{tr}K^{\circ}_{2,S}$ starts increasing which suggests that hydrophobic–hydrophobic interactions also start showing their effect due to decrease in number of contacts of non-polar groups with water, in addition to the tendency to disrupt the hydration sphere of charged end centers which results in increase in $\Delta_{tr}K^{\circ}_{2,S}$. Thus there is a balance between the various competing forces, exhibiting almost linear behavior of $\Delta_{tr}K^{\circ}_{2,S}$ in case of L-leucine. In the higher concentration range the more positive $\Delta_{tr}K^{\circ}_{2,S}$ values further strengthens our earlier view that there is tendency of cooperative aggregation of side chain of amino acids with non-polar part of *n*-propanol.

In the lower concentration range of *n*-propanol the positive $\Delta_{tr}B$ values may be attributed to the more structured medium in the presence of *n*-propanol in this region. As described earlier the $\Delta_{tr}B$ values decrease after passing through maximum and become negative which may be due to the cooperative aggregation between the side chains of the amino acids and *n*-propanol as discussed in case of volume and compressibility results.

The values of *B*-coefficients for the amino acids reflect the net structural effects of the charged end and hydrophobic groups on the amino acids [11a,b,30]. These two effects can be separated by noting that *B*-coefficients are linear in n_C i.e.:

$$B = B(^{+}NH_3, COO^{-}) + n_C B(CH_2) \quad (7)$$

where n_C is number of carbon atoms and the regression parameters, $B(^{+}NH_3, COO^{-})$ the zwitterionic group and $B(CH_2)$ the methylene group, are the contributions to the *B*-coefficients. The contributions of the other alkyl side chains of the amino acids were calculated from the $B(CH_2)$

Table 7
Contribution to the viscosity *B*-coefficient from zwitterionic groups, CH_2 and other alkyl side chains of amino acids in water and in aqueous solutions of *n*-propanol at 298.15 K

Group	*B*-coefficient ($dm^3\,mol^{-1}$)					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
NH_3^+, COO^-	0.032 ± 0.012	0.038 ± 0.021	0.023 ± 0.020	0.028 ± 0.010	0.038 ± 0.016	0.050 ± 0.017
CH_2–	0.104 ± 0.004	0.110 ± 0.008	0.108 ± 0.007	0.099 ± 0.003	0.089 ± 0.005	0.078 ± 0.005
CH_3CH–	0.208 ± 0.006	0.220 ± 0.011	0.216 ± 0.010	0.198 ± 0.004	0.178 ± 0.006	0.156 ± 0.007
CH_3CH_2CH–	0.311 ± 0.007	0.330 ± 0.014	0.324 ± 0.012	0.297 ± 0.005	0.267 ± 0.007	0.234 ± 0.009
$(CH_3)_2CHCH$–	0.415 ± 0.008	0.440 ± 0.016	0.432 ± 0.014	0.396 ± 0.006	0.356 ± 0.008	0.312 ± 0.010
$(CH_3)_2CHCH_2CH$–	0.519 ± 0.009	0.550 ± 0.018	0.540 ± 0.016	0.495 ± 0.007	0.445 ± 0.009	0.340 ± 0.011

Table 8
Contribution to the viscosity *B*-coefficient of transfer $\Delta_{tr}B$ from zwitterionic groups, CH_2 and other alkyl side chains of amino acids in aqueous solutions of *n*-propanol at 298.15 K

Compound	$\Delta_{tr}B$ ($dm^3\,mol^{-1}$)				
	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
NH_3^+, COO^-	0.006 ± 0.024	−0.009 ± 0.023	−0.004 ± 0.016	0.006 ± 0.020	0.018 ± 0.021
CH_2–	0.006 ± 0.009	0.004 ± 0.008	−0.005 ± 0.005	−0.015 ± 0.006	−0.026 ± 0.006
CH_3CH–	0.012 ± 0.012	0.008 ± 0.012	−0.010 ± 0.007	−0.030 ± 0.008	−0.052 ± 0.009
CH_3CH_2CH–	0.018 ± 0.016	0.012 ± 0.014	−0.015 ± 0.009	−0.045 ± 0.010	−0.078 ± 0.011
$(CH_3)_2CHCH$–	0.024 ± 0.018	0.016 ± 0.016	−0.020 ± 0.010	−0.060 ± 0.011	−0.104 ± 0.013
$(CH_3)_2CHCH_2CH$–	0.030 ± 0.020	0.020 ± 0.018	−0.025 ± 0.011	−0.075 ± 0.013	−0.130 ± 0.014

value, where $B(CH_2)$ value characterizes the mean value of CH_3 and CH groups [30]. These results are given in Table 7. The contribution of alkyl side chain *B*(R) of amino acid to *B*-coefficient increases with increase in alkyl side chain. The transfer *B*-coefficients of zwitterionic end group $B(^+NH_3, COO^-)$ and alkyl side chain group *B*(R) of amino acids from water to cosolvent solution have been calculated as follows:

$$\Delta_{tr}B(^+NH_3, COO^-)/\Delta_{tr}B(R) = B(^+NH_3, COO^-)/B(R)\ (\text{in aqueous } n\text{-propanol}) - B(^+NH_3, COO^-)/B(R)\ (\text{in water}) \quad (8)$$

$\Delta_{tr}B(R)(^+NH_3, COO^-)$ or $\Delta_{tr}(B)$ values are given in Table 8 and illustrated in Fig. 3. The $\Delta_{tr}B(^+NH_3, COO^-)$ values do not show any regular behavior and have little contribution (very small negative and positive values) up to $m_B \approx 4.0$ and have small positive contribution at $m_B = 5.0$.

The contribution of $\Delta_{tr}B(R)$ to $\Delta_{tr}B$ values are positive in lower concentration range of *n*-propanol and becomes zero at $m_B \approx 2.5$ for all amino acids irrespective to the size of side chain, and then becomes negative at a higher concentration. Both the positive as well as negative magnitude of $\Delta_{tr}B(R)$ increases with the alkyl side chain of the amino acids. Similar trends of $\Delta_{tr}B(R)$ for these amino acids in aqueous 1,2-propandiol (viscosities reported [10] earlier) have also been observed, where cross over occurs at $m_B \approx 3.5$ (Fig. 4). Shift of cross over from $m_B \approx 2.5$ (in *n*-propanol) to $m_B \approx 3.5$ (in 1,2-propandiol) may be due to the additional –OH group. Another significant feature which can be observed from Figs. 3 and 4 that the contribution of $\Delta_{tr}B(^+NH_3, COO^-)$ is positive over the concentration studied in case of 1,2-propandiol and the magnitude is more than in case of *n*-propanol. Apparently it can also be seen that the magnitude of side chain contribution $\Delta_{tr}B(R)$ are more in case of *n*-propanol than in case of 1,2-propandiol. These features may be attributed to the difference in number of –OH groups.

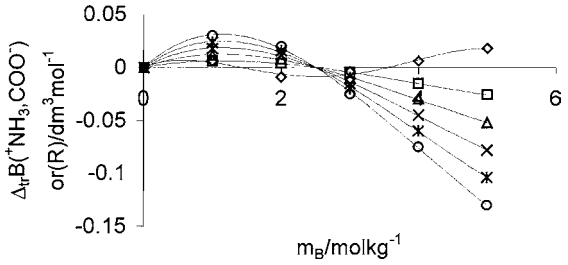

Fig. 3. $\Delta_{tr}B(^+NH_3, COO^-)$ or $\Delta_{tr}B(R)$ vs concentration of *n*-propanol: (◇) $^+NH_3, COO^-$; (□) $–CH_2$; (△) $–CHCH_3$; (×) $–CHCH_2CH_3$; (✕) $–CHCH(CH_3)_2$; (○) $–CHCH_2CH(CH_3)_2$.

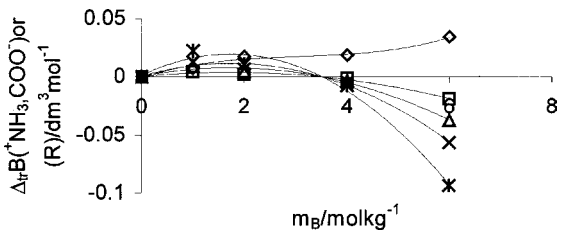

Fig. 4. $\Delta_{tr}B(^+NH_3, COO^-)$ or $\Delta_{tr}B(R)$ vs concentration of 1,2-propandiol: (◇) $^+NH_3, COO^-$; (□) $–CH_2$; (△) $–CHCH_3$; (×) $–CHCH_2CH_3$; (✕) $–CHCH_2CH(CH_3)_2$.

Table 9
Activation free energy, $\Delta\mu^{\circ}_{1^{\#}}$ and average molar volume, V°_{1} of aqueous *n*-propanol solution at 298.15 K

m_B (mol kg^{-1})	$\Delta\mu^{\circ}_{1^{\#}}$ (kJ mol^{-1})	V°_{1} (cm^3 mol^{-1})
0	9.16	18.07
1.0	9.90	18.97
2.0	10.53	19.84
3.0	11.03	20.68
4.0	11.42	21.52
5.0	11.72	22.36

According to Eyring's simple model [31], the average activation free energy of a single solute in a pure solvent can be calculated from the following equation:

$$\eta_0 = \left(\frac{hN_A}{V^{\circ}_1}\right) \exp\left(\frac{\Delta\mu^{\circ}_{1^{\#}}}{RT}\right) \quad (9)$$

where h, N_A, T, R are Plank's constant, Avogadro's number, temperature and gas constant, respectively; and V°_1 the average molar volume of aqueous *n*-propanol solution at 298.15 K, calculated from the density data [25c]. The $\Delta\mu^{\circ}_{1^{\#}}$ and V°_1 values are given in Table 9.

The activation free energy ($\Delta\mu^{\circ}_{2^{\#}}$) for viscous flow of amino acids and diglycine both in aqueous and mixed aqueous solutions is related to *B*-coefficient as reported by Feakins et al. [32] and used by Wadi et al. [11b] and Yan et al. [30] as follows:

$$B = \frac{V^{\circ}_1 - V^{\circ}_2}{1000} + \frac{V^{\circ}_1(\Delta\mu^{\circ}_{2^{\#}} - \Delta\mu^{\circ}_{1^{\#}})}{1000RT} \quad (10)$$

which can be rearranged as

$$\Delta\mu^{\circ}_{2^{\#}} = \Delta\mu^{\circ}_{1^{\#}} + \left(\frac{RT}{V^{\circ}_1}\right)[1000B - (V^{\circ}_1 - V^{\circ}_2)] \quad (11)$$

The $\Delta\mu^{\circ}_{2^{\#}}$ values are given in Table 10. The $\Delta\mu^{\circ}_{2^{\#}}$ values contain the change in the free energy of activation of solvent molecules in the presence of solute and have also the contribution from the movement of solutes.

According to transition state theory [32b], every solvent molecule in 1 mol of solution must pass through the transition state and also interact more or less strongly with solute molecules. Thus $\Delta\mu^{\circ}_{2^{\#}}$ includes the free energy of transfer of solute from ground state to transition state solvents ($\Delta G_2{}^{\circ}(1 \rightarrow 1')$) and the free energy of solute through its own viscous transition state ($\Delta G_2{}^{\circ}(2 \rightarrow 2')$). The ($\Delta G_2{}^{\circ}(1 \rightarrow 1')$) values (given in Table 11) have been obtained from the $\Delta\mu^{\circ}_{2^{\#}}$ values and ($\Delta G_2{}^{\circ}(2 \rightarrow 2')$), which is equal to $\Delta\mu^{\circ}_{1^{\#}}$, by the methods reported elsewhere [30,32b]. The positive $\Delta\mu^{\circ}_{2^{\#}}$ and ($\Delta G_2{}^{\circ}(1 \rightarrow 1')$) values, much larger in comparison to $\Delta\mu^{\circ}_{1^{\#}}$ suggest that the formation of transition state is less favored in the presence of amino acids and diglycine. This may be because of the breaking and distortion of intermolecular bonds. The ($\Delta G_2{}^{\circ}(1 \rightarrow 1')$) values increase from glycine to L-leucine indicating that the more energy is required for the amino acids with longer alkyl side chains for the transfer from ground state solvent to transition state solvent. Accordingly more solute–solvent bonds must be broken to form transition state.

Further the ($\Delta G_2{}^{\circ}(1 \rightarrow 1')$) values decrease with increase in concentration of *n*-propanol and this decrease becomes more prominent in case of amino acids with longer alkyl side chains e.g. the effect is more in case of L-leucine at $m_B = 5.0$. Thus the effect of concentration of *n*-propanol and size of alkyl side chain are reinforcing each other in transferring the solute from ground state solvent to transition state solvent. Hence the $\Delta\mu^{\circ}_{2^{\#}}$ values should also increase gradually from glycine to L-leucine and decrease with increase in concentration of *n*-propanol. Further the interactions of charged end groups of different amino acids in *n*-propanol are same and similar is the case for different amino acids with water. The increase in $\Delta\mu^{\circ}_{2^{\#}}$ values with increase in the side chain of amino acids can be attributed to the difference in interactions of different alkyl side chains of amino acids with water and in those with *n*-propanol in the solvent mixture. Similar effects have been observed in case of volume and compressibility studies. Frank and Wen have reported [33] the similar increase in interactions of amino acid and water with the increase in size of non-polar part of amino acids. Palecz has reported [34] the endothermic enthalpic pair interaction coefficients between L-amino acids and electrolyte in water and magnitude increase in the order glycine < alanine < amino butyrate < valine < leucine. Similarly in the present case, it may be inferred that the interactions of amino acids with water or *n*-propanol increase with increase in non-polar part of alkyl side chain as $\Delta\mu^{\circ}_{2^{\#}}$ values increase gradually from glycine to L-leucine.

Table 10
Activation free energy $\Delta\mu^{\circ}_{2^{\#}}$ for viscous flow of amino acids in water and in aqueous *n*-propanol solutions at 298.15 K

Compound	$\Delta\mu^{\circ}_{2^{\#}}$ (kJ mol^{-1})					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	32.22 ± 0.55	33.22 ± 0.65	29.61 ± 1.00	28.49 ± 0.96	27.11 ± 0.70	26.51 ± 0.55
DL-α-Alanine	49.80 ± 0.55	51.77 ± 1.17	47.87 ± 1.12	44.48 ± 0.72	43.04 ± 0.70	40.31 ± 0.78
DL-α-Amino-*n*-butyric acid	64.71 ± 0.68	62.53 ± 0.91	59.55 ± 0.50	56.20 ± 0.96	53.88 ± 0.58	50.71 ± 0.89
L-Valine	79.36 ± 0.68	76.61 ± 1.04	72.58 ± 0.87	69.13 ± 0.84	63.66 ± 2.44	59.23 ± 0.89
L-Leucine	107.21 ± 1.10	102.94 ± 2.48	94.13 ± 2.87	84.61 ± 2.76	76.89 ± 2.87	68.66 ± 2.11
Diglycine	65.44 ± 0.88	59.58 ± 0.78	55.20 ± 2.00	53.58 ± 0.84	52.58 ± 1.05	51.02 ± 0.67

Table 11
Thermodynamic activation parameter of transfer for the amino acids and diglycine from ground state to transition state in aqueous *n*-propanol solutions at 298.15 K[a]

Compound	$\Delta G_2^\circ(1 \rightarrow 1')$ (kJ mol^{-1})					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Glycine	23.06	23.32	19.08	17.46	15.69	14.79
DL-α-Alanine	40.24	41.87	37.34	33.45	31.62	28.59
DL-α-Amino-*n*-butyric acid	55.55	52.63	49.02	45.17	42.46	38.99
L-Valine	70.20	66.71	62.05	58.10	52.24	47.51
L-Leucine	98.05	93.04	83.60	73.58	65.47	56.94
Diglycine	56.28	49.69	44.67	42.55	41.16	39.30

[a] As the major contribution to uncertainty in $\Delta G_2^\circ(1 \rightarrow 1')$ values comes from $\Delta\mu_{2^\#}^\circ$, thus the uncertainties in this case are almost same as for $\Delta\mu_{2^\#}^\circ$.

Table 12
Contribution of zwitterionic group ($^+NH_3, COO^-$) and CH_2 group to the activation free energies of the amino acids at 298.15 K

Group	$\Delta\mu_{2^\#}^\circ$ (kJ mol^{-1})					
	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
($^+NH_3, COO^-$)	12.80 ± 4.54	16.13 ± 2.89	14.62 ± 2.74	15.52 ± 1.26	16.86 ± 1.64	18.12 ± 1.91
CH_2	17.95 ± 1.37	16.43 ± 1.06	15.39 ± 0.99	13.69 ± 0.38	12.02 ± 0.46	10.32 ± 0.58

Further $\Delta_{tr}\mu_{2^\#}^\circ$ ($\Delta_{tr}\mu_{2^\#}^\circ = \Delta\mu_{2^\#}^\circ$(*n*-propanol) − $\Delta\mu_{2^\#}^\circ$(in water)) is negative for the amino acids studied at different concentrations of *n*-propanol except for glycine and DL-α-alanine at $m_B = 1.0$ only. Further the magnitude of $\Delta_{tr}\mu_{2^\#}^\circ$ increases with increase in concentration of *n*-propanol and alkyl side chain of amino acids e.g. the magnitude is maximum for L-leucine at $m_B = 5.0$. Similar distinct behavior for L-leucine has been observed from volume, compressibility and viscosity *B*-coefficients in aqueous *n*-propanol solutions. This shows that the ground state in aqueous *n*-propanol is more structured for glycine and DL-α-alanine at $m_B = 1.0$, while the reverse is true (ground state in water is more structured) at higher concentration and for rest of studied amino acids with longer alkyl side chains. In case of glycine and DL-α-alanine at $m_B = 1.0$ the interactions between charged end groups and *n*-propanol increase the solvent structure where as at higher concentration and for amino acids with longer alkyl side chains, the interactions of charged end groups with *n*-propanol does not increase the solvent structure in aqueous *n*-propanol to the extent done by hydrophobic or non-polar alkyl side chains in water. Thus, the ground state in water becomes more structured than in *n*-propanol.

Further the $\Delta\mu_{2^\#}^\circ$ varies linearly with n_C, as observed in case of V_2° and *B*-coefficients of amino acids. The regression of $\Delta\mu_{2^\#}^\circ$ data using Eq. (12) gives $\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ and $\Delta\mu_{2^\#}^\circ(CH_2)$ as the respective contribution of $^+NH_3, COO^-$ and CH_2 groups:

$$\Delta\mu_{2^\#}^\circ = \Delta\mu_{2^\#}^\circ(^+NH_3, COO^-) + n_C\Delta\mu_{2^\#}^\circ(CH_2) \quad (12)$$

The $\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ and $\Delta\mu_{2^\#}^\circ(CH_2)$ values are given Table 12. Like $B(^+NH_3, COO^-)$, the $\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ does not show any regular trend at different concentration of *n*-propanol. However, $\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ values in *n*-propanol are overall larger than in water. The $\Delta\mu_{2^\#}^\circ(CH_2)$ values are less than in water and decrease continuously with increase in concentration of *n*-propanol. Further the $\Delta_{tr}\mu_{2^\#}^\circ(^+NH_3, COO^-)$ ($\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ in *n*-propanol − $\Delta\mu_{2^\#}^\circ(^+NH_3, COO^-)$ in water) at lower concentration is larger than $\Delta_{tr}\mu_{2^\#}^\circ(CH_2)$ ($\Delta\mu_{2^\#}^\circ(CH_2)$ in *n*-propanol − $\Delta\mu_{2^\#}^\circ(CH_2)$ in water). This again strengthens the view that for glycine and DL-α-alanine the ground state is more structured at lower concentration of *n*-propanol. Where as at higher concentration the $\Delta_{tr}\mu_{2^\#}^\circ(CH_2)$ is larger than $\Delta_{tr}\mu_{2^\#}^\circ(^+NH_3, COO^-)$ indicating that the ground state in water is more structured at higher concentration and for amino acids with longer alkyl side chains.

Further, it is observed that *B*-coefficients for amino acids in aqueous *n*-propanol also show linear correlation with V_2° values. The regression coefficients A_1 and A_2 are given in Table 13:

$$B = A_1 + A_2V_2^\circ \quad (13)$$

As reported by Yan et al. [30], the A_2 values reflect the size and shape of solute and lies between zero and 2.5 for unsolvated spherical species. Table 13 shows the A_2 values are

Table 13
Parameters A_1 and A_2 of Eq. (13) for amino acids in water and in aqueous *n*-propanol solutions at 298.15 K

m_B (mol kg^{-1})	A_1 (dm^3 mol^{-1})	A_2	R	Standard deviation
0	0.149 ± 0.034	6.5 ± 0.4	0.993	0.02
1.0	0.168 ± 0.050	7.2 ± 0.7	0.988	0.03
2.0	0.187 ± 0.027	7.2 ± 0.7	0.996	0.02
3.0	0.169 ± 0.006	6.6 ± 0.1	1.000	0.01
4.0	0.142 ± 0.025	5.9 ± 0.3	0.995	0.02
5.0	0.101 ± 0.032	5.1 ± 0.4	0.991	0.02

larger than 2.5. Further the A_2 values at lower concentration of *n*-propanol are larger than A_2 values in water and the reverse is true at higher concentration again indicating the increase in CH_2–*n*-propanol interactions at higher concentration and hence the decrease in solvent structure around CH_2 group. The similar conclusion has been drawn earlier from $\Delta_{tr}\mu^{\circ}_{2\#}(CH_2)$ data.

The formalism based on McMillan Mayer theory of solutions, proposed by Kozak et al. [35] and further discussed by Friedman and Krishnan [36] and Franks et al. [37] has been used to include the solute–cosolute interactions in the solvation sphere. According to this at infinite dilution $\Delta_{tr}K^{\circ}_{2,S}$ can be expressed as

$$\Delta_{tr}K^{\circ}_{2,S} = 2K_{AB}m_B + 3K_{ABB}m_B^2 + K_{ABBB}m_B^3 + \cdots \quad (14)$$

where the K_{AB}, K_{ABB}, and K_{ABBB} are, respectively, the pair, triplet and quartet interaction coefficients. The $\Delta_{tr}K^{\circ}_{2,S}$ data have been fitted to above equation to obtain the K_{AB} and K_{ABB} which are given in Table 14. Further the K_{AB} values are positive for the studied amino acids and diglycine. The K_{AB} values decrease from glycine to DL-α-alanine and DL-α-amino-*n*-butyric acid and then starts increasing with further increase in side chain i.e. from L-valine to L-leucine. These trends are same as that of $\Delta_{tr}K^{\circ}_{2,S}$ in the lower concentration range of *n*-propanol. The K_{AB} values for diglycine are less than for glycine. The K_{ABB} is negative for glycine and positive for the remaining amino acids and diglycine, and it increases from glycine to DL-α-alanine to DL-α-amino-*n*-butyric acid to L-valine, and decreases sharply for L-leucine. These trends for K_{AB} further strengthen our view that ion/hydrophilic-hydrophilic interactions are dominating in case of glycine and with increase in side chain, the hydration sphere is disrupted in case of DL-α-alanine and DL-α-amino-*n*-butyric acid which results in less K_{AB} values. With further increase in side chain, in case of L-valine and L-leucine the hydrophobic–hydrophobic interactions starts dominating over the tendency to disrupt the hydration sphere and thus become more prominent in the latter cases. These results are again in line with the view that there is tendency of cooperative aggregation of non-polar side chains of amino acids with *n*-propanol at higher concentration.

Table 14
Interaction coefficients K_{AB} and K_{ABB} of some amino acids and diglycine in aqueous *n*-propanol solutions at 298.15 K[a]

Compound	$10^{15} \times K_{AB}$ ($m^3\,mol^{-2}\,Pa^{-1}\,kg$)	$10^{15} \times K_{ABB}$ ($m^3\,mol^{-3}\,Pa^{-1}\,kg^2$)	R
Glycine	3.5316	−0.0975	0.998
DL-α-Alanine	1.9396	0.1036	0.997
DL-α-Amino-*n*-butyric acid	1.8350	0.1360	0.964
L-Valine	1.9448	0.2439	0.985
L-Leucine	4.2645	0.0456	0.999
Diglycine	2.5849	0.1364	0.976

[a] K_{AB} and K_{ABB} are the pair and triplet interaction coefficient, respectively.

The hydration numbers (n_H) have been calculated from both compressibility and viscosity data. From the compressibility data, the n_H values were calculated using the method reported by Millero et al. [26] i.e.:

$$n_H = -\frac{K^{\circ}_{(elec)}}{K^{\circ}_S V^{\circ}} \quad (15)$$

and further they replaced $K^{\circ}_{(elec)}$ by $K^{\circ}_{2,S}$ (amino acid), and calculated n_H values from the following equation:

$$n_H = -\frac{K^{\circ}_{2,S}(\text{amino acid})}{K^{\circ}_S V^{\circ}} \quad (16)$$

where K°_S is the compressibility of pure water or aqueous-*n*-propanol solvent and V° the molar volume of bulk water or bulk solvent. According to viscosity method [38,39], the effective volume as $m \rightarrow 0$ is called the limiting value of effective flow volume (V°_H), is related to *B*-coefficient by following equation:

$$B = 2.5V^{\circ}_H \quad (17)$$

Kalgud et al. have suggested [40] that the volume of solvation sheath (Vs) and n_H can be calculated as follows:

$$V_S = V^{\circ}_H - V^{\circ}_2 \quad (18)$$

$$n_H = \frac{V_S}{V^{\circ}} \quad (19)$$

where V° is the molar volume of bulk water or bulk solvent. The n_H calculated from both the methods are given in Table 15. The n_H values in water, calculated from compressibility data agree well with the literature values [26]. The n_H values in water increase with increase in side chain of the amino acids. Further the n_H values calculated from compressibility data are less in aqueous *n*-propanol solutions as compared to water and decrease with the increase in concentration. While the n_H values calculated from viscosity data are higher in aqueous *n*-propanol solutions at $m_B = 1.0$ and then decrease with increase in concentration of *n*-propanol. Earlier we have calculated [25c] the n_H values from partial molar volume data, where the behavior of n_H values in case of glycine and DL-α-alanine is closer to that observed from the compressibility method and in case of L-valine and L-leucine the n_H behavior is almost similar to that from viscosity data. It should be noted that n_H values are model dependent. The decreasing trend of n_H values from the compressibility data, being more sensitive to hydration characteristics confirm that the solute–cosolvent interactions become stronger as discussed earlier for these systems.

3.2. Thermal denaturation of lysozyme

The observed absorbance as a function of temperature has been utilized to calculate fraction of protein denatured

Table 15
Hydration number n_H of some amino acids in water and in aqueous *n*-propanol solutions at 298.15 K

Compound	Water	$m_B = 1.0$	$m_B = 2.0$	$m_B = 3.0$	$m_B = 4.0$	$m_B = 5.0$
Compressibility method						
Glycine	3.34	2.57	1.68	1.09	0.32	–[a]
DL-α-Alanine	3.17	2.67	2.18	1.28	0.57	–
DL-α-Amino-*n*-butyric acid	3.16	2.97	2.35	0.88	0.46	–
L-Valine	3.71	3.24	2.42	1.63	–	–
L-Leucine	3.87	2.82	1.72	0.44	–	–
Viscosity method						
Glycine	0.78	0.96	0.40	0.21	–	–
DL-α-Alanine	2.21	2.70	2.14	1.68	1.53	1.12
DL-α-Amino-*n*-butyric acid	2.99	3.38	3.08	2.65	2.41	1.93
L-Valine	4.34	4.56	4.17	3.79	3.04	2.44
L-Leucine	6.79	7.59	6.48	5.13	4.01	2.77

[a] Negative n_H values have not been included.

ΔA as

$$\Delta A = \frac{A_N - A}{A_N - A_D} \quad (20)$$

where A_N is absorbance of pure native state, A_D the absorbance of pure denatured state and A is the absorbance observed at any temperature. The evaluation of thermodynamic parameters is based on equilibrium constant for reversible two-state transition native ↔ denatured. The absorbance vs temperature data were processed using Exam Program of Kirchhoff [41] which gives least squares fits of the data to a two-state model to calculate denaturation temperature (T_m) and ΔH_m. The T_m and ΔH_m values are reported in Table 16.

Plot of ΔA vs temperature in Figs. 5–7 represent the thermal denaturation profile of lysozyme in aqueous and mixed aqueous solutions of various cosolvents (*n*-propanol, 1,2-propandiol, glycerol), studied by change in absorbance at ≈280 nm as a function of temperature. The T_m value (325.7 K) of lysozyme obtained in water at pH 2, is in good agreement with the literature values of 325.8 K at pH 2.3 (DSC study [42]), 326.8 K at pH 2.12 (CD study [43]) and 325.0 K at pH 2.12 (UV-visible study [44]). The reversibility of the thermal denaturation of lysozyme under these conditions was deduced from the recovery of the thermal denaturation profile upon reheating which again confirms the two-state nature of denaturation. The change in denaturation temperature in the presence of cosolvent ΔT_m was calculated as

$$\Delta T_m = T_m \text{ (in mixed aqueous solution)} - T_m \text{ (in water)} \quad (21)$$

The concentration dependence of ΔT_m shown in Fig. 8, clearly indicates that *n*-propanol slightly stabilize the protein at lower concentration where as it destabilize at higher concentration. For 1,2-propandiol, although ΔT_m is positive

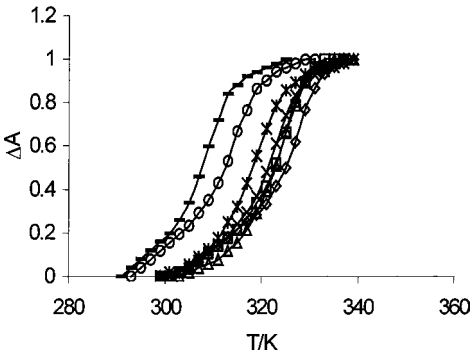

Fig. 5. Thermal denaturation profile of lysozyme in the presence of *n*-propanol: (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (▬) $m_B = 2.0$.

Table 16
Thermodynamic parameters of lysozyme in aqueous and in aqueous solutions of *n*-propanol, 1,2 propandiol and glycerol at pH 2

m_B	T_m (K)	ΔH_m (kJ mol^{-1})	ΔS_m (kJ mol^{-1} K^{-1})	$\Delta\Delta G^\circ$ (kJ mol^{-1})
Water	325.7 ± 0.1	326 ± 5	1.00 ± 0.02	0
n-Propanol				
0.1	328.3 ± 0.2	402 ± 8	1.22 ± 0.02	3.8
0.3	325.7 ± 0.1	370 ± 10	1.14 ± 0.03	0
0.5	324.2 ± 0.2	361 ± 7	1.11 ± 0.02	−1.7
0.7	322.4 ± 0.2	360 ± 9	1.11 ± 0.03	−3.8
1.0	319.3 ± 0.1	342 ± 12	1.07 ± 0.04	−7.3
1.5	316.2 ± 0.2	303 ± 7	0.96 ± 0.02	−10.1
2.0	309.4 ± 0.2	270 ± 7	0.87 ± 0.02	−17.1
1,2-Propandiol				
0.1	330.6 ± 0.2	429 ± 7	1.30 ± 0.02	6.1
0.3	330.7 ± 0.1	445 ± 12	1.35 ± 0.04	6.4
0.5	330.7 ± 0.2	422 ± 11	1.28 ± 0.03	6.1
0.7	330.0 ± 0.1	412 ± 6	1.25 ± 0.02	5.1
1.0	329.7 ± 0.2	417 ± 9	1.26 ± 0.03	5.0
1.5	329.2 ± 0.1	416 ± 5	1.26 ± 0.02	4.3
2.0	329.1 ± 0.2	411 ± 8	1.25 ± 0.02	4.1
Glycerol				
0.1	329.9 ± 0.2	466 ± 5	1.41 ± 0.02	5.5
0.3	330.2 ± 0.1	474 ± 6	1.43 ± 0.02	5.9
0.5	330.7 ± 0.2	470 ± 9	1.42 ± 0.03	6.4
0.7	330.3 ± 0.2	473 ± 7	1.43 ± 0.02	6.0
1.0	331.2 ± 0.1	479 ± 8	1.45 ± 0.02	7.2
1.5	331.4 ± 0.3	492 ± 6	1.48 ± 0.02	7.7
2.0	331.6 ± 0.1	496 ± 7	1.49 ± 0.02	8.0

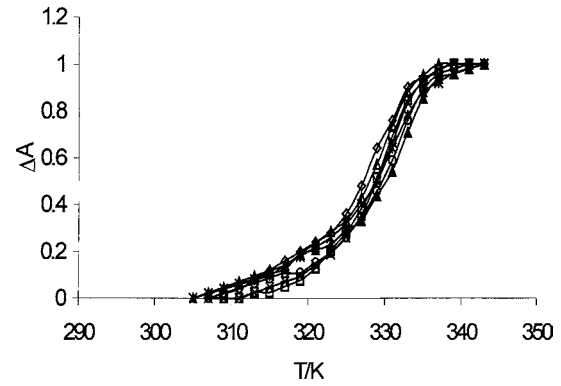

Fig. 6. Thermal denaturation profile of lysozyme in the presence of 1,2-propandiol: (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (▬) $m_B = 2.0$.

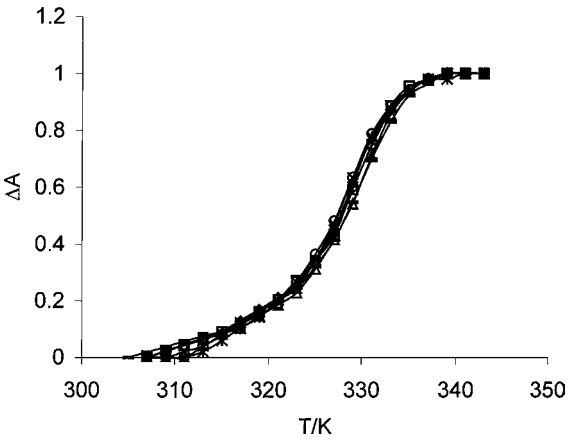

Fig. 7. Thermal denaturation profile of lysozyme in the presence of glycerol: (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (▬) $m_B = 2.0$.

for the concentration range studied, but shows a slight decreasing trend with the increase in concentration and positive ΔT_m in case of glycerol is almost independent of the concentration. A comparison of ΔT_m of lysozyme in *n*-propanol, 1,2-propandiol and glycerol, all with same number of carbon atoms, shows that *n*-propanol reduces the transition/denaturation temperature most, and with replacement of one-H atom of *n*-propanol with –OH its denaturation capacity decreased sharply and further an additional –OH in glycerol stabilize the protein. Similar trends for α-lactalbumin (having loose structure in comparison to lysozyme) have been reported [45] in *n*-propanol and 1,2-propandiol, where the presence of an additional –OH group on alcohol reduces its effect of lowering the denaturation temperature. During N ↔ D conversions, new extensive non-polar hydrophobic and polar peptide groups get exposed to the solvent and the distance between the charges increases greatly. The effect of various cosolvents on protein stability is defined by a balance between their preferential interactions with the two end states of protein [46–48]. It has been reported that non-polar parts of alcohols interact favorably with the hydrophobic side chains of proteins exposed on the denaturation. It has also been shown that the organization of water in the presence of alcohols [49,50] or direct binding of alcohols to non-polar parts of protein enhances the stability of the secondary structure of proteins [51]. Further the larger fall in denaturation temperature by alcohols with greater number of $–CH_2–$ groups has been assigned to the increased hydrophobic interactions [45,52]. From the present studies the negative $\Delta_{tr}\mu^{\circ}_{2\#}$ values and smaller values of A_2 coefficient in *n*-propanol than in water, for amino acids with longer alkyl side chain e.g. L-leucine, again strengthens the view that the CH_2–alcohol interactions also increase at higher concentration of alcohol. Stabilization effect of ethylene glycol for certain proteins has been explained [53,54] on the basis of the preferential hydration of the proteins, which increase with increasing number of hydroxyl groups of the cosolvent molecules. The preferential hydration favors the compact native state and disfavors an increase in surface area of the protein [45].

The change in enthalpy at denaturation temperature in the presence of cosolvent, ΔH_m vs concentration plot (Fig. 9) ($\Delta\Delta H_m = \Delta H_m$ (in mixed aqueous solution) $- \Delta H_m$ (in water)) shows a complex dependence on the solvent composition. The $\Delta\Delta H_m$ first increases with cosolvent concentration in case of *n*-propanol and 1,2-propandiol and then starts decreasing with further increase in concentration, which is comparatively sharp in case of *n*-propanol. The $\Delta\Delta H_m$ in case of glycerol is almost independent of concentration, similar to ΔT_m. The maximum value of $\Delta\Delta H_m$ increases from *n*-propanol to 1,2-propandiol to glycerol, which may be attributed to the increase in –OH groups. These trends

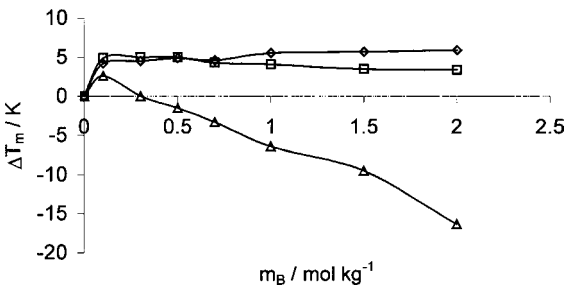

Fig. 8. ΔT_m vs concentration of various cosolvents: (△) *n*-propanol; (□) 1,2-propandiol; (◇) glycerol.

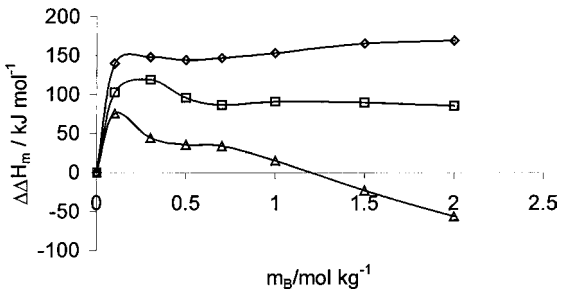

Fig. 9. $\Delta\Delta H_m$ vs concentration of various cosolvents: (△) *n*-propanol; (□) 1,2-propandiol; (◇) glycerol.

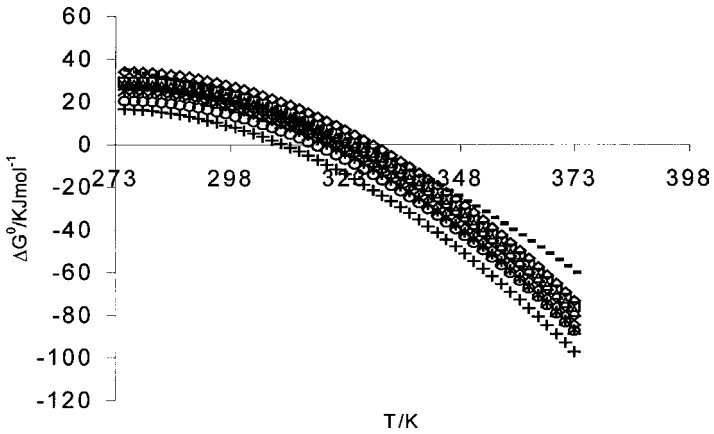

Fig. 10. Temperature dependence of ΔG° of lysozyme in water and in the presence of *n*-propanol: (▬) water; (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (+) $m_B = 2.0$.

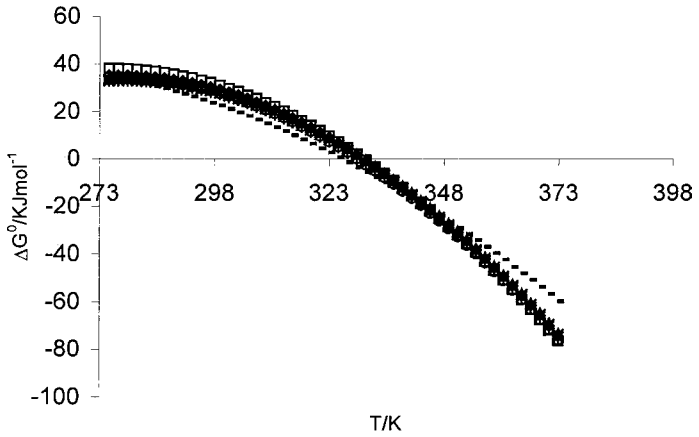

Fig. 11. Temperature dependence of ΔG° of lysozyme in water and in the presence of 1,2-propandiol: (▬) water; (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (+) $m_B = 2.0$.

indicate the preferential stabilization of the unfolded state of lysozyme through the stronger interactions of *n*-propanol with the hydrophobic side chains exposed on denaturation of protein. Whereas preferential hydration effect results in increase in denaturation temperature in latter two cases, thus favoring the compact native state. It may further be seen that preferential hydration effect is more in case of glycerol than 1,2-propandiol.

Further, ΔH_m varies linearly with T_m (plot not shown), thus ΔC_p has been calculated from the slope, which is used for the evaluation of other thermodynamic parameters. Gibbs free energy of stabilization/destabilization caused by presence of cosolvents can be calculated using the following Gibbs–Helmholtz equation [45]:

$$\Delta G^\circ = \frac{\Delta H_\mathrm{m}(T_\mathrm{m} - T)}{T_\mathrm{m}} + T\Delta C_p \ln\left(\frac{T_\mathrm{m}}{T}\right) + \Delta C_p(T - T_\mathrm{m}) \quad (22)$$

Figs. 10–12 show a temperature dependence of the standard Gibbs free energies of denaturation for lysozyme at various concentrations of cosolvents studied. It can be seen from these plots that destabilization effect of *n*-propanol increase with its concentration, whereas stabilization has been observed in case of 1,2-propandiol and glycerol. Fig. 13 shows comparison of ΔG° of denaturation of lysozyme in these cosolvents at $m_B = 2.0$ and in water, which represent stabilizing/destabilizing capacity of these cosolvents. It is clear that *n*-propanol destabilizes while 1,2-propandiol and glycerol slightly stabilizes the lysozyme.

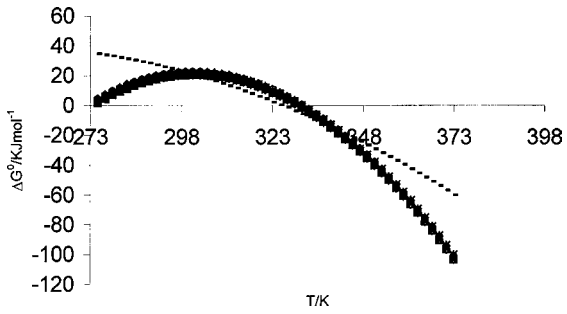

Fig. 12. Temperature dependence of ΔG° of lysozyme in water and in the presence of glycerol: (▬) water; (◇) $m_B = 0.1$; (□) $m_B = 0.3$; (△) $m_B = 0.5$; (×) $m_B = 0.7$; (✕) $m_B = 1.0$; (○) $m_B = 1.5$; (+) $m_B = 2.0$.

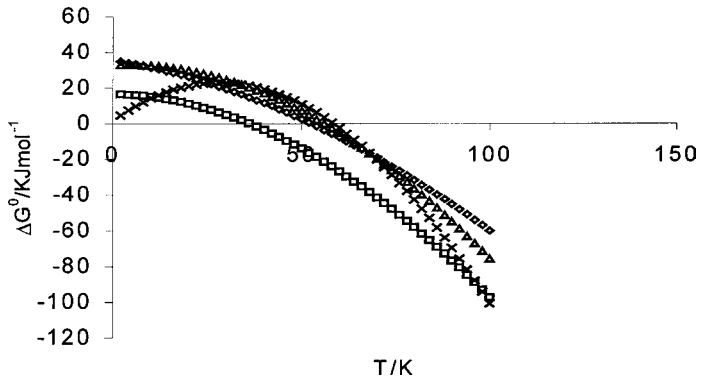

Fig. 13. Temperature dependence of ΔG° of lysozyme in water and at $m_B = 2.0$ of various cosolvents: (◇) water; (□) *n*-propanol; (△) 1,2-propandiol; (×) glycerol.

At the denaturation temperature of lysozyme in water (325.7 K) where equilibrium constant is unity and ΔG° is zero, the standard free energy changes of stabilization/destabilization, $\Delta\Delta G^\circ$ in the presence of cosolvents have also been calculated which are given in Table 16 and illustrated in Fig. 14. This shows that $\Delta\Delta G^\circ$ is positive at lower concentration of *n*-propanol, where it stabilizes the native protein, and becomes negative after $m_B \approx 0.3$ and decrease sharply with increase in concentration. The $\Delta\Delta G^\circ$ is positive for glycerol and 1,2-propandiol with slight negative slope in latter case. Further the values of $\Delta\Delta G^\circ$ at $m_B = 2.0$ are -17.1, 4.1 and 8.0 kJ mol^{-1} for *n*-propanol, 1,2-propandiol and glycerol, respectively, i.e. with the increase in –OH group the $\Delta\Delta G^\circ$ increases, which is similar to that found in the case of $\Delta\Delta H_\mathrm{m}$ and ΔT_m. Thus with the increase in hydrophilic character of the alcohol, the effect on stabilization of native protein also increases. Similarly the $\Delta\Delta S_m$ (at denaturation temperature where ΔG° is zero) values (Table 16) also increase from *n*-propanol to 1,2-propandiol to glycerol.

The data from reversible transition between N ↔ D can be used for calculation of preferential interactions [55] of these cosolvents with lysozyme as

$$\Delta\Gamma_{AB} = \Delta\Gamma_{\mathrm{D}B} - \Delta\Gamma_{\mathrm{N}B} = -\left[\frac{\Delta H_\mathrm{m}(\partial T_\mathrm{m}/\partial x_B)_\mathrm{pH}}{RT_\mathrm{m}^2(\partial \ln a_B/\partial x_B)_{T_\mathrm{m}}}\right] \quad (23)$$

where $\Delta\Gamma_{AB}$ is the preferential interaction parameter which is the measure of denaturational change of preferential solvation of lysozyme (*A*) by these cosolvents (*B*) at T_m, ΔH_m the enthalpy absorbed upon transition under these conditions, *R* the gas constant, $(\partial T_\mathrm{m}/\partial x_B)_\mathrm{pH}$ the rate of variation in T_m on increasing x_B (mole fraction) at constant pH, a_B the activity of the solvents which is assumed nearly equal to concentration (mole fraction) of the solvent for the studied range. The term $(\partial \ln a_B/\partial x_B)_{T_\mathrm{m}}$ in the denominator is the solution non-ideality. $\Delta\Gamma_{AB}$ values are given in Table 17 and illustrated in Fig. 15, which give the comparison of the denaturational change in the preferential solvation of lysozyme by these cosolvents. The $\Delta\Gamma_{AB}$ values are negative for glycerol and positive for 1,2-propandiol and *n*-propanol, where the magnitude is more in case of *n*-propanol.

Table 17
Preferential interaction parameter $\Delta\Gamma_{AB}$ accompanying the thermal unfolding of lysozyme in the presence of *n*-propanol, 1,2-propandiol and glycerol at pH 2

Mole fraction	$\Delta\Gamma_{AB}$		
	Glycerol	1,2-Propandiol	*n*-Propanol
0.0018	−0.049	0.047	0.437
0.0054	−0.148	0.146	1.227
0.0089	−0.242	0.229	1.993
0.0124	−0.341	0.313	2.794
0.0177	−0.489	0.453	3.861
0.0263	−0.745	0.673	5.193
0.0348	−0.993	0.882	6.394

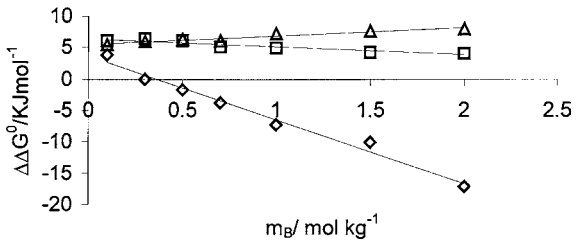

Fig. 14. $\Delta\Delta G^\circ$ vs concentration of various cosolvents: (◇) *n*-propanol; (□) 1,2-propandiol; (△) glycerol.

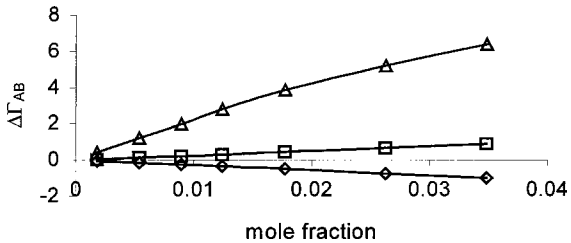

Fig. 15. $\Delta\Gamma_{AB}$ against mole fraction of various cosolvents: (◇) *n*-propanol; (□) 1,2-propandiol; (△) glycerol.

The positive values of $\Delta\Gamma_{AB}$ in case of *n*-propanol and 1,2-propandiol indicate the accumulation of these cosolvent molecules in the immediate vicinity of the protein, while negative value of $\Delta\Gamma_{AB}$ parameter is the resultant of preferential exclusion of glycerol molecules from the surface of protein. Further higher $\Delta\Gamma_{AB}$ values for *n*-propanol in comparison to 1,2-propandiol indicate that former has stronger preferential interactions as it is concentrated more strongly in the vicinity of protein than in bulk water. This leads to the shift of N → D equilibrium of protein to the right to greater extent and hence is more effective in lowering the transition temperature of the protein, which further indicate that the alcohols stabilize the secondary structure of the protein [56].

The change in surface tension of water in the presence of cosolvents has been suggested to play a significant role in the stabilization of protein in aqueous solutions and their preferential hydration [57]. It has been generally observed that the compound that increase the surface tension of the water, gets depleted in the interface leading to preferential exclusion of cosolvent from the surface of protein. Preferential exclusion corresponds to an excess of water over the bulk solvent in the vicinity of protein. It has been shown that the effect of sugars [13,58] on the surface tension of water has been the main force responsible for stabilization of protein and their preferential hydration. Presently determined surface tension σ values, of 1,2-propandiol solutions measured by drop weight method are shown in Fig. 16. Each experimental point represents an average of five or six measurements. Surface tension data for *n*-propanol solutions have been taken from literature [29]. Surface tension of both these cosolvents vary non-linearly with the increase in concentration. Thus, the $(\partial\sigma/\partial m_B)_{T,P}$ was obtained by measuring the tangent as a function of *n*-propanol or 1,2-propandiol concentration.

These data have been used to calculate the preferential interaction parameters [56] using the following Gibbs adsorption isotherm, which defines the excess concentration of a solute at an interface [57] i.e.:

$$\left(\frac{\partial m_B}{\partial m_A}\right)^{\text{Surface tension}}_{T,P,\mu B} = -\frac{Sa_B}{RT(\partial\sigma/\partial m_B)_{T,P}} \quad (24)$$

where m_A and S represent the molality and surface area of protein. The μ_B, a_B, T, P and R values are the chemical potential, activity of cosolvent assumed equal to the cosolvent concentration, temperature, pressure and gas constant, respectively. The surface area value of 6844 $A^{\circ 2}$ from X-ray crystallography data of Acharya et al. [59] for lysozyme has been used. $(\partial m_B/\partial m_A)_{T,P,\mu B}$ values calculated at $m_B = 0.5$ are 216.9 and 89.7 for lysozyme in case of *n*-propanol and 1,2-propandiol, respectively. The values of $(\partial m_B/\partial m_A)_{T,P,\mu B}$ for the interactions of *n*-propanol with lysozyme are larger than for 1,2-propandiol hence leads to stronger interactions of the former with the D state of protein as compared to N state and, thus shifts the equilibrium towards right. The positive values of $(\partial m_B/\partial m_A)_{T,P,\mu B}$ for both the cosolvents indicate the accumulation of these cosolvents in the immediate vicinity of the protein which is more in case of *n*-propanol (surface tension data for glycerol could not be collected due to its viscous nature). Similar conclusion has been drawn from the preferential interaction parameters calculated from the thermal denaturation data. These findings are consistent with those reported [53,54,56] earlier, that increase in hydroxyl groups on alcohol favors the preferential hydration of protein, which leads to stabilization effect on some proteins. It should be noted that the $(\partial m_B/\partial m_A)_{T,P,\mu B}$ values represent only a relative picture as they have been calculated from surface tension or the alteration of the surface free energy of water in the presence of cosolvent.

It has been reported that at lower concentration, the hydrophobic parts of alcohol interact selectively with non-polar groups exposed upon denaturation [52]. This leads to weakening of the hydrophobic interactions between non-polar groups of protein. The hydrophobic bond rupture has been reported as an exothermic process [60]. Thus, the maxima in $\Delta\Delta H_m$ vs concentration plot (Fig. 9) may be attributed to the reduction in exothermic contribution of hydrophobic bond rupture to the total ΔH_m [52]. Further as mentioned earlier this maxima increase from *n*-propanol to 1,2-propandiol to glycerol, which suggest that, the contribution from the endothermic rupture of polar interactions increase with the increase in hydroxyl group. As discussed in the previous section the positive $\Delta_{tr}B$ values and negative $\Delta_{tr}V^{\circ}_2$ values for the studied amino acids in the lower concentration of *n*-propanol which increase with the increase in side chain of amino acids, are also suggestive of the above view that *n*-propanol interact selectively with the non-polar parts of the side chains of amino acids. When the methylene groups are present in equimolar quantities the polyhydric alcohol stabilize the native structure of protein in contrast to monohydric alcohols [52] as polyhydric alcohol–water interactions are stronger than monohydric alcohol–water. With introduction of second or third –OH group on the monohydric alcohol, their interactions with water increase, whereas hydrophobic properties are less pronounced i.e. the increase in the number of hydroxyl groups shifts the balance of competing influences in favor of aqueous behavior [49]. From our viscometric studies the higher $\Delta_{tr}B(^{+}NH_3, COO^{-})$ values in case of 1,2-propanoldiol as compared to the values in case of *n*-propanol for the studied amino acids also support

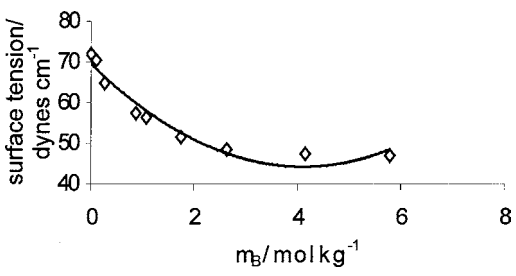

Fig. 16. Surface tension against the concentration of 1,2-propandiol.

the view that with the increase in number of –OH group their interaction with water increase and the reverse is true for the magnitude of $\Delta_{tr}B(R)$ values which are indicative of less pronounced hydrophobicity. Since glycerol interacts favorably with water by entering into the water structure, its presence in aqueous solutions may lead to increase in hydrophobicity of protein [54]. The present trends further strengthen the view that hydrophilic effect of polyhydric alcohol result in increase in the hydrophobic effect of the protein, which has been considered the main factor for the stability of the protein. This gets support from the studies on the thermal denaturation of ribonucleus and lysozyme in the presence of polyhydric alcohol where Gerlsma and Sturr have suggested [44] that there is no direct molecular interaction between proteins and polyhydric alcohols. As discussed earlier the polyhydric alcohol–water interactions are stronger than the monohydric alcohol–water interactions, the former remains (glycerol) excluded from the surface of the protein, whereas the latter (*n*-propanol) gets accumulated and interacts favorably with the hydrophobic side chains exposed on denaturation.

In summary from the studies on amino acids (model compounds) and lysozyme (protein) in the aqueous solutions, it may be said that some parallelism seems to exist in the various interactions operating in these systems. The negative $\Delta_{tr}V_2^{\circ}$ values and positive $\Delta_{tr}B$ values for the studied amino acids in the lower concentration range of aqueous *n*-propanol solutions also indicate that *n*-propanol interacts selectively with non-polar parts of the amino acids. The changes in thermodynamic parameters with the increase in non-polar side chain of amino acids in aqueous *n*-propanol, particularly in case of L-leucine have been attributed to hydrophobic–hydrophobic interactions/cooperative aggregation which are in line with the view that alcohols interact with non-polar side chains exposed upon denaturation and thus stabilize the secondary structure of the proteins. This strengthens the view that hydrophobic interactions play the key role in controlling the protein stability. Although the study on more amino acids with different side chains is needed to further rationalize these results.

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