Int J Thermophys (2010) 31:572–584
DOI 10.1007/s10765-010-0742-8

# Physicochemical Behavior of Some Amino Acids/Glycylglycine in Aqueous D-Galactose Solutions at Different Temperatures

**Anwar Ali · Rajan Patel · Shahjahan · Nizamul Haque Ansari**

Received: 29 January 2009 / Accepted: 17 April 2010 / Published online: 8 May 2010


**Abstract** The apparent molar volumes ($\overline{V_2}$) for glycine (Gly), L-alanine (Ala), phenylalanine (Phe), and glycylglycine (Gly-Gly) in 0.10 m aqueous D-galactose solutions have been determined from density measurements at (298.15, 303.15, 308.15, and 313.15) K. The data for ($\overline{V_2}$) were utilized to estimate the partial molar volume at infinite dilution ($\overline{V_2^0}$), and experimental slope ($S_v^*$). The transfer volume, ($\overline{V_{2\,(tr)}^0}$), and hydration number, ($n_H$) were also evaluated. The viscosity data were used to evaluate *A*- and *B*-coefficients of the Jones–Dole equation, the free energy of activation of viscous flow per mole of the solvent $\left(\Delta\mu_1^{0*}\right)$ and the solute $\left(\Delta\mu_2^{0*}\right)$. The molar refractivity ($R_D$) was calculated from refractive index data. The results were discussed in terms of hydrophilic–ionic, hydrophilic–hydrophobic, and hydrophobic–hydrophobic interactions, and structure-making/-breaking ability of the solute (AAs/peptide) in aqueous D-galactose solutions.

**Keywords** Amino acids · Density · D-galactose · Glycylglycine · Interactions · Refractive index · Viscosity

## 1 Introduction

Investigators have shown interest in the study of aqueous solutions of hydrophilic non-electrolytes because of their weak non-bonding intermolecular interactions, and

A. Ali (✉) · Shahjahan · N. H. Ansari
Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi 110 025, India
e-mail: anwarali.chem@gmail.com

R. Patel
Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia (Central University), New Delhi 110 025, India

they are interesting from a biological viewpoint due to their low specificity [1]. Carbohydrates are the typical non-electrolytes having hydrophilic hydroxyl groups. The hydration of carbohydrates largely depends on the position of axial and equatorial hydroxyl groups, the equatorial position of hydroxyl groups being more favorable [2,3]. If a carbohydrate molecule is introduced in water, hydrogen bonds with water molecules will depend to a large extent on the spacing and orientation of the polar groups of the carbohydrate molecules relative to the –OH geometry in water [4].

Studies reveal that these polyhydroxy compounds help in stabilizing the native conformation of globular proteins [5–7]. The changes in the conformation of proteins are difficult to study as a result of their complex structural organization. However, constituents of proteins such as amino acids, peptides, and their derivatives are widely used to study conformational and configurational changes in proteins.

A literature survey reveals that different techniques such as X-rays, crystallography [8,9], NMR spectra, computer calculation [10,11], and chromatography [12,13] have been used to study carbohydrate–protein interactions. However, studies of carbohydrate–amino acid/peptide interactions using thermodynamic tools are rare [14,15].

## 2 Experimental

Glycine (E. Merck, Germany), L-alanine and phenylalanine (Thomas Baker Chem. Ltd. Mumbai, India), glycylglycine (Acros Organics, Belgium), and D-galactose (Himedia Laboratories Ltd, India) of high purities (each having a mass fraction of 0.99) were used as obtained, except for drying over $P_2O_5$ in a desiccator. Doubly distilled water was used for the preparation of solutions on a molality basis by weighing on a Precisa XB-220A (Swiss-make) electronic balance, with a precision up to $\pm 0.0001$ g. All necessary precautions were taken during the preparation of solutions to avoid moisture contamination and evaporation. A stock solution of 0.10 $m$ D-galactose in water was used as a solvent for preparing amino acid and glycylglycine (Gly-Gly) solutions.

The density measurements were carried out using a single stem pycnometer made of Borosil glass having a bulb capacity of $8 \times 10^{-6}$ m$^3$ by a method described elsewhere [16,17]. A Ubbelohde type suspended level viscometer was employed for measurement of the viscosity. The viscometer was calibrated with double-distilled water, toluene, and acetone. The flow times at 298.15 K, 303.15 K, 308.15 K, and 313.15 K for pure water were 456.6 s, 410.4 s, 372.0 s, and 339.1 s, respectively. The viscometer containing a test solution was allowed to stand for about 25 min in the thermostated water bath in order to minimize thermal fluctuations in the solution. At least three sets of flow times for each solution were taken, and the average value is used as the experimental flow time. The refractive indices of solutions were measured with the help of a thermostated Abbe refractometer using sodium D line of 589.26 nm wavelength. Calibration of the refractometer was carried out by measuring the refractive indices of doubly distilled water and pure toluene at the desired temperature. The uncertainties in the measurements of $\rho$, $\eta$, and $n_D$ were estimated to be 0.0001 g $\cdot$ cm$^{-3}$, $3 \times 10^{-6}$ Pa $\cdot$ s, and 0.0001, respectively. For all the measurements,

the temperature of the solution was maintained in an electronically controlled water bath (Julabo, Model: MD, Germany) and the estimated uncertainty in temperature measurement was ±0.02 K.

## 3 Results and Discussion

The experimental densities, viscosities, and refractive indices for glycine (Gly), alanine (Ala), phenylalanine (Phe), and glycylglycine (Gly-Gly) in 0.10 m aqueous D-galactose solutions at four different temperatures ($T = 298.15$ K, 303.15 K, 308.15 K, and 313.15 K) are reported in Table 1.

The volumetric property has been regarded as a sensitive structural tool for understanding interactions in solutions [18]. The apparent molar volumes of amino acids and Gly-Gly ($\overline{V_2}$) were calculated using the following relation:

$$\overline{V_2} = \frac{M}{\rho} - \frac{1000\,(\rho - \rho_0)}{m\rho\rho_0} \tag{1}$$

where $M$ is the molar mass of the solute (amino acid/Gly-Gly), $m$ is its molality, and $\rho$ and $\rho_0$ are the densities of the solution and solvent (aqueous D-galactose), respectively. The molality dependence of $\overline{V_2}$ for dilute solutions of the amino acids/peptide can be represented by the linear equation,

$$\overline{V_2} = \overline{V_2^0} + S_v^* m \tag{2}$$

where $\overline{V_2^0}$ is the partial molar volume of the solute (amino acid/peptide) at infinite dilution and $S_v^*$ is the experimental slope [16]. The computed values of $\overline{V_2^0}$ and $S_v^*$ are given in Table 2. The $\overline{V_2^0}$ values give insight into the solute–solvent interactions, whereas $S_v^*$ represents the solute–solute interactions. A close perusal of Table 2 reveals that values of $\overline{V_2^0}$ are large and positive and are much larger than the small negative values of $S_v^*$, thereby, suggesting the presence of strong solute–solvent interactions in an aqueous D-galactose solution. Furthermore, the strength of solute (amino acid/peptide)–solvent (D-galactose/water) interactions follows the trend,

$$\text{Phe} > \text{Gly-Gly} > \text{Ala} > \text{Gly}$$

which in turn, is the order in which $\overline{V_2^0}$ increases from Gly to Phe. This may be attributed to the increased hydrophobicity from Gly to Phe. The $\overline{V_2^0}$ values are also found to increase with temperature which may be attributed to the reduction in the electrostriction of the solvent molecules. The values of $S_v^*$ (Table 2) are smaller than the $\overline{V_2^0}$ values, suggesting the presence of weak solute–solute interactions.

The standard partial molar volumes of transfer $\overline{V_{2\,(\mathrm{tr})}^0}$ at infinite dilution for AAs/peptide from aqueous to aqueous D-galactose have been determined from $\overline{V_2^0}$ data using the equation,

**Table 1** Values of density, $\rho$, viscosity, $\eta$, and refractive index, $n_D$, of glycine, alanine, phenylalanine, and glycylglycine in 0.1 *m* aqueous D-galactose solution at different temperatures

| $m$(mol · kg$^{-1}$) | $T$ (K) | | | |
|---|---|---|---|---|
| | 298.15 | 303.15 | 308.15 | 313.15 |
| Gly + aqueous D-galactose | | | | |
| | | $\rho$ (kg · m$^{-3}$) | | |
| 0.00 | 1003.9 | 1002.3 | 1001.0 | 999.5 |
| 0.01 | 1004.2 | 1002.6 | 1001.2 | 999.7 |
| 0.05 | 1005.5 | 1003.8 | 1002.3 | 1000.7 |
| 0.10 | 1007.4 | 1005.5 | 1003.9 | 1002.1 |
| 0.15 | 1009.4 | 1007.4 | 1005.7 | 1003.8 |
| 0.20 | 1011.6 | 1009.4 | 1007.5 | 1005.5 |
| | | $\eta$ (mPa · s) | | |
| 0.00 | 0.9284 | 0.8345 | 0.7518 | 0.6858 |
| 0.01 | 0.9324 | 0.8371 | 0.7533 | 0.6865 |
| 0.05 | 0.9391 | 0.8422 | 0.7570 | 0.6891 |
| 0.10 | 0.9456 | 0.8474 | 0.7612 | 0.6924 |
| 0.15 | 0.9515 | 0.8522 | 0.7651 | 0.6958 |
| 0.20 | 0.9567 | 0.8564 | 0.7689 | 0.6987 |
| | | $n_D$ | | |
| 0.00 | 1.3345 | 1.3340 | 1.3335 | 1.3329 |
| 0.01 | 1.3347 | 1.3341 | 1.3337 | 1.3332 |
| 0.05 | 1.3353 | 1.3347 | 1.3343 | 1.3337 |
| 0.10 | 1.3362 | 1.3356 | 1.3351 | 1.3345 |
| 0.15 | 1.3372 | 1.3366 | 1.3361 | 1.3355 |
| 0.20 | 1.3382 | 1.3375 | 1.3370 | 1.3363 |
| Ala + aqueous D-galactose | | | | |
| | | $\rho$ (kg · m$^{-3}$) | | |
| 0.00 | 1003.9 | 1002.3 | 1001.0 | 999.5 |
| 0.01 | 1004.5 | 1002.8 | 1001.4 | 999.8 |
| 0.05 | 1005.7 | 1003.9 | 1002.4 | 1000.7 |
| 0.10 | 1007.0 | 1005.2 | 1003.6 | 1001.8 |
| 0.15 | 1008.2 | 1006.3 | 1004.7 | 1002.9 |
| 0.20 | 1009.4 | 1007.5 | 1005.8 | 1004.0 |
| | | $\eta$ (mPa · s) | | |
| 0.00 | 0.9284 | 0.8345 | 0.7518 | 0.6858 |
| 0.01 | 0.9323 | 0.8373 | 0.7536 | 0.6866 |
| 0.05 | 0.9435 | 0.8468 | 0.7614 | 0.6926 |
| 0.10 | 0.9561 | 0.8580 | 0.7714 | 0.7010 |
| 0.15 | 0.9686 | 0.8690 | 0.7814 | 0.7100 |
| 0.20 | 0.9810 | 0.8797 | 0.7909 | 0.7188 |

**Table 1** continued

| $m\left(\text{mol}\cdot\text{kg}^{-1}\right)$ | $T$ (K) | | | |
|---|---|---|---|---|
| | 298.15 | 303.15 | 308.15 | 313.15 |
| | | $n_D$ | | |
| 0.00 | 1.3345 | 1.3340 | 1.3335 | 1.3329 |
| 0.01 | 1.3347 | 1.3341 | 1.3337 | 1.3332 |
| 0.05 | 1.3354 | 1.3348 | 1.3343 | 1.3338 |
| 0.10 | 1.3363 | 1.3357 | 1.3352 | 1.3347 |
| 0.15 | 1.3372 | 1.3366 | 1.3361 | 1.3356 |
| 0.20 | 1.3382 | 1.3376 | 1.3370 | 1.3365 |
| Phe + aqueous D-galactose | | | | |
| | | $\rho$ (kg · m$^{-3}$) | | |
| 0.00 | 1003.9 | 1002.4 | 1001.0 | 0999.5 |
| 0.01 | 1004.4 | 1002.7 | 1001.3 | 0999.8 |
| 0.04 | 1005.9 | 1004.1 | 1002.5 | 1000.8 |
| 0.08 | 1008.2 | 1006.2 | 1004.3 | 1002.4 |
| 0.12 | 1010.9 | 1008.7 | 1006.5 | 1004.3 |
| | | $\eta$ (mPa · s) | | |
| 0.00 | 0.9284 | 0.8345 | 0.7518 | 0.6858 |
| 0.01 | 0.9406 | 0.8427 | 0.7569 | 0.6883 |
| 0.04 | 0.9642 | 0.8614 | 0.7713 | 0.6990 |
| 0.08 | 0.9919 | 0.8849 | 0.7903 | 0.7149 |
| 0.12 | 1.0198 | 0.9084 | 0.8106 | 0.7323 |
| | | $n_D$ | | |
| 0.00 | 1.3345 | 1.3340 | 1.3335 | 1.3329 |
| 0.01 | 1.3347 | 1.3342 | 1.3338 | 1.3332 |
| 0.04 | 1.3355 | 1.3349 | 1.3344 | 1.3338 |
| 0.08 | 1.3367 | 1.3361 | 1.3355 | 1.3349 |
| 0.12 | 1.3380 | 1.3373 | 1.3367 | 1.3360 |
| Gly-Gly + aqueous D-galactose | | | | |
| | | $\rho$ (kg · m$^{-3}$) | | |
| 0.00 | 1003.9 | 1002.4 | 1001.0 | 999.5 |
| 0.01 | 1004.4 | 1002.8 | 1001.4 | 999.9 |
| 0.05 | 1006.7 | 1005.0 | 1003.3 | 1001.6 |
| 0.10 | 1010.1 | 1008.2 | 1006.3 | 1004.3 |
| 0.15 | 1013.8 | 1011.7 | 1009.6 | 1007.3 |
| 0.20 | 1017.9 | 1015.6 | 1013.2 | 1010.8 |
| | | $\eta$ (mPa · s) | | |
| 0.00 | 0.9284 | 0.8345 | 0.7518 | 0.6858 |
| 0.01 | 0.9324 | 0.8371 | 0.7532 | 0.6865 |
| 0.05 | 0.9446 | 0.8465 | 0.7606 | 0.6921 |

**Table 1** continued

| $m$ (mol · kg$^{-1}$) | $T$ (K) | | | |
|---|---|---|---|---|
| | 298.15 | 303.15 | 308.15 | 313.15 |
| 0.10 | 0.9587 | 0.8589 | 0.7707 | 0.7006 |
| 0.15 | 0.9731 | 0.8711 | 0.7815 | 0.7100 |
| 0.20 | 0.9875 | 0.8838 | 0.7926 | 0.7205 |
| | | $n_D$ | | |
| 0.00 | 1.3345 | 1.3339 | 1.3335 | 1.3329 |
| 0.01 | 1.3347 | 1.3341 | 1.3336 | 1.3331 |
| 0.05 | 1.3356 | 1.3350 | 1.3344 | 1.3338 |
| 0.10 | 1.3368 | 1.3361 | 1.3355 | 1.3348 |
| 0.15 | 1.3382 | 1.3375 | 1.3367 | 1.3360 |
| 0.20 | 1.3397 | 1.3389 | 1.3381 | 1.3373 |

$$\overline{V_{2\,(\mathrm{tr})}^{0}} = \overline{V_{2\,(\mathrm{aq.\,galactose})}^{0}} - \overline{V_{2\,(\mathrm{aq})}^{0}} \tag{3}$$

The values of $\overline{V_{2\,(\mathrm{tr})}^{0}}$ are summarized in Table 2. The data of $\overline{V_{2\,(\mathrm{aq})}^{0}}$ for Gly and Ala at 298.15 K, 308.15 K, and 313.15 K, and for Phe and Gly-Gly at 298.15 K and 308.15 K were taken from the literature [19,20]. In general, the interactions that are expected to occur between the AAs/peptide and aqueous D-galactose can be classified as follows:

(1) Hydrophilic–ionic group interactions between the –OH groups of D-galactose and zwitterionic ($NH_3^+$, $COO^-$) centers of AAs/peptide.
(2) Hydrophilic–hydrophobic group interactions between the –OH groups of D-galactose and the non-polar side groups of AAs/peptide.
(3) Hydrophobic–hydrophobic group interactions between the non-polar group of D-galactose and non-polar side groups of AAs/peptide.

The interactions of type (1) contribute positively to the transfer volumes due to the overlap of hydration co-spheres of the charged ionic centers ($NH_3^+$ and $COO^-$) and of hydrophilic –OH group of D-galactose, leading to the decrease in the structure-breaking ability of the ion and reduction in the electrostriction of water molecules by these charged ionic centers. As a result of the overlap of a hydration co-sphere of –OH groups of D-galactose and a hydration co-sphere of a polar groups of AAs/peptide, type (2) interactions lead to negative volumes of transfer. Similarly, interactions of type (3) also contribute negatively to the transfer volumes, as the overlap of two hydrophobic hydration co-spheres relaxes some water molecules from the solvation sphere to bulk, giving rise to a negative change in volume [21]. Thus, the observed positive $\overline{V_{2\,(\mathrm{tr})}^{0}}$ values (Table 2) suggest the dominance of interactions of type (1) over types (2) and (3) in the systems under study.

The standard partial molar volume of non-elecrolytes (AAs and peptide) results from the combination of the intrinsic volume of the solute and volume changes due to its interaction with the solvent [22]. Teresawa et al. [23] suggested that the intrinsic

**Table 2** Values of $\overline{V_2^0}$, $S_v^*$, and $\overline{V_{2\,(tr)}^0}$ of glycine, alanine, phenylalanine, and glycylglycine in aqueoue D-galactose solutions at different temperatures

| | $T$ (K) | | | |
|---|---|---|---|---|
| | 298.15 | 303.15 | 308.15 | 313.15 |
| Gly + aqueous D-galactose | | | | |
| $10^5 \times \overline{V_2^0}$ ($m^3 \cdot mol^{-1}$) | 4.990 | 5.298 | 5.607 | 5.793 |
| $10^5 \times S_v^*$ ($m^3 \cdot mol^{-2} \cdot kg$) | −3.062 | −3.113 | −3.161 | −2.957 |
| $10^5 \times \overline{V_{2\,(aq)}^0}$ ($m^3 \cdot mol^{-1}$) | 4.320[a] | – | 4.380[a] | 4.400[a] |
| $10^5 \times \overline{V_{2\,(tr)}^0}$ ($m^3 \cdot mol^{-1}$) | 0.670 | – | 1.227 | 1.393 |
| Ala + aqueous D-galactose | | | | |
| $10^5 \times \overline{V_2^0}$ ($m^3 \cdot mol^{-1}$) | 8.475 | 8.596 | 8.709 | 8.833 |
| $10^5 \times S_v^*$ ($m^3 \cdot mol^{-2} \cdot kg$) | −1.907 | −2.004 | −2.045 | −2.133 |
| $10^5 \times \overline{V_{2\,(aq)}^0}$ ($m^3 \cdot mol^{-1}$) | 6.050[a] | – | 6.100[a] | 6.120[a] |
| $10^5 \times \overline{V_{2\,(tr)}^0}$ ($m^3 \cdot mol^{-1}$) | 2.425 | – | 2.609 | 2.713 |
| Phe + aqueous D-galactose | | | | |
| $10^5 \times \overline{V_2^0}$ ($m^3 \cdot mol^{-1}$) | 12.562 | 13.332 | 14.119 | 14.592 |
| $10^5 \times S_v^*$ ($m^3 \cdot mol^{-2} \cdot kg$) | −5.514 | −6.178 | −6.482 | −6.203 |
| $10^5 \times \overline{V_{2\,(aq)}^0}$ ($m^3 \cdot mol^{-1}$) | 12.132[a] | – | 12.280[a] | – |
| $10^5 \times \overline{V_{2\,(tr)}^0}$ ($m^3 \cdot mol^{-1}$) | 0.430 | – | 1.840 | – |
| Gly-Gly + aqueous D-galactose | | | | |
| $10^5 \times \overline{V_2^0}$ ($m^3 \cdot mol^{-1}$) | 8.846 | 9.363 | 9.869 | 10.385 |
| $10^5 \times S_v^*$ ($m^3 \cdot mol^{-2} \cdot kg$) | −5.975 | −6.343 | −6.328 | −6.384 |
| $10^5 \times \overline{V_{2\,(aq)}^0}$ ($m^3 \cdot mol^{-1}$) | 7.623[b] | – | 7.710[b] | – |
| $10^5 \times \overline{V_{2\,(tr)}^0}$ ($m^3 \cdot mol^{-1}$) | 1.223 | – | 2.158 | – |

[a] Ref. [19]
[b] Ref. [20]

volume is made up of two types of contributions, namely, the van der Waals volume [24] ($V_{vw}$) and the volume associated with the voids/empty spaces present therein [24] as

$$V_{2(int)} = V_{vw} + V_{void} \tag{4}$$

Shahidi et al. [25] evaluated the contribution of a solute molecule toward its $\overline{V_2^0}$ by modifying the above equation as

$$\overline{V_2^0} = V_{vw} + V_{void} - n\sigma_s \tag{5}$$

where $\sigma_s$ is the shrinkage in volume due to the interaction of hydrogen bonding groups present in the solute with water molecules, and $n$ is the number of hydrogen bonding sites in the molecule. Therefore, $\overline{V_2^0}$ of AAs/Gly-Gly can be written as

$$\overline{V_2^0} = V_{\text{vw}} + V_{\text{void}} - V_{\text{Shrinkage}} \tag{6}$$

If it is assumed that $V_{\text{vw}}$ and $V_{\text{void}}$ remain unchanged in water as well as in an aqueous D-galactose solution then the positive volume of transfer for AAs/peptide can be rationalized in terms of the decrease in volume shrinkage, $\sigma_s$, in the presence of D-galactose molecules in aqueous solutions [26].

Furthermore, the standard partial molar volumes of the AAs/peptide can be expressed by a simple model [22];

$$\overline{V_2^0} = \overline{V_{2\,(\text{int})}^0} + \overline{V_{2\,(\text{elect})}^0} \tag{7}$$

where $\overline{V_{2\,(\text{int})}^0}$ is the intrinsic volume of AAs/peptide and $\overline{V_{2\,(\text{elect})}^0}$ is the electrostriction partial molar volume because of the hydration of the AAs/peptide. According to Millero et al. [27], the value of $\overline{V_{2\,(\text{int})}^0}$ for AAs and Gly-Gly can be estimated from the respective crystal molar volumes, $\overline{V_{2\,(\text{cryst})}^0}$;

$$\overline{V_{2\,(\text{int})}^0} = \left(\frac{0.7}{0.634}\right) \overline{V_{2\,(\text{cryst})}^0} \tag{8}$$

where 0.7 is the packing density for molecules in an organic crystal and 0.634 is the packing density for randomly packed spheres. $\overline{V_{2\,(\text{cryst})}^0}$ can be evaluated using the following relation:

$$\overline{V_{2\,(\text{cryst})}^0} = \frac{M}{\rho_{(\text{cryst})}} \tag{9}$$

where $\rho_{(\text{cryst})}$ is the crystal density of the solute (AAs and Gly-Gly) determined by using the method adopted by Berlin and Pallansch [28]. $\overline{V_{2\,(\text{elect})}^0}$ can be calculated form the experimentally measured $\overline{V_2^0}$ values,

$$\overline{V_{2\,(\text{elect})}^0} = \overline{V_2^0} - \overline{V_{2\,(\text{int})}^0} \tag{10}$$

The decrease in volume due to electrostriction of water molecules can be related to the number of water molecules ($n_{\text{H}}$) hydrated to AAs/Gly-Gly molecules.

Thus, the values of the hydration number $n_{\text{H}}$ (at 298.15 K) are evaluated by using the equation [29],

$$n_{\text{H}} = \frac{\overline{V_{2\,(\text{elect})}^0}}{\overline{V_{\text{E}}^0} - \overline{V_{\text{B}}^0}} \tag{11}$$

where $\overline{V_{\text{E}}^0}$ is the molar volume of electrostricted water and $\overline{V_{\text{B}}^0}$ is the molar volume of bulk water at 298.15 K. It is assumed that for every water molecule taken from

the bulk phase to the region near the AAs/Gly-Gly, the reduction in volume occurs by $\left(\overline{V_{\mathrm{E}}^{0}}-\overline{V_{\mathrm{B}}^{0}}\right)$ for electrolyte solutions. Millero et al. [27] estimated the value of $\overline{V_{\mathrm{E}}^{0}}-\overline{V_{\mathrm{B}}^{0}}$ to be nearly equal to $-3.3\,\mathrm{cm}^{3}\cdot\mathrm{mol}^{-1}$. The computed $n_{\mathrm{H}}$ values at 298.15 K are found to be 15.72, 21.74, 29.18, and 42.03 for Gly, Ala, Gly-Gly, and Phe, respectively. The hydration number of AAs/Gly-Gly in aqueous D-galactose follows the sequence,

$$\text{Phe} > \text{Gly-Gly} > \text{Ala} > \text{Gly}$$

This shows that the value of $n_{\mathrm{H}}$ tends to increase as the molar mass of AAs/Gly-Gly increases from Gly to Phe.

The $A$- and $B$-coefficients of viscosity were obtained by the analysis of viscosity data using the Jones–Dole [30] equation;

$$\eta_{\mathrm{r}}=\frac{\eta}{\eta_{0}}=1+Am^{1/2}+Bm \tag{12}$$

where $\eta_{\mathrm{r}}$ is the relative viscosity; $\eta$ and $\eta_{0}$ are the respective viscosities of solution (AAs/Gly-Gly + aqueous D-galactose) and solvent (aqueous D-galactose). $A$ is the Falkenhagen coefficient and $B$ is the Jones–Dole coefficient. The values of viscosity coefficients $A$ and $B$ were obtained from the intercept and slope of the plot $(\eta_{\mathrm{r}}-1)/m^{1/2}$ against $m^{1/2}$. $A$ determines the solute–solute interactions, whereas $B$ is a measure of structural modification due to solute–solvent interactions [31,32]. The values of $A$- and $B$-coefficients are summarized in Table 3. The large positive values of $B$-coefficients as compared to $A$-coefficients suggest the predominance of solute–solvent interactions over solute–solute interactions, thereby, supporting the behavior of $\overline{V_{2}^{0}}$ and $S_{\mathrm{v}}^{*}$ for the systems under study.

The positive values of the $B$-coefficient correspond to the structure-making behavior, and negative values correspond to the structure-breaking behavior of the solute [33]. Thus, positive $B$ values for all the AAs and Gly-Gly, suggest that AAs and Gly-Gly in aqueous D-galactose act as structure-makers.

According to Feakins et al. [34], the $B$-coefficient of viscosity depends on the molar volume of the solvent according to the equation,

$$B=\frac{\left(\overline{V_{1}^{0}}-\overline{V_{2}^{0}}\right)}{1000}+\frac{\overline{V_{1}^{0}}\left(\Delta\mu_{2}^{0*}-\Delta\mu_{1}^{0*}\right)}{1000RT} \tag{13}$$

where $\overline{V_{1}^{0}}$ is the partial molar volume of the solvent, and $\Delta\mu_{1}^{0*}$ and $\Delta\mu_{2}^{0*}$ are the respective free energies per mole of the solvent and solute. Eyring and co-workers [35] proposed that the free energy of activation of viscous flow per mole of solvent, $\Delta\mu_{1}^{0*}$ can be calculated as

$$\Delta\mu_{1}^{0*}=RT\ln\left(\frac{\eta_{0}\overline{V_{1}^{0}}}{hN_{\mathrm{A}}}\right) \tag{14}$$

**Table 3** Values of $A$, $B$, $\Delta\mu_1^{0*}$, and $\Delta\mu_2^{0*}$ of glycine, alanine, phenylalanine, and glycylglycine in aqueous D-galactose solutions at different temperatures

| $T$ (K) | $10^2 \times A$ ($dm^{3/2} \cdot mol^{-1/2}$) | $10^2 \times B$ ($dm^3 \cdot mol^{-1}$) | $\Delta\mu_1^{0*}$ ($kJ \cdot mol^{-1}$) | $\Delta\mu_2^{0*}$ ($kJ \cdot mol^{-1}$) |
|---|---|---|---|---|
| Gly + aqueous D-galactose | | | | |
| 298.15 | 3.569 | 7.323 | 9.288 | 23.560 |
| 303.15 | 2.289 | 8.132 | 9.179 | 25.206 |
| 308.15 | 1.174 | 8.784 | 9.066 | 26.681 |
| 313.15 | 0.137 | 9.234 | 8.978 | 27.753 |
| Ala + aqueous D-galactose | | | | |
| 298.15 | 1.794 | 24.300 | 9.288 | 51.398 |
| 303.15 | 0.873 | 25.270 | 9.179 | 53.429 |
| 308.15 | −0.170 | 26.569 | 9.066 | 55.964 |
| 313.15 | −1.678 | 27.779 | 8.978 | 58.461 |
| Phe + aqueous D-galactose | | | | |
| 298.15 | 6.996 | 61.463 | 9.288 | 107.518 |
| 303.15 | 3.366 | 63.802 | 9.179 | 113.192 |
| 308.15 | 3.390 | 63.671 | 9.066 | 115.569 |
| 313.15 | −3.009 | 64.507 | 8.978 | 118.904 |
| Gly-Gly + aqueous D-galactose | | | | |
| 298.15 | 1.441 | 28.453 | 9.288 | 57.553 |
| 303.15 | 0.018 | 29.246 | 9.179 | 59.983 |
| 308.15 | −1.148 | 29.344 | 9.066 | 61.483 |
| 313.15 | −2.181 | 29.423 | 8.978 | 63.006 |

where $R$, $h$, and $N_A$ are the universal gas constant, Planck's constant, and Avogadro's number, respectively.

Rearrangement of Eq. 13 gives $\Delta\mu_2^{0*}$ as

$$\Delta\mu_2^{0*} = \Delta\mu_1^{0*} + \left(\frac{RT}{\overline{V_1^0}}\right)\left[1000B - \left(\overline{V_1^0} - \overline{V_2^0}\right)\right] \tag{15}$$

The values of $\Delta\mu_1^{0*}$ and $\Delta\mu_2^{0*}$ are also listed in Table 3. Table 3 shows that $\Delta\mu_2^{0*}$ values are positive and larger than $\Delta\mu_1^{0*}$. This suggests that the formation of the transition state is less favored in the presence of AAs/peptide, i.e., the interactions between solute (AAs/Gly-Gly) and solvent (D-galactose + water) molecules in the ground state are stronger than in the transition state. The $\Delta\mu_2^{0*}$ values follow the order,

$$\text{Gly} < \text{Ala} < \text{Gly-Gly} < \text{Phe}$$

This reflects that the solute having a longer alkyl chain requires more energy in transferring from the ground state to the transition state. Similar results were also

**Table 4** Values of $R_D$ of glycine, alanine, phenylalanine, and glycylglycine in aqueous D-galactose solutions at different temperatures

| $m$ (mol · kg$^{-1}$) | $T$ (K) | | | |
|---|---|---|---|---|
| | 298.15 | 303.15 | 308.15 | 313.15 |
| | $10^6 \times R_D$ (m$^3$ · mol$^{-1}$) | | | |
| Gly + aqueous D-galactose | | | | |
| 0.00 | 3.7632 | 3.7639 | 3.7638 | 3.7633 |
| 0.01 | 3.7637 | 3.7645 | 3.7649 | 3.7652 |
| 0.05 | 3.7656 | 3.7666 | 3.7673 | 3.7679 |
| 0.10 | 3.7684 | 3.7694 | 3.7703 | 3.7713 |
| 0.15 | 3.7716 | 3.7730 | 3.7741 | 3.7750 |
| 0.20 | 3.7740 | 3.7752 | 3.7768 | 3.7780 |
| Ala + aqueous D-galactose | | | | |
| 0.00 | 3.7632 | 3.7639 | 3.7638 | 3.7633 |
| 0.01 | 3.7631 | 3.7633 | 3.7645 | 3.7654 |
| 0.05 | 3.7662 | 3.7669 | 3.7674 | 3.7687 |
| 0.10 | 3.7712 | 3.7718 | 3.7727 | 3.7744 |
| 0.15 | 3.7765 | 3.7775 | 3.7784 | 3.7801 |
| 0.20 | 3.7828 | 3.7838 | 3.7841 | 3.7858 |
| Phe + aqueous D-galactose | | | | |
| 0.00 | 3.7632 | 3.7639 | 3.7638 | 3.7633 |
| 0.01 | 3.7638 | 3.7647 | 3.7660 | 3.7657 |
| 0.04 | 3.7669 | 3.7676 | 3.7685 | 3.7687 |
| 0.08 | 3.7716 | 3.7730 | 3.7740 | 3.7750 |
| 0.12 | 3.7758 | 3.7769 | 3.7791 | 3.7802 |
| Gly-Gly + aqueous D-galactose | | | | |
| 0.00 | 3.7632 | 3.7639 | 3.7638 | 3.7633 |
| 0.01 | 3.7635 | 3.7638 | 3.7640 | 3.7641 |
| 0.05 | 3.7645 | 3.7650 | 3.7652 | 3.7655 |
| 0.10 | 3.7655 | 3.7660 | 3.7664 | 3.7668 |
| 0.15 | 3.7664 | 3.7671 | 3.7678 | 3.7683 |
| 0.20 | 3.7678 | 3.7683 | 3.7692 | 3.7700 |

reported for Gly, Ala, and valine in aqueous urea [36] and also for Gly, Ala, serine, and valine in aqueous glucose solutions [16].

According to Feakins et al. [34], the greater the value of $\Delta\mu_2^{0*}$ the greater is the structure-making ability of the solute. Therefore, all three AAs and Gly-Gly act as structure-makers in aqueous D-galactose solution. This again supports the behavior of the properties discussed earlier.

The refractive index data have been used to compute the molar refractivity, $R_D$, using the Lorentz–Lorenz equation,

$$R_{\mathrm{D}} = \left(\frac{n_{\mathrm{D}}^2 - 1}{n_{\mathrm{D}}^2 + 2}\right)\left(\frac{\sum_{i=1}^{3} x_i M_i}{\rho}\right) \tag{16}$$

where $x_i$ and $M_i$ are the mole fraction and molar mass of the $i$th component of the mixtures, respectively. The values of $R_{\mathrm{D}}$ are given in Table 4. As $R_{\mathrm{D}}$ is directly proportional to the molecular polarizability, Table 4 shows that the overall polarizability of the three AAs and one peptide increases with increasing amount of the solute in the mixture. It has been shown [37] that the quantity $\left(n_{\mathrm{D}}^2 - 1\right)/\left(n_{\mathrm{D}}^2 + 2\right)^{5/3}$ is proportional to the cohesive energy density of the liquids and liquid mixtures and, hence, molar refractions are related to internal forces present among the entities of a liquid mixture. Thus, increase in $R_{\mathrm{D}}$ with increase in the amount of AAs/peptide can be attributed to the increased interaction between solute and solvent molecules in the mixtures.

**Acknowledgment** One of the authors (AA) is thankful to the University Grants Commission (U.G.C.), Govt. of India, for a grant of a major research project.

## References

1. V. Abate, G. Barone, G. Castronuovo, V. Elin, V. Savino, J. Chem. Soc. Faraday Trans. I **80**, 759 (1984)
2. F. Franks, M.J. Tait, A. Suggett, S. Ablett, P.A. Quickenden, J. Solution Chem. **1**, 131 (1972)
3. F. Franks, D.S. Reid, A. Suggett, J. Solution Chem. **2**, 99 (1973)
4. F. Franks, *Water* (The Royal Society of Chemistry, London, 1983), p. 135
5. J.F. Back, D. Oakenfull, M.B. Smith, Biochemistry **18**, 5191 (1979)
6. H. Uedaira, Bull. Chem. Soc. Jpn. **53**, 2451 (1980)
7. S. Li, W. Sang, R. Lin, J. Chem. Thermodyn. **34**, 1761 (2002)
8. M.E. Newcomer, B.A. Lewis, F.A. Quiocho, J. Biol. Chem. **254**, 13218 (1981)
9. F.A. Quiocho, N.K. Vyas, Nature **310**, 381 (1984)
10. K. Bock, Pure Appl. Chem. **55**, 605 (1983)
11. H. Thogersen, R.U. Lemieux, K. Bock, B. Meyer, Can. J. Chem. **60**, 44 (1982)
12. K.A. Karlsson, Pure. Appl. Chem. **59**, 1477 (1987)
13. T. Osawa, T. Tsuji, Annu. Rev. Biochem. **56**, 21 (1987)
14. E.V. Parfenyuk, O.I. Davydova, N.S. Levedeva, J. Solution Chem. **33**, 1 (2004)
15. C. Zhao, P. Ma, J. Li, J. Chem. Thermodyn. **37**, 37 (2005)
16. A. Ali, S. Hyder, S. Sabir, D. Chand, A.K. Nain, J. Chem. Thermodyn. **38**, 136 (2006)
17. A. Ali, A.K. Nain, Bull. Chem. Soc. Jpn. **75**, 681 (2002)
18. J. Wang, Z. Yan, K. Zhuo, D. Liu, Z. Phys. Chem. **214**, 333 (2000)
19. T.S. Banipal, P. Kapoor, J. Indian Chem. Soc. **76**, 431 (1999)
20. J.F. Reading, I.D. Watson, G.R. Hedwig, J. Chem. Thermodyn. **22**, 159 (1990)
21. A.K. Mishra, J.C. Ahluwalia, J. Phys. Chem. **88**, 86 (1984)
22. F. Franks, M.A. Quickenden, D.S. Reid, B. Watson, Trans. Faraday Soc. Trans. 1 **66**, 582 (1970)
23. S. Teresawa, H. Hsuki, S. Arakawa, J. Phys. Chem. **79**, 2345 (1975)
24. A. Bondi, J. Phys. Chem. **68**, 441 (1964)
25. F. Shahidi, P.G. Farrell, J.T. Edwards, J. Solution Chem. **5**, 807 (1976)
26. R. Bhat, J.C. Ahluwalia, J. Phys. Chem. **89**, 1099 (1985)
27. F.J. Millero, A.L. Surdo, C. Shin, J. Phys. Chem. **82**, 784 (1978)
28. E. Berlin, M.J. Pallansch, J. Phys. Chem. **72**, 1887 (1968)
29. F.J. Millero, G.K. Lepple, E.V. Haff, J. Phys. Chem. **78**, 1636 (1974)
30. G. Jones, M. Dole, J. Am. Chem. Soc. **51**, 2950 (1929)
31. D. Feakins, D.J. Freemantle, K.G. Lawrence, J. Chem. Soc. Faraday Trans. I **70**, 795 (1974)
32. R.H. Stokes, R. Milles, *International Encyclopedia of Physical Chemistry and Chemical Physics* (Pergamon, New York, 1965)
33. M.A. Motin, J. Chem. Eng. Data **49**, 94 (2004)

34. D. Feakins, F.M. Canning, W.E. Waghorne, K.G. Lawrence, J. Chem. Soc. Faraday Trans. **89**, 3381 (1993)
35. S. Glasstone, K.J. Laidlar, H. Eyring, *The Theory of Rate Processes* (McGraw Hill, New York, 1941), p. 477
36. A. Pal, S. Kumar, J. Mol. Liq. **109**, 23 (2004)
37. Y. Marcus, *Introduction to Liquid State Chemistry* (Wiley, New York, 1977), p. 16