

# Refractive Indices and Isentropic Compressibilities of Glycylglycine–FeCl<sub>2</sub> in Aqueous Ethanol Mixtures

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The density and refractive index studies have been carried out for glycylglycine–FeCl<sub>2</sub> in aqueous ethanol mixtures at four different temperatures in the range  $T = (288.15 \text{ to } 318.15) \text{ K}$ . A comparative study of the refractive indices obtained experimentally and those calculated by means of Gladstone–Dale and Lorentz–Lorenz relation has been made. Among them, the Gladstone–Dale equation afforded similar values to those obtained experimentally. Isentropic compressibilities,  $\kappa_S$ , and excess molar isentropic compressibilities,  $K_{S,m}^E$ , were estimated using the Kirkwood model. Using the Redlich–Kister type equations, excess partial molar isentropic compressibilities,  $K_{S,i}^E$ , were also obtained. The observed negative values of  $K_{S,2}^E$  in the water-rich region are due to the structural disorderliness. As infinitely dilute water is hydrogen-bonded to ethanol and glycylglycine, the variation of strength of interaction is not sensed by  $K_{S,1}^E$ .

## Introduction

Hydration is an important phenomenon which helps in determining the native structure of proteins. Various functional groups of proteins upon interaction with water and other solvents contribute to the formation of stable folded structures associated with proteins.<sup>1</sup> A direct study of protein thermodynamics is somewhat difficult in aqueous and mixed solvent systems. To overcome this, a useful approach is to study small molecules such as amino acids and peptides in simple aqueous alcohol mixtures unraveling the molecular interactions existing between solute and solvents, which can mimic specific aspects of protein structure. The effect of electrolytes on the structure and function of both proteins and nucleic acids has been widely studied in terms of their structure-making and structure-breaking properties.<sup>2</sup> Salt-induced electrostatic forces are known to play a vital role in modifying the amino acid structure by affecting the properties like solubility, denaturation, and activity of enzymes.<sup>3</sup> It is well-known that water forms the basis of all living organisms. Similarly, ethanol has long been used as a powerful disinfectant in medicine and as an extraction solvent in pharmaceutical and food industry. It is also present in food and beverage products either naturally or added for its functional properties. In this regard, the model compound approach<sup>4,5</sup> is a good source for numerous studies involving amino acids and peptides which are the basic building blocks of proteins. On the other hand, transition metal ions play a significant role in plant growth, lipid metabolism, and regulation of physiological systems. Thermodynamic properties of model compounds with transition metal salt in aqueous ethanol media provide useful information on solute–solvent, solute–solute, and solvent–solvent interactions enhancing our understanding of more complex biochemical processes.<sup>6–8</sup> Previous workers<sup>9–14</sup> have focused mainly on thermodynamic, volumetric, and ultrasonic velocity studies of aqueous ethanol mixtures. However, studies involving dipeptides and metal salts in aqueous ethanol mixtures are very

scant. Also, it is well-known that density and refractive index measurements of binary systems provide essential analytical information for industrial purposes. Hence, in continuation of our earlier studies<sup>15–19</sup> involving dipeptide and transition metal salts, we make an attempt to understand the physicochemical behavior of glycylglycine + FeCl<sub>2</sub> in aqueous ethanol mixtures through ultrasonic velocity, density, and refractive index measurements. From these experimental data, isentropic compressibilities and excess molar isentropic compressibilities are also reported and interpreted in terms of intermolecular interactions occurring in solution.

## Experimental Section

**Materials.** Glycylglycine (CAS 556-50-3) and iron(II) chloride tetrahydrate (CAS 13478-10-9) of mass fraction purity 0.99 were purchased from Sigma-Aldrich, Germany. Commercially available glycylglycine of the highest purity was used without further purification. Iron(II) chloride tetrahydrate was used after drying for 72 h in a vacuum desiccator at room temperature. Deionized, double-distilled degassed water with a specific conductance of less than  $1.29 \cdot 10^{-6} \Omega^{-1} \cdot \text{cm}^{-1}$  was used for the preparation of solutions. Ethanol (CAS 64-17-5) was purchased from Changshu Yangyuan Chemicals, China and had mass fraction purity 0.999. Ethanol was further distilled and used in our experiments to ensure maximum purity. The solutions were prepared on a weight basis by using a Mettler balance having a precision of  $\pm 0.01 \text{ mg}$ . Care was taken to avoid evaporation and contamination during mixing. In our studies, glycylglycine and FeCl<sub>2</sub> were kept constant at  $0.020 \text{ mol} \cdot \text{kg}^{-1}$  and  $0.25 \text{ mol} \cdot \text{kg}^{-1}$ , respectively, and the composition of ethanol was varied in terms of mole fraction ( $x_1$ ). The estimated uncertainty for the mole fraction of ethanol was found to be  $< 1 \cdot 10^{-4}$ . To prevent the formation of air bubbles, all solutions were preheated in sealed Eppendorf tubes to 5 °C above the measurement temperature before filling the ultrasonic and densimetric cells.

**Methods.** Ultrasonic velocities of pure components and their mixtures were measured by a variable-path fixed-frequency interferometer supplied by Mittal Enterprises, New Delhi (model

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83). It consists of a high-frequency generator and a measuring cell. The measurements of ultrasonic velocities are made at a fixed frequency of 2 MHz. The volume of the measuring cell was 7 mL. The calibration of ultrasonic interferometer was made by measuring the velocity in AR grade benzene and carbon tetrachloride. The maximum estimated error in ultrasonic velocity measurements was  $\pm 0.08\%$ . The temperature was controlled by circulating water around the liquid cell from a thermostatically controlled adequately stirred water bath with an uncertainty of  $\pm 0.01$  K. Densities were measured using the (Mettler Toledo) density 30PX digital densitometer having an accuracy of  $\pm 1 \cdot 10^{-3} \text{ kg} \cdot \text{m}^{-3}$ . Refractive indices were measured using automatic digital refractometer RX-7000 $\alpha$ , Atago Co. Ltd., Japan, having an accuracy of  $\pm 0.0001\%$ . The densitometer and refractometer were calibrated using double-distilled water. The sample and reference resonator cells with minimum volumes of  $0.5 \text{ cm}^3$  were thermostatted to an accuracy of  $\pm 0.01$  K, and a previously described differential technique was employed for all measurements.<sup>20</sup> The ultrasonic velocity and density for aqueous ethanol solutions of glycylglycine-FeCl<sub>2</sub> were measured at four different temperatures,  $T = (288.15, 298.15, 308.15, \text{ and } 318.15) \text{ K}$ . Each measurement was repeated thrice, and the reported values are an average of all three measurements.

## Results and Discussion

The experimental values of ultrasonic velocity,  $u$ , density,  $\rho$ , refractive index,  $n_D$ , and calculated values of isentropic compressibility,  $\kappa_S$ , at  $T = (288.15 \text{ to } 318.15) \text{ K}$  are reported in Table 1. Using the Newton-Laplace equation given in eq 1, the isentropic compressibilities,  $\kappa_S$ , were obtained.

$$\kappa_S/\text{Pa}^{-1} = \frac{1}{u^2 \rho} \quad (1)$$

The variation of  $\kappa_S$  values as a function of temperature and composition at four different temperatures are shown in Figure 1. An increase in the magnitude of  $\kappa_S$  values can be attributed to the hydrophilic effect.<sup>21</sup> Although the temperature effect is rather small, displacement toward the more water-rich region takes place. Hence, two opposing temperature-dependent effects get canceled out.

**Refractive Indices.** The refractive indices of binary mixtures are correlated using the well-known Lorentz-Lorenz equation:<sup>22</sup>

$$\frac{n_L^2 - 1}{n_L^2 + 2} = \phi_1 \frac{n_{D1}^2 - 1}{n_{D1}^2 + 2} + \phi_2 \frac{n_{D2}^2 - 1}{n_{D2}^2 + 2} \quad (2)$$

where  $n_L$  is the refractive index of the mixture according to the Lorentz-Lorenz formula,  $\Phi_1$  and  $\Phi_2$  are the volume fractions of ethanol and water, calculated from volume determinations based on the mass and density measurements, and  $n_{D1}$  and  $n_{D2}$  are the refractive indices of ethanol and water, respectively.

To determine the refractive indices of binary liquid mixtures, we have adopted an empirical equation proposed by Gladstone-Dale:<sup>23</sup>

$$\frac{n_G - 1}{\rho} = w_1 \left( \frac{n_{D1} - 1}{\rho_1} \right) + w_2 \left( \frac{n_{D2} - 1}{\rho_2} \right) \quad (3)$$

where  $n_G$  is the refractive index of the mixture according to the Gladstone-Dale formula,  $\rho$  is the experimental density of the

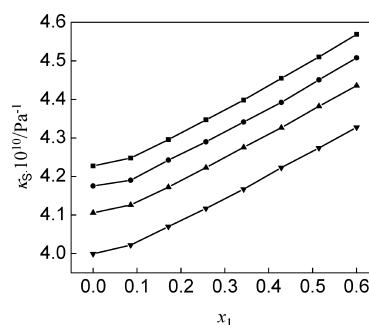
**Table 1.** Mole Fraction of Ethanol,  $x_1$ , Ultrasonic Velocity,  $u$ , Density,  $\rho$ , Isentropic Compressibility,  $\kappa_S$ , and Refractive Indices,  $n_D$ ,  $n_G$  (Gladstone-Dale),  $n_L$  (Lorentz-Lorenz), and  $\Delta n_G$ , of Glycylglycine-FeCl<sub>2</sub> (1) in Aqueous Ethanol Mixtures (2) at  $T = (288.15 \text{ to } 318.15) \text{ K}$

$x_1$	$u$ $\text{m} \cdot \text{s}^{-1}$	$\rho \cdot 10^{-3}$ $\text{kg} \cdot \text{m}^{-3}$	$\kappa_S \cdot 10^{10}$ $\text{Pa}^{-1}$	$n_D$	$n_G$	$n_L$	$\Delta n_G$
$T/\text{K} = 288.15$							
0.0000	1518.30	1.0261	4.22761	1.3591	1.3591	1.3589	0.0000
0.0857	1522.16	1.0161	4.24759	1.3620	1.3618	1.3483	0.0002
0.1715	1525.34	1.0006	4.29543	1.3649	1.3642	1.3502	0.0007
0.2572	1529.25	0.9837	4.34691	1.3678	1.3675	1.3523	0.0003
0.3430	1533.76	0.9665	4.39828	1.3707	1.3700	1.3568	0.0007
0.4288	1537.32	0.9498	4.45491	1.3735	1.3729	1.3589	0.0006
0.5145	1541.54	0.9331	4.50985	1.3763	1.3755	1.3616	0.0008
0.6003	1545.43	0.9164	4.56895	1.3791	1.3783	1.3634	0.0008
$T/\text{K} = 298.15$							
0.0000	1528.06	1.0257	4.17541	1.3576	1.3576	1.3574	0.0000
0.0857	1533.14	1.0153	4.19027	1.3605	1.3602	1.3467	0.0003
0.1715	1536.25	0.9988	4.24226	1.3633	1.3630	1.3478	0.0003
0.2572	1540.48	0.9823	4.28987	1.3661	1.3657	1.3501	0.0004
0.3430	1544.37	0.9658	4.34120	1.3689	1.3682	1.3523	0.0007
0.4288	1548.74	0.9492	4.39223	1.3717	1.3710	1.3554	0.0007
0.5145	1552.19	0.9326	4.45056	1.3745	1.3737	1.3587	0.0008
0.6003	1556.42	0.9158	4.50760	1.3773	1.3765	1.3617	0.0008
$T/\text{K} = 308.15$							
0.0000	1541.34	1.0252	4.10577	1.3540	1.3540	1.3538	0.0000
0.0857	1545.43	1.0148	4.12592	1.3569	1.3567	1.3404	0.0002
0.1715	1549.67	0.998	4.17245	1.3597	1.3593	1.3432	0.0004
0.2572	1553.53	0.9812	4.22283	1.3625	1.3620	1.3472	0.0005
0.3430	1557.26	0.9644	4.27583	1.3653	1.3648	1.3487	0.0005
0.4288	1561.81	0.9476	4.32632	1.3681	1.3675	1.3514	0.0006
0.5145	1565.79	0.9308	4.38204	1.3709	1.3701	1.3543	0.0008
0.6003	1569.68	0.915	4.43564	1.3737	1.3729	1.3588	0.0008
$T/\text{K} = 318.15$							
0.0000	1562.37	1.0243	3.99949	1.3516	1.3516	1.3513	0.0000
0.0857	1566.56	1.0131	4.02211	1.3545	1.3543	1.3389	0.0002
0.1715	1570.39	0.9963	4.07002	1.3573	1.3570	1.3411	0.0003
0.2572	1574.73	0.9795	4.11702	1.3601	1.3596	1.3456	0.0005
0.3430	1578.86	0.9627	4.16698	1.3629	1.3623	1.3487	0.0006
0.4288	1582.28	0.9459	4.22268	1.3657	1.3650	1.3514	0.0007
0.5145	1586.94	0.9291	4.27382	1.3685	1.3677	1.3532	0.0008
0.6003	1590.14	0.9139	4.32744	1.3713	1.3704	1.3565	0.0009

mixture,  $w_1$  and  $w_2$  are the mass fractions of ethanol and water, and  $n_{D1}$  and  $n_{D2}$  are the refractive indices of ethanol and water, respectively.

To determine the formula that best predicts the refractive indices of the studied system, we have made a comparison of the values obtained from eqs 2 and 3.

On the other hand, calculations have also been made to identify the differences between the refractive index obtained experimentally,  $n_D$ , and that calculated from the equation of Gladstone-Dale,  $n_G$ , that is,  $\Delta n_G = n_D - n_G$ .



**Figure 1.** Composition dependence of isentropic compressibilities,  $\kappa_S$ , of glycylglycine-FeCl<sub>2</sub> in aqueous ethanol mixtures at different temperatures: ■, 288.15 K; ●, 298.15 K; ▲, 308.15 K; ▼, 318.15 K.

On the basis of the above etiquette, the values of the experimental refractive indices,  $n_D$ , and the expected values according to the equations of Gladstone–Dale,  $n_G$ , and Lorentz–Lorenz,  $n_L$ , as a function of mole fraction of ethanol are shown in Table 1. The calculated values of Gladstone–Dale equation provide a very good correlation with the experimental values. In contrast, the values obtained with the Lorentz–Lorenz expression differ greatly from the experimental values.

This observation could be explained by taking into account the volume contraction occurring in mixtures of alcohols and water involving dipeptide and transition metal salt, and further, the density of the mixture is considered in the Gladstone–Dale equation. As the volume contraction in this case is significant, with an increase in temperature, the discrepancy between the experimental values and those obtained from the Lorentz–Lorenz expression decreases. Accordingly, Gladstone–Dale equation is the best refractive index predicting equation. The differences,  $\Delta n_G$ , between the experimental refractive index values,  $n_D$ , and the values obtained by Gladstone–Dale equation,  $n_G$ , have been calculated.

**Excess Molar and Excess Partial Molar Isentropic Compressibility.** The molar isentropic compressibility is defined by  $K_{S,m}^E = V_m \kappa_S = -(\partial V_m / \partial p)_S$ , where the molar volume of the mixture is denoted by  $V_m$ . A comparison of the deviation of the excess molar isentropic compressibility for real mixtures from those of a well-defined ideal state are shown in the plots of  $K_{S,m}^E$  as a function of composition. As a powerful tool, the excess partial molar property allows the separation of  $K_{S,m}^E$  into individual contributions of the components in the equilibrium mixture. However, important insights on changes in various features such as packing effects, aggregation pattern, solvation produced by modifications in composition, temperature, and pressure can be obtained by excess partial molar isentropic compressibility,  $K_{S,m}^E$ .<sup>24,25</sup> Further, to estimate the excess quantity,  $K_{S,m}^E$ , defined by eq 6,  $K_{S,m}$  values were calculated and used in this work.

$$K_{S,m}^E / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = K_{S,m} - K_{S,m}^{\text{id}} \quad (6)$$

where  $K_{S,m}^{\text{id}}$  is the molar isentropic compressibility of a thermodynamically ideal mixture at the same temperature, pressure, and composition as  $K_{S,m}$  and  $K_{S,m}^E$ . For the experimental compositions at different temperatures, this quantity was estimated using eq 7.

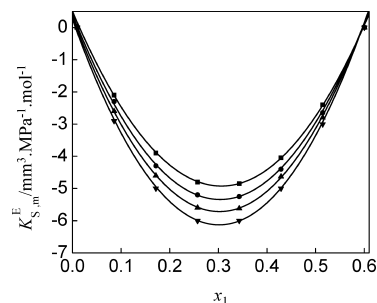
$$K_{S,m}^{\text{id}} / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = K_{T,m}^{\text{id}} - T(E_{p,m}^{\text{id}})^2 / C_{p,m}^{\text{id}} \quad (7)$$

where  $K_{T,m}^{\text{id}}$ ,  $E_{p,m}^{\text{id}}$ , and  $C_{p,m}^{\text{id}}$  are the ideal molar isothermal compression, isobaric expansion, and isobaric heat capacity, respectively. By the following simple mixing rule, ideal molar values of these Gibbsian properties<sup>26</sup> were obtained.

$$Q_m^{\text{id}} = x_1 Q_{m,1}^* + x_2 Q_{m,2}^* \quad (8)$$

where  $Q_m^{\text{id}}$  stands for  $K_{T,m}^{\text{id}}$ ,  $E_{p,m}^{\text{id}}$ , or  $C_{p,m}^{\text{id}}$ .  $Q_{m,1}^*$  and  $Q_{m,2}^*$  are the corresponding molar properties of pure water and ethanol, respectively.

As a function of ethanol concentration, the estimated excess molar isentropic compressibilities are plotted in Figure 2 at  $T = (288.15 \text{ to } 318.15) \text{ K}$ . With an increase in temperature,



**Figure 2.** Composition dependence of excess molar isentropic compressibility,  $K_{S,m}^E$ , for glycyglycine–FeCl<sub>2</sub> in an aqueous ethanol mixture at different temperatures: ■, 288.15 K; ●, 298.15 K; ▲, 308.15 K; ▼, 318.15 K.

**Table 2.** Least Squares Fitting Coefficients,  $B_k$ , of Equation 9 and Corresponding Standard Deviations of the Fits,  $\sigma_{\text{fit}}$ , for Glycyglycine–FeCl<sub>2</sub> (1) in Aqueous Ethanol Mixture (2) at Different Temperatures

coefficients	$T/K = 288.15$	$T/K = 298.15$	$T/K = 308.15$	$T/K = 318.15$
$B_0$	-32.4458	-32.3672	-32.2893	-32.1659
$B_1$	17.4867	16.8945	16.2564	15.6385
$B_2$	-9.2398	-8.8876	-8.6627	-8.2148
$B_3$	21.0874	19.5672	17.9899	15.8366
$B_4$	-18.3636	-16.0368	-14.1278	-12.9235
$\sigma_{\text{fit}}$	0.067	0.063	0.061	0.058

the moderate negative values decrease. To represent the  $K_{S,m}^E$  values, the Redlich–Kister fitting method as given in eq 9 was applied:

$$K_{S,m}^E / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = x_1 x_2 \sum_{k=0}^{k=n} B_k (2x_2 - 1)^k \quad (9)$$

The  $F$ -test indicated that five terms ( $n = 4$ ) is the appropriate number, and correspondingly, the number of  $B_k$  parameters were varied. Figure 2 also presents these analytical correlations. The standard deviation of the fits along with the values of the  $B_k$  parameter is shown in Table 2. The excess partial molar isentropic compressibility of both components,  $K_{S,i}^E$ , was obtained from eq 10 to separate the contributions of each component to the excess molar isentropic compressibility.

$$K_{S,i}^E / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = K_{S,m}^E + (1 - x_i) \frac{dK_{S,m}^E}{dx_i} \quad (10)$$

eq 9 was differentiated with respect to  $x_2$  to evaluate the derivative shown in the right-hand side of eq 10. Figure 3 shows the resulting excess partial molar isentropic compressibility of water and ethanol at extreme temperatures, (288.15 and 318.15) K. A good interpretation for the excess partial molar isentropic compressibility values was given by the Kirkwood model.<sup>27</sup> At all mixture compositions, a better packing of the components in the liquid mixture than in their pure states is indicated by the negative  $K_{S,i}^E$  values found for both components. The principal reasons for the marked negative values of  $K_{S,2}^E$  in the water-rich region may be the structural disorderliness caused due to the presence of FeCl<sub>2</sub> and the hydrophobic effect of the CH<sub>2</sub> groups in the molecule. The zwitterionic state of the glycyglycine is the most favorable form in water. Figure 3 shows that, at high

**Table 3.** Limiting Excess Partial Molar Compressibility,  $K_{S,i}^{E,\infty}$ , and Standard Deviations,  $\sigma_i$ , for Glycylglycine–FeCl<sub>2</sub> (1) in Aqueous Ethanol Mixture (2) at Different Temperatures

$T$ K	$K_{S,1}^{E,\infty}$ $\text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1}$	$\sigma_1$ $\text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1}$	$K_{S,2}^{E,\infty}$ $\text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1}$	$\sigma_2$ $\text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1}$
288.15	-23.83	0.89	-85.32	1.48
298.15	-23.52	0.92	-82.91	2.83
308.15	-23.30	0.86	-78.45	2.79
318.15	-23.17	0.88	-73.74	2.56

temperatures, the  $K_{S,2}^{E,\infty}$  values present a neat displacement to less negative values in the water-rich region and the  $K_{S,1}^{E,\infty}$  curves for the two temperatures close to one another. This may happen in the water-rich region and at slightly higher compositions due to solute–solvent interactions and hydrophobic effect that varies with temperature becoming more evident at low temperatures.

**Limiting Partial Molar Isentropic Compressibility.** The limiting excess partial molar isentropic compressibility of water in ethanol,  $K_{S,1}^{E,\infty}$ , and ethanol in water,  $K_{S,2}^{E,\infty}$ , can be easily obtained using the concept of partial molar properties from eq 9. The excess apparent molar isentropic compressibility of both components,  $K_{S,\phi,i}^E$ , can be expressed as a function of  $K_{S,m}^{E,\infty}$  by eq 11:

$$K_{S,\phi,i}^E = \frac{K_{S,m}^{E,\infty}}{x_i} \quad (11)$$

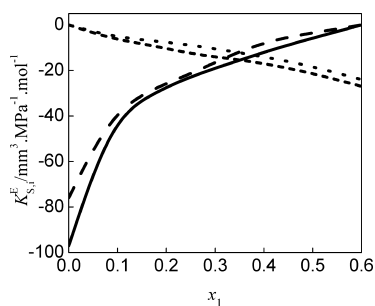
Substituting in eq 11  $K_{S,m}^{E,\infty}$  in eq 9, and as  $x_i$  tends to zero, the limiting excess partial molar isentropic compressibility can be expressed in terms of the  $B_k$  coefficients. Maham et al.<sup>28</sup> first obtained the resulting eqs 12 and 13.

$$\lim_{x_1 \rightarrow 0} K_{S,\phi,1}^E = K_{S,1}^{E,\infty} / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = \sum_k B_k \quad (12)$$

$$\lim_{x_2 \rightarrow 0} K_{S,\phi,2}^E = K_{S,2}^{E,\infty} / \text{mm}^3 \cdot \text{MPa}^{-1} \cdot \text{mol}^{-1} = \sum_{k=\text{even}} B_k - \sum_{k=\text{odd}} B_k \quad (13)$$

The standard deviations calculated on the basis of the standard deviation of  $B_k$  coefficients are presented in Table 3 along with the  $K_{S,i}^{E,\infty}$  values.

Within their standard uncertainty, the negative values of  $K_{S,1}^{E,\infty}$  seem to be constant. In relation to the pure state, a better packing of water in an ethanol environment is observed. This quantity would not sense the variation of strength of interaction, if



**Figure 3.** Composition dependence of the excess partial molar isentropic compressibility,  $K_{S,i}^E$ , in a glycylglycine–FeCl<sub>2</sub> aqueous ethanol system: ethanol, —, at 288.15 K and ---, at 318.15 K; water, ···, at 288.15 K and - · -, at 318.15 K.

infinitely dilute water is hydrogen-bonded to ethanol and glycylglycine as  $K_{S,1}^{E,\infty}$  is consistent with temperature. In contrast, the corresponding limiting excess partial molar isobaric expansion,  $K_{P,1}^{E,\infty}$  increases with temperature and the consistency of  $K_{S,1}^{E,\infty}$  is not apparent,<sup>29</sup> indicating that the strength of solute–solvent interaction decreases as the temperature increases. Consequently, the process of mixing is sensed differently by the two parent properties. On the other hand, the two aforementioned factors, namely, the changes in ethanol binding when it is infinitely diluted in water and the solute–solvent interactions, are due to the larger negative values of  $K_{S,2}^{E,\infty}$ . A decrease in the intrinsic compression of the ethanol molecule is seen from the first factor, and at the same time, in relation to the pure state, the second effect would stiffen the surrounding water molecules. At infinite dilution, the driving force for hydrophobic hydration depends on solute–solvent hydrophilic interactions rather than on enhancing the water structure.<sup>30–32</sup> As the hydrophobic hydration increases with temperature, weakening of the solute–solvent interaction (H-bonding) prevails, indicating a net increase in the temperature dependences for ethanol. Otherwise,  $K_{S,1}^{E,\infty}$  decreases with the increase in temperature, corresponding to infinitely diluted water in ethanol. In the ethanol-rich region, this trend reflects the behavior of water. Nevertheless, water–ethanol hydrogen bonding would prevail in solvents with a higher density of –OH groups, whereas in mixtures of solvents, water clustering would not be favorable as polar alkyl groups dominate the accessible surface area.

## Conclusions

The experimental studies of ultrasonic velocity, density, and refractive index have been used to calculate excess parameters. A comparison of the values of experimental refractive index and calculated values obtained from Gladstone–Dale and Lorentz–Lorenz equations gives a clear indication that only the Gladstone–Dale equation predicts the refractive indices best. Negative  $K_{S,i}^{E,\infty}$  values indicate a better packing of the components of the mixture. The weakening of the solute–solvent interaction exists upon the increase of hydrophobic hydration with temperature.

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Received for review July 30, 2010. Accepted August 17, 2010. The authors thank the DRDO, Govt. of India, for financial support in the form of an R & D project grant.

JE100789K