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Molecular interactions of glycine and L-alanine + citrate buffer solutions at different temperatures: Volumetric, viscometric, and FTIR approach

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Densities,ρ, and viscosities,$\eta$of glycine/L-alanine in water and in (0.10, 0.50, 1.00) $\mathrm{mol \cdot kg^{-1}}$ aqueous trisodium citrate buffer (Na_3CB) solutions of pH 7.40 at T = (298.15 to 318.15) K have been measured by using vibrating tube digital density meter and suspended level Ubbelohde capillary viscometer, respectively, at atmospheric pressure *i.e.*101.3 kPa. Thermodynamic parameters, *i.e.*, ϕ_v^o, $\Delta_{tr}\phi_v$, viscosity B-coefficients, and $\Delta_{tr}B$ obtained from density and viscosity measurements have been used to unravel predominant forces among glycine/L-alanine and aqueous Na_3CB solutions. Activation free energies, $\Delta\mu_2^{o\#}$ for the viscous flow of solutions, have been obtained by applying transition state theory to the viscosity B-coefficients values. The results are further supported by FTIR studies for glycine/L-alanine in water and aqueous Na_3CB solutions of pH 7.40, revealing the intermolecular hydrogen bonding in these systems. Moreover, the results obtained from volumetric, viscometric, and spectroscopic studies provide valuable information on intermolecular interactions, influencing their efficacy in biological applications.

Keywords: Amino acids, Citrate buffer, Partial molar volume, Viscosity B-coefficients, Activation free energies

Organic salts (such as citrates, phosphates, acetates, etc.) play a vital role in governing the configurational and functional behaviour[1-4] of biologically important molecules such as proteins, nucleic acids, lipids, and enzymes. Proteins are complex bio-molecules; therefore, their organic salts behaviour is influenced by various interactions, *i.e.*, H-bonding, electrostatic interactions, van der Waals interactions, etc[5]. Thus, study of these interactions among organic salts and proteins is essential to understanding their physiological properties in aqueous and mixed aqueous solutions. Thus complexity associated with the structure of proteins can be simplified by considering the basic structural units, *i.e.*, amino acids (hereafter abbreviated as AA) and peptides[6]. The functional properties of the AA depend upon various factors like concentration, temperature, the environment of the surrounding molecules, and the pH of the solution. A small change in pH frequently results in an intense change in the conformational stability of protein[7,8]. Therefore, buffers are significant in living organisms to maintain proper homeostasis among the cells[9]. Citrate buffer, one of the vital buffers, plays a crucial role in the biochemical activities of nearly all living organisms. Due to low toxicity and high chemical and thermal stability[10], citrate buffers are suitable for biochemical and medicinal research to maintain accurate pH values (as buffering agents) in various enzymatic reactions and body fluids[11]. Citrate buffer has wide applications, *i.e.*, used to reduce gastric acidity, food preservatives, preservative material for blood banks, form citrate complex with calcium ions in the blood (*i.e.*, effectively decreasing the level of ionized free calcium), and also used to disrupt clotting mechanism[12]. Therefore, interactions between citrate buffer and AA help elucidate the conformation behaviour of protein molecules.

Extensive studies on AA in pure aqueous and mixed aqueous citrate salt solutions have been undertaken[13-20]. Still, no report has been found on the volumetric, viscometric, and spectroscopic properties of amino acids in aqueous trisodium citrate buffer solutions (hereafter abbreviated as Na_3CB) of pH 7.40. Hence, in the present paper, we report a systematic study on the volumetric, viscometric, and FTIR studies of AA (*i.e.*, glycine/L-alanine) in the water of pH 7.40 (zwitterionic)) and in (0.10, 0.50, 1.00) $\mathrm{mol \cdot kg^{-1}}$$Na_3CB$ solutions of pH 7.40 at different temperatures, T = (298.15 to 318.15) K with interval of 10 K, including physiological temperature at atmospheric pressure.

Experimental Details

Materials

Glycine, L-alanine, trisodium citrate dihydrate, and anhydrous citric acid were procured from M/S S.D. Fine Chem. Ltd., India. All details regarding the samples used in the present work are given in Table S1. Chemicals in the current work were used without any purification and then dried in a vacuum oven for 24 h, kept in a desiccator over anhydrous $CaCl_2$ before use. Deionized double distilled and degassed water with specific conductance $<1\times 10^{-6}\mathrm{S}\cdot\mathrm{cm}^{-1}$was used to prepare all the solutions. The conductance of water was checked using SYSTRONICS Conductivity meter-306, India, having a cell constant of $0.10\ \mathrm{cm}^{-1}$.

Methods

Experimental solutions were prepared afresh on a molality, $(m/\mathrm{mol}\cdot\mathrm{kg}^{-1})$ basis using METTLER TOLEDO ME204, India balance with precision of ±0.1 mg. The pH was measured with SYSTRONICS digital pH meter-335, India having repeatability of ±0.01. Calibration of pH meter was done with standard buffer solutions of pH (7.00 and 9.20). Accuracy in pH measurements was found to be ±0.02. Na_3CB of pH 7.40 was prepared by adding equimolar (means the same amount of moles) concentrations of trisodium citrate dihydrate (sodium salt of citric acid) and anhydrous citric acid (a weak acid). The solution densities were measured using a vibrating-tube density meter (DMA 4500M, Anton Paar, Austria). Working of the density meter was also checked by measuring the densities of aqueous sodium chloride (NaCl) solutions at T=298.15 K, which agreed well with the literature values[21]. The accuracy and precision of an average of triplicate density measurements were observed to be $\pm4\times10^{-4}\ \mathrm{g}\cdot\mathrm{cm}^{-3}$ and $\pm2\times10^{-4}\ \mathrm{g}\cdot\mathrm{cm}^{-3}$, respectively. The density meter has a built-in thermostat to maintain the required temperatures within ±0.03 K over the T= (273.15 to 363.15) K.

Viscosity measurements were carried out using a suspended level Ubbelohde viscometer having a flow time of more than 300 s for water at T=298.15 K. The viscometer was calibrated with double distilled degassed distilled water from T=(288.15, 298.15, 308.15, 310.15 and 318.15) K. Flow time measurements were performed with RACER digital electronic stopwatch with a resolution of ±0.01 s. At least an average of four readings reproducible to 0.01 s was obtained and further used as flow time for the experimental solutions. All measurements were carried out in a constant temperature viscobath (Siskin profichill, India) with temperature-controlled up to ±0.01 K. The measured viscosities were accurate to ±1%. The uncertainties calculated from triplicate measurements were ±0.30%.

FTIR spectra were obtained by using PerkinElmer (MODEL SPECTRUM TWO, India), which possesses diamond ATR crystal (Attenuated Total Reflectance) with the range of (8300-350) cm^{-1}. FTIR spectra for the experimental solutions were recorded in the wavenumber range from (4000-450) $\mathrm{cm}^{-1}$with a spectral resolution of 4 cm^{-1}.

Results and Discussion

Volumetric properties

Apparent molar volumes,$\phi_v$of AA in water (Table S2) and in (0.10, 0.50, 1.00) $\mathrm{mol}\cdot\mathrm{kg}-1$aqueous Na_3CB solutions of pH 7.40 summarized in Table 1 were calculated from experimentally measured densities at T= (298.15 to 318.15) K with the interval of 10 K including physiological temperature (*i.e.*, T = 310.15 K) at atmospheric pressure by employing the following equation:

$$\phi_v = {}^{M}\!/_{\rho} - \left[{}^{1000(\rho-\rho_0)}\!/_{m\rho\rho_0}\right] \qquad \ldots(1)$$

where M $(\mathrm{g}\cdot\mathrm{cm}^{-1})$ is the molar mass of the AA, $\rho_o$and ρ $(\mathrm{g}\cdot\mathrm{cm}^{-3})$ are the densities of the solvent and solution, respectively, and $m(\mathrm{mol}\cdot\mathrm{kg}^{-1})$ is the molality of the solutions. The ρ values of glycine and L-alanine in the water of pH 7.40 agree well with the earlier reported values of glycine/L-alanine in water. Comparative plots of densities versus molalities for AA in the water of pH 7.40 (present work) and literature values[22-35] at T = (298.15, 308.15, 310.15, and 318.15) K are given as Fig. 1(a-d) and 2(a-d), respectively. The influence of pH on the charge distribution of these systems involves a more vital interaction between citrate ions and ammonium ions. The amino and carboxyl groups of AA dissociate in aqueous Na_3CB solutions and form negatively and positively charged ions as:

Dissociation of amino acids[7]

$$\mathrm{NH_2RCOOH} \leftrightarrow \mathrm{NH_3^+RCOO^-} \qquad \ldots(i)$$

Dissociation of Na_3CB in water[7]

$$\mathrm{Na_3Cit} \rightleftarrows \mathrm{Cit^{3-}} + 3\mathrm{Na^+} \qquad \ldots(ii)$$

Table 1 — Densities, ρ and apparent molar volumes, ϕ_v of glycine and L-alanine in different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50 and 1.00) mol·kg^{-1}of pH 7.40 at different temperatures, T = (298.15 to 318.15) K

m/ (mol kg^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})
	Glycine in 0.10 mol·kg^{-1}aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.00999		1.00674		1.00600		1.00277	
0.0999	1.01303	44.36	1.00975	44.71	1.00899	44.92	1.00574	45.16
0.2960	1.01888	44.49	1.01532	44.91	1.01474	45.05	1.01145	45.31
0.3950	1.02177	44.57	1.01836	45.02	1.01758	45.13	1.01427	45.39
0.4892	1.02449	44.63	1.02104	45.09	1.02026	45.18	1.01692	45.46
0.5963	1.02753	44.71	1.02403	45.18	1.02324	45.28	1.01989	45.53
0.6822	1.02994	44.77	1.02641	45.23	1.02561	45.33	1.02224	45.59
0.7732	1.03244	44.87	1.02887	45.33	1.02807	45.42	1.02468	45.68
0.9726	1.03790	44.95	1.03423	45.44	1.03345	45.49	1.03003	45.75
	L-alanine in 0.10 mol kg^{-1} aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.01531		1.01205		1.01131		1.00807	
0.0942	1.01788	61.12	1.01459	61.54	1.01384	61.67	1.01058	61.99
0.2918	1.02314	61.24	1.01978	61.69	1.01902	61.79	1.01573	62.07
0.4010	1.02597	61.32	1.02258	61.75	1.02181	61.85	1.01851	62.11
0.5134	1.02882	61.41	1.02539	61.85	1.02462	61.93	1.02130	62.20
0.5856	1.03064	61.42	1.02718	61.88	1.02641	61.96	1.02307	62.24
0.6911	1.03321	61.54	1.02974	61.95	1.02865	62.05	1.02560	62.33
0.8152	1.03619	61.63	1.03271	62.02	1.03192	62.11	1.02856	62.38
0.9003	1.03821	61.68	1.03471	62.07	1.03392	62.15	1.03055	62.41
1.0139	1.04090	61.71	1.03733	62.13	1.03654	62.21	1.03315	62.48
	Glycine in 0.50 mol kg^{-1} aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.08476		1.08069		1.07981		1.07611	
0.1966	1.09001	46.29	1.08590	46.55	1.08501	46.61	1.08128	46.83
0.2974	1.09264	46.35	1.08851	46.61	1.08761	46.69	1.08387	46.89
0.3997	1.09526	46.43	1.09112	46.67	1.09019	46.80	1.08644	46.99
0.4879	1.09749	46.48	1.09331	46.77	1.09236	46.91	1.08861	47.09
0.6013	1.10032	46.54	1.09610	46.85	1.09514	46.99	1.09135	47.20
0.7101	1.10301	46.58	1.09875	46.90	1.09778	47.03	1.09396	47.27
0.8059	1.10530	46.66	1.10100	47.00	1.10006	47.09	1.09623	47.32
	L-alanine in 0.50 mol kg^{-1} aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.08304		1.07901		1.07813		1.07444	
0.0919	1.08526	61.53	1.08123	61.69	1.08035	61.72	1.07664	
0.1867	1.08751	61.59	1.08347	61.79	1.08258	61.87	1.07887	62.15
0.2982	1.09009	61.70	1.08605	61.88	1.08515	61.97	1.08143	62.23
0.3922	1.09220	61.82	1.08815	62.02	1.08725	62.10	1.08354	62.31
0.4852	1.09426	61.90	1.09019	62.13	1.08929	62.20	1.08557	62.42
0.5976	1.09670	61.99	1.09264	62.19	1.09173	62.27	1.08800	62.49
0.6821	1.09846	62.10	1.09442	62.27	1.09350	62.36	1.08977	62.57
0.8018	1.10093	62.21	1.09685	62.42	1.09593	62.50	1.09221	62.69
0.8863	1.10262	62.30	1.09852	62.53	1.09760	62.60	1.09387	62.80
1.0083	1.10499	62.43	1.10091	62.64	1.10000	62.70	1.09626	62.90

(*Contd*.)

Table 1 — Densities, ρ and apparent molar volumes, ϕ_v of glycine and L-alanine in different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50 and 1.00) mol·kg^{-1}of pH 7.40 at different temperatures, T = (298.15 to 318.15) K (*Contd*.)

m/ (mol kg^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})	ρ/ (g cm^3)	ϕ_v/ (cm^3 mol^{-1})
	Glycine in 1.00 mol kg^{-1} aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.16715		1.16240		1.16140		1.15728	
0.2007	1.17166	47.64	1.16688	47.87	1.16586	47.98	1.16171	48.20
0.2940	1.17368	47.75	1.16889	47.98	1.16787	48.06	1.16371	48.27
0.4078	1.17609	47.86	1.17129	48.08	1.17025	48.18	1.16609	48.37
0.5069	1.17809	48.03	1.17333	48.17	1.17226	48.30	1.16811	48.46
0.5851	1.17966	48.11	1.17489	48.27	1.17381	48.40	1.16966	48.55
0.6933	1.18175	48.26	1.17701	48.38	1.17591	48.52	1.17176	48.66
0.7991	1.18376	48.37	1.17907	48.45	1.17796	48.58	1.17382	48.72
0.9014	1.18568	48.46	1.18099	48.54	1.17989	48.66	1.17574	48.80
	L-alanine in 1.00 mol kg^{-1} aqueous Na_3CB solutions of pH=7.40							
	T=298.15 K		T=308.15 K		T=310.15 K		T=318.15 K	
0.0000	1.16686		1.16211		1.16112		1.15700	
0.1963	1.17004	64.28	1.16529	64.49	1.16429	64.57	1.16017	64.76
0.2912	1.17151	64.37	1.16674	64.63	1.16574	64.70	1.16150	64.88
0.4107	1.17330	64.48	1.16853	64.73	1.16753	64.79	1.16339	65.01
0.5061	1.17470	64.54	1.16991	64.81	1.16891	64.87	1.16482	65.10
0.5967	1.17594	64.67	1.17113	64.96	1.17013	65.02	1.16523	65.21
0.6950	1.17727	64.77	1.17244	65.08	1.17144	65.13	1.16720	65.32
0.7924	1.17853	64.88	1.17373	65.15	1.17273	65.21	1.16859	65.42
0.9091	1.18005	64.96	1.17525	65.22	1.17422	65.30	1.17010	65.50
1.0154	1.18127	65.12	1.17646	65.39	1.17550	65.41	1.17131	65.66

The standard uncertainties are as follows:$u(m)$ = ±2×10^{-4} mol kg^{-1}, $u(\rho)$= ±4.0×10^{-4} g cm^{-3}, $u(\phi_v)$ = 0.10 cm^3 mol^{-1}, $u(T)$ = ±0.03 K (Level of confidence for standard uncertainty is 0.68).

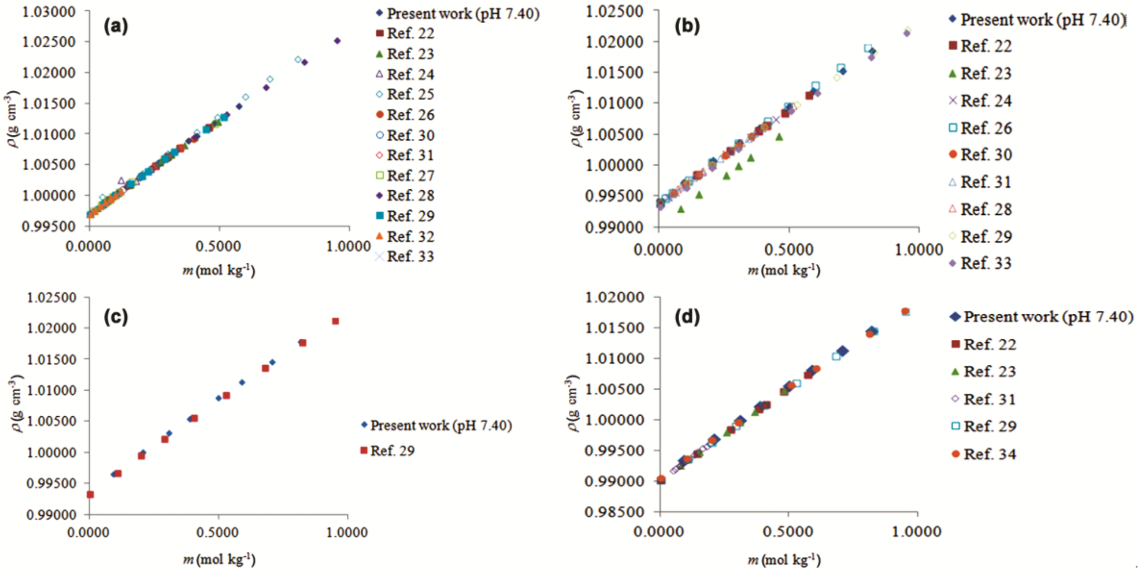

Fig. 1 — (a) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of glycine in the water of pH 7.40 (Present work) with literature values of glycine in water[22-33] at T= 298.15 K; (b) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of glycine in the water of pH 7.40 (Present work) with literature values of glycine in water[22-24,26,28-31,33] at T/K = 308.15; (c) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of glycine in the water of pH 7.40 (Present work) with literature values of glycine in water[29] at T/K = 310.15 and (d) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of glycine in the water of pH 7.40 (Present work) with literature values of glycine in water[22,23,29,31,34] at T=318.15 K

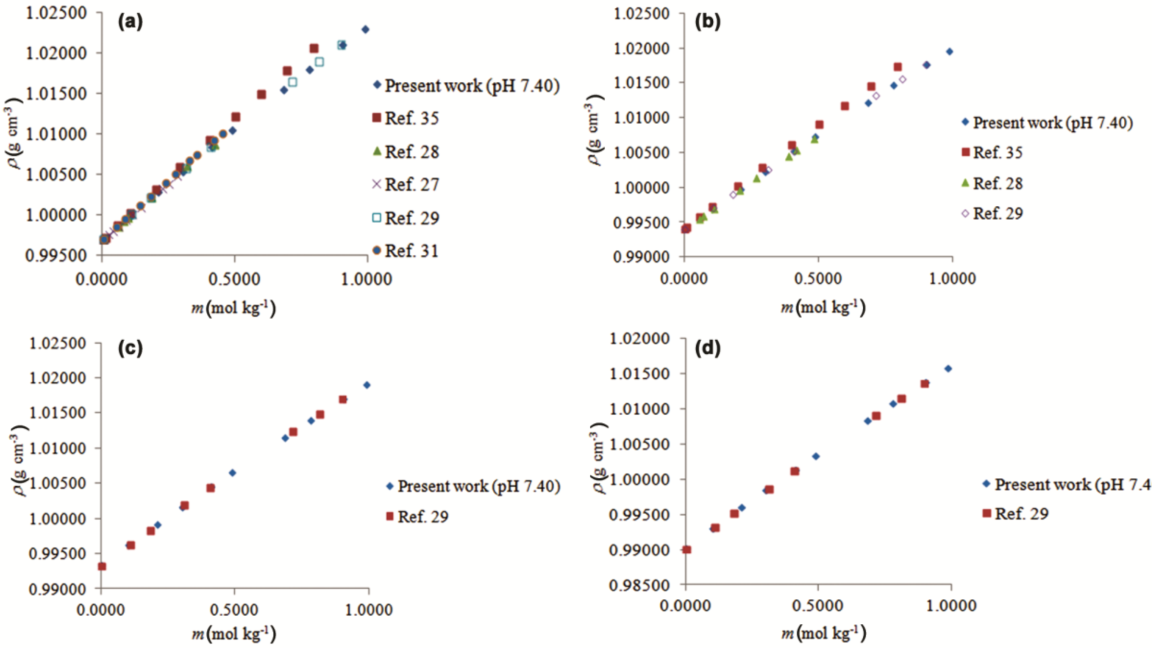

Fig. 2 — (a) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of L-alanine in the water of pH 7.40 (Present work) with literature values of L-alanine in water[27-29,31,35] at T=298.15 K; (b) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of L-alanine in the water of pH 7.40 (Present work) with literature values of L-alanine in water[28,29,35] at T/K = 308.15; (c) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of L-alanine in the water of pH 7.40 (Present work) with literature values of L-alanine in water[29] at T/K = 310.15 and (d) Plots of densities, ρ (g ·cm^{-3}) versus molality, m (mol· kg^{-1})of L-alanine in the water of pH 7.40 (Present work) with literature values of L-alanine in water[29] at T=318.15 K

$$\mathrm{H_3Cit} \rightleftarrows \mathrm{H^+} + \mathrm{H_2Cit^-} \qquad \ldots\text{(iii)}$$

$$\mathrm{H_2Cit^-} \rightleftarrows \mathrm{H^+} + \mathrm{HCit^{2-}} \qquad \ldots\text{(iv)}$$

Presently, the observedϕ_v values for both AA (Table 1) may be attributed due to the strong solute-solvent interactions between charged groups of AA (*i.e.,* $\mathrm{NH_3^+}$ and $\mathrm{COO^-}$as given in reactions (i)) and ions of trisodium citrate (*i.e.*, sodium ions, citrate ions, formed due to the dissociation of Na_3CB in water, as given in reactions (ii) to (iv)). Representative plots of ϕ_v versus m for AA in water and aqueous Na_3CB solutions at T=310.15 K at pH 7.40 are illustrated in Fig. 3. ϕ_v values for AA in water and aqueous Na_3CB increase with a temperature rise may be attributed due to increased thermal motion. In contrast, an increase in ϕ_v values with concentration may be due to the presence of dissociated Na_3CB ions in an aqueous medium at pH 7.40, which led to strong solute-solvent interactions among these systems.

Solute-solute interactions are least at infinite dilution, and apparent molar volume, ϕ_v, become equivalent to partial molar volume, ϕ_v^o. Theϕ_v^o values have been determined by least-squares fitting of the following equations to the correspondingϕ_v data and are summarized in Table 2

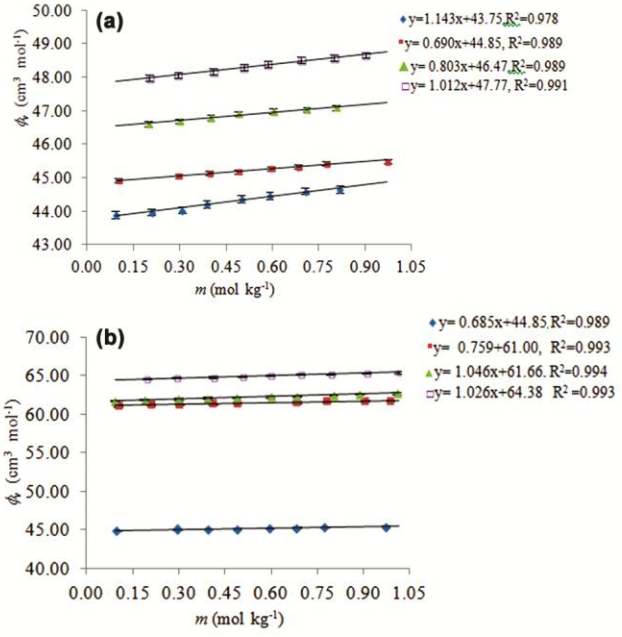

Fig. 3 — Representative plots of apparent molar volume, ϕ_v versus molality, m for (a) glycine in water and in (▪0.10, ▲ 0.50, and □ 1.00) mol·kg^{-1} aqueous Na_3CB solutions of pH 7.40 and (b) L-alanine in ♦ water and in (▪0.10, ▲ 0.50 and □ 1.00) mol·kg^{-1} aqueous Na_3CB solutions of pH 7.40 at T=310.15 K.

Table 2 — Partial molar volumes, ϕ_v^o of glycine and L-alanine in the water of pH 7.40, in different concentrations of aqueous Na_3CB solutions (*i.e.*, 0.10, 0.50, and 1.00) $mol \cdot kg^{-1}$ of pH 7.40 at temperatures, T = (298.15 to 318.15) K, *a* and *b* are the fitting parameters in equation 3

T/K ↓	ϕ_v^o/ ($cm^3\ mol^{-1}$)	R^2	*a*	*b*	SD	ϕ_v^o/ ($cm^3\ mol^{-1}$)	R^2	*a*	*b*	SD
	Glycine in the water of pH=7.40					L-alanine in the water of pH=7.40				
298.15	43.21±0.02 $(43.18±0.06)^{a,b}$	0.99	1.07±0.04	-	0.02	60.38±0.01	0.99	0.79±0.02	-	0.02
308.15	43.67±0.02	0.99	1.14±0.05	-	0.03	60.96±0.01	0.99	0.75±0.01	-	0.01
310.15	43.76±0.03	0.99	1.15±0.07	-	0.04	61.01±0.01	0.99	0.76±0.02	-	0.02
318.15	44.10±0.02 $(44.09±0.06)^{a,b}$	0.99	1.06±0.05	-	0.03	61.28±0.01	0.99	0.75±0.02	-	0.02
	Glycine in0.10 mol kg^{-1} aqueous Na_3CB of pH=7.40					L-alanine in0.10 mol kg^{-1} aqueous Na_3CB of pH=7.40				
298.15	44.29±0.01	0.99	0.70±0.03	-	0.02	61.05±0.02	0.99	0.68±0.03	-	0.02
308.15	44.66±0.02	0.99	0.84±0.04	-	0.03	61.50±0.01	0.99	0.64±0.02	-	0.01
310.15	44.86±0.02	0.99	0.69±0.03	-	0.02	61.62±0.01	0.99	0.60±0.01	-	0.01
318.15	45.11±0.02	0.99	0.70±0.03	-	0.02	61.92±0.01	0.99	0.56±0.02	-	0.02
	Glycine in 0.50 mol kg^{-1}aqueous Na_3CB of pH=7.40					L-alanine in 0.50 mol kg^{-1}aqueous Na_3CB of pH=7.40				
298.15	46.18±0.01	0.99	0.59±0.02	-	0.01	61.42±0.01	0.99	0.99±0.02	-	0.01
308.15	46.40±0.02	0.99	0.74±0.03	-	0.02	61.59±0.01	0.99	1.04±0.02	-	0.02
310.15	46.47±0.03	0.99	0.81±0.06	-	0.03	61.66±0.02	0.99	1.05±0.03	-	0.02
318.15	46.66±0.02	0.99	0.85±0.04	-	0.02	61.96±0.01	0.99	0.92±0.02	-	0.02
	Glycine in 1.00 mol kg^{-1}aqueous Na_3CB of pH= 7.40					L-alanine in 1.00 mol kg^{-1}aqueous Na_3CB of pH= 7.40				
298.15	47.40±0.02	0.99	1.21±0.03	-	0.02	64.07±0.02	0.99	1.01±0.03	-	0.02
308.15	47.69±0.01	0.99	0.90±0.02	-	0.02	64.30±0.02	0.99	1.06±0.04	-	0.03
310.15	47.78±0.02	0.99	1.01±0.04	-	0.02	64.38±0.02	0.99	1.03±0.03	-	0.02
318.15	48.02±0.01	0.99	0.88±0.02	-	0.02	64.56±0.01	0.99	1.07±0.02	-	0.02

R^2 represents regression coefficient; SD represents standard deviation
The standard uncertainties are as follows: $u(m)$ = ±2×10^{-4} mol kg^{-1}, $u(\rho)$= ±4.0×10^{-4}g cm^{-3}, $u(\phi_v)$ = 0.10 $cm^3\ mol^{-1}$, $u(T)$ = ±0.03 K (Level of confidence for standard uncertainty is 0.68)

$$\phi_v = \phi_v^0 + S_v m \qquad \ldots(2)$$

where $\phi_v^o\,(cm^3 \cdot mol^{-1})$ is partial molar volume, S_v is the experimental slope, and m $(mol \cdot kg^{-1})$ is the molality of the solution. ϕ_v versus m dependence was linear for the presently studied system. It is also evident from Table 2 that ϕ_v^o values of AA in water and aqueous Na_3CB solutions increase with an increase in the concentration of aqueous Na_3CB solutions. This may be attributed due to (a) the presence of positively and negatively charged hydration sphere of NH_3^+ and COO^- ions within the same molecule which can disrupt the hydration sphere of each other ion; (b) the presence of most stabilizing and hydrating anion, *i.e.*, citrate ion (the order of hydration of anions in Hofmeister series[36] is given below), hydrogen and sodium ions.

strongly hydrated anions
weakly hydrated anions

$$F^- > SO_4{}^{2-} > HPO_3^{2-} > CH_3COO^- > C_6H_5O_7^{2-} > Cl^- > NO_3^- > Br^- > I^- > ClO_4^-$$

Further, ϕ_v^o values of AA in water as well as in aqueous Na_3CB solutions of pH 7.40 also increase with a rise in temperature, which may be due to reduction in electrostriction, *i.e.*, discharge of some solvent molecules from loose solvation layers of the charged AA (*i.e.*, glycine and L-alanine) in Na_3CB solutions[37]. This trend of ϕ_v^o values in these systems specifies that the solvation behaviour of AA in aqueous Na_3CB solutions is concentration and temperature.

Partial molar volumes of transfer, $\Delta_{tr}\phi_v$ for AA from water to aqueous Na_3CB solutions at infinite dilution have been calculated by using the following equation are tabulated in Table 3:

$$\Delta_{tr}\phi_v\ (water \rightarrow aqueous\ Na_3CB\ solutions) = \phi_v^o\ (aqueous\ Na_3CB\ solutions) - \phi_v^o\ (water) \qquad \ldots(3)$$

Table 3 — Partial molar volume of transfer, $\Delta_{tr}\,\phi_v$ of AA in different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50 and 1.00) mol kg^{-1} of pH 7.40 at T = (298.15 to 318.15) K

$\Delta_{tr}\,\phi_v$/ (cm^3 mol^{-1})			
T=298.15 K	T=308.15 K	T=310.15 K	T=318.15 K
Glycine in 0.10 mol kg^{-1}aqueous Na_3CB			
1.08±0.02	0.99±0.03	1.10±0.04	1.01±0.03
Glycine in 0.50 mol kg^{-1}aqueous Na_3CB			
2.97±0.02	2.73±0.03	2.71±0.04	2.56±0.03
Glycine in1.00 mol kg^{-1}aqueous Na_3CB			
4.19±0.03	4.02±0.02	4.02±0.04	3.92±0.02
L-alanine in 0.10 mol kg^{-1}aqueous Na_3CB			
0.67±0.02	0.54±0.01	0.61±0.01	0.64±0.01
L-alanine in 0.50 mol kg^{-1}aqueous Na_3CB			
1.04±0.01	0.63±0.01	0.65±0.02	0.68±0.01
L-alanine in 1.00 mol kg^{-1}aqueous Na_3CB			
4.08±0.02	4.19±0.02	4.21±0.02	4.25±0.01

± are the standard errors

Positive $\Delta_{tr}\,\phi_v$ values have been observed for AA in different concentrations of aqueous Na_3CB solutions at the studied pH. $\Delta_{tr}\,\phi_v$values for AA in aqueous Na_3CB solutions decreases in the order: $1.00\,\mathrm{mol\cdot kg^{-1}}$>$0.50\ \ \mathrm{mol\cdot kg^{-1}}$>$0.10\ \ \mathrm{mol\cdot kg^{-1}}$. Trend obtained in $\Delta_{tr}\,\phi_v$ values may be attributed due to the overlap of the hydration sphere of $\mathrm{Na^+}$ (from Na_3CB solution) with those of $\mathrm{COO^-}$of AA and those of $(\mathrm{H_2Cit^-, HCit^{2-}})$ with the hydration sphere of $\mathrm{NH_3^+}$ ions. As a result of these interactions, the water molecules are relaxed to the bulk state, leading to positive $\Delta_{tr}\,\phi_v$values.

The co-sphere overlap model developed by Gurney[38], customized by Frank and Evans[39], delivers information regarding solute-solvent interactions. Overlapping ions/polar groups with hydrophilic groups would lead to positive transfer volume, whereas the overlap of ion or non-polar group interactions with that of a hydrophobic group will result in negative $\Delta_{tr}\,\phi_v$ values. The following type of interactions may be possible in the studied ternary system: (a) ion-dipolar/ion-ion interactions occurring between zwitterionic centers of AA (*i.e.*$-\mathrm{NH_3^+}$, and $-\mathrm{COO^-}$) and ions of Na_3CB (*i.e.*, Na^+, $\mathrm{H_2Cit^-, HCit^{2-}}$);(b) ion-hydrophobic interactions between ions of Na_3CB (*i.e.*, $\mathrm{H_2Cit^-, HCit^{2-}}$), and non-polar parts of amino acids, respectively; (c) hydrophobic-hydrophobic interactions among the non-polar groups of AA and non-polar groups of Na_3CB. Positive $\Delta_{tr}\,\phi_v$ values at pH 7.40 for both the AA indicate the dominance of type (a) interactions (*i.e.* ion-dipolar/ion-ion interactions). The results obtained from $\Delta_{tr}\,\phi_v$values further strengthen our results obtained from ϕ_v^o values.

The magnitude of ϕ_v^ovalues of AA in Na_3CB can be formulated by considering the altered equation of Shahidi and Farrell[40]:

$$\phi_v = V_{v,w} + V_{void} - V_{shrinkage} \qquad \ldots(4)$$

where$V_{v,w}$ is the van der Waals volume, V_{void} is the volume related to void, and $V_{shrinkage}$ is the volume because shrinkage emerges from electrostriction of solvent molecules brought about by hydrophilic groups present in solute. Assuming that $V_{v,w}$, and V_{void} are not significantly affected by the presence of aqueous Na_3CB solutions. Thus, negative $\Delta_{tr}\,\phi_v$values can be ascribed due to an increase in the $V_{shrinkage}$ in aqueous Na_3CB solutions or may be owed to the dominance of ionic hydration, *i.e.*, the electrostriction of neighbouring water molecules due to these charged centers will be enhanced. In contrast, positive $\Delta_{tr}\,\phi_v$ values may be attributed to a decrease in volume due to shrinkage resulting from the more robust interactions between zwitterionic centers and most stabilizing citrate ions (in Hofmeister series) moieties.

To study the effect of temperature onϕ_v^o, the partial molar expansibilities, $(\partial\phi_v^o/\partial T)_P$and their second-order derivative, $(\partial^2\phi_v^o/\partial T^2)_P$have been calculated by fitting the following equation to the respective data

$$\phi_v^0 = a + bT + cT^2 \qquad \ldots(5)$$

where$\phi_v^o(\mathrm{cm^3\cdot mol^{-1}})$is the partial molar volume, (a, b and c) are constants, and T(K) is the absolute temperature. The values of $(\partial\phi_v^o/\partial T)_P$ and $(\partial^2\phi_v^o/\partial T^2)_P$ are summarized in Table 4. At pH 7.40, the $(\partial\phi_v^o/\partial T)_P$ values decrease with increasing temperature for AA in water and in aqueous Na_3CB solutions (except for L-alanine in (0.50 and $1.00\,\mathrm{mol\cdot kg^{-1}}$). Hepler[41] suggested the following thermodynamic relation by which qualitative information related to hydration of a solute could be assessed from thermal expansion:

$$(\partial C_P^0/\partial P)_T = -T(\partial^2\phi_v^0/\partial T^2)_P \qquad \ldots(6)$$

where$C_P^o(\mathrm{J\cdot K^{-1}\cdot mol^{-1}})$is the partial molar heat capacity. The sign of $(\partial C_P^o/\partial P)_T$ values and its temperature dependence provides a direct probe of AA structure making or breaking properties.

Table 4 — Partial molar expansibilities,$(\partial\phi_v^o/\partial T)_P$and their second-order derivative $(\partial^2\phi_v^o/\partial T^2)_P$of glycine and L-alanine in water and different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50, and 1.00) $\mathrm{mol\cdot kg^{-1}}$of pH 7.40 at temperatures, T = (298.15 to 318.15) K

$(\partial\phi_v^o/\partial T)_P$/ $\mathrm{cm^3\ mol^{-1}\ K^{-1}}$				$((\partial^2\phi_v^o/\partial T^2)_P$ $\mathrm{cm^3\ mol^{-1}\ K^{-2}}$
T=298.15 K	T=308.15 K	T=310.15 K	T=318.15 K	
Glycine in water				
0.0596	0.0467	0.0441(0.046)[a,b]	0.0338(0.030)[a,b]	-0.0013±0.0001
Glycine in 0.10 mol $\mathrm{kg^{-1}}$ aqueous Na_3CB				
0.0592	0.0463	0.0438	0.0335	-0.0013±0.0002
Glycine in 0.50 mol $\mathrm{kg^{-1}}$aqueousNa_3CB				
0.0378	0.0277	0.0268	0.0235	-0.0004±0.0001
Glycine in 1.00 mol $\mathrm{kg^{-1}}$aqueousNa_3CB				
0.0359	0.0298	0.0298	0.0237	-0.0006±0.0004
L-alanine in water				
0.0823	0.0780	0.0759	0.0717	-0.0021±0.0004
L-alanine in 0.10 mol $\mathrm{kg^{-1}}$ aqueousNa_3CB				
0.0548	0.0427	0.0402	0.0305	-0.0012±0.0005
L-alanine in 0.50 mol $\mathrm{kg^{-1}}$ aqueousNa_3CB				
0.0213	0.0239	0.0244	0.0265	0.0003±0.0003
L-alanine in 1.00 mol $\mathrm{kg^{-1}}$aqueousNa_3CB				
0.0229	0.0232	0.0234	0.0236	0.0004±0.0004

[a]Reference[2]
[a]Reference[14]

According to Eqn (6)[41], the structure-breaking solute should have negative$(\partial^2\phi_v^o/\partial T^2)_P$values, whereas positive$(\partial^2\phi_v^o/\partial T^2)_P$values suggest that the solute behaves like a structure maker. From Table 4, the negative $(\partial^2\phi_v^o/\partial T^2)_P$values at pH 7.40 indicate that glycine /L-alanine act as a structure breaker in the studied systems, which may be attributed due to the reduction in the formation of AA solvation shells in water as well as in 0.1 $\mathrm{mol\cdot kg^{-1}}$ Na_3CB results into a net breaking of the solution "structure." On the other hand, positive $(\partial^2\phi_v^o/\partial T^2)_P$values for AA in (0.50 and 1.00) $\mathrm{mol\cdot kg^{-1}}$ aqueous Na_3CB of pH 7.40 indicate that AA acts as structure breaker by forming AA solvation shells, where the solvent molecules are tightly held in bulk solution making the solution more "structured" in these systems.

Viscometric properties

Viscosities, η of AA in water and in (0.10, 0.50, 1.00) $\mathrm{mol\cdot kg^{-1}}$ aqueous Na_3CB solutions of pH 7.40 and at T = (298.15, 308.15, 310.15 and 318.15) K were calculated from the following equation:

$$\eta = \left(at - \frac{b}{t}\right)\rho \times 100 \qquad \ldots(7)$$

where$\rho(\mathrm{g\cdot cm^{-3}})$is the density of solution (AA+ aqueous Na_3CB), $t(\mathrm{s})$ is the flow time and a and b are the viscometer constants (where a = 0.00002873 $\mathrm{mPa\cdot m^3\cdot kg^{-1}}$ and b = 0.0075959 $\mathrm{mPa\cdot s^2\cdot m^3\cdot kg^{-1}}$). Plots of viscosity, η/mPa·s versus molality, m/mol·kg^{-1} of glycine/L-alanine in the water of pH 7.40 (Table S3) show good agreement with the literature values of glycine/L-alanine in water at T = 298.15 K[24,32,33,35], are presented as Figs 4(a) and 4(b), respectively. The uncertainty in calculated viscosity values is found to be ±0.30%. Viscosities of the solutions increase with an increase in the concentration of AA and an increase in the concentration of aqueous Na_3CB at the studied pH. This may be ascribed to collisions among the solute-solvent molecules increases, which further results in loss of kinetic energy. Hence, the molecules tend to bundle together to enhance the viscosity in these solutions. Moreover, with a temperature rise, the movement among the molecules increases, which results in a decrease in viscosity.

The relative viscosities, η_r of the experimental solutions were calculated by using the equation: $\eta_r = \eta/\eta_o$, where η and η_o are the viscosities of (AA+ aqueous Na_3CB) solution and solvent (water/ aqueous Na_3CB), respectively. η values of AA in aqueous Na_3CB solutions of pH 7.40 are given in Table 5. It can be seen from Tables (S3, 5) that η

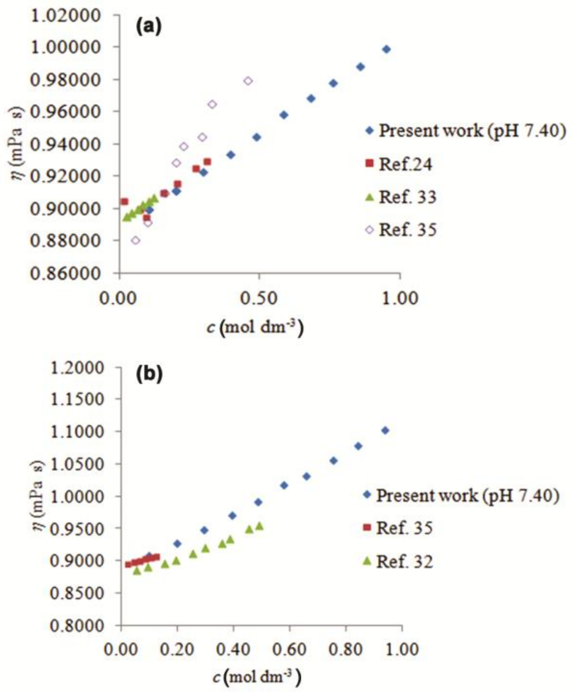

Fig. 4 — (a) Plots of viscosities, η (mPa·s) versus concentration, *c* (mol·dm^{-3}) of glycine in the water of pH 7.40 (Present work) with literature values of glycine in pure water[24,33,35] at T=298.15 K and (b) Plots of viscosities, η (mPa·s) versus molality, *c* (mol·dm^{-3}) of L-alanine in the water of pH 7.40 (Present work) with literature values of L-alanine in pure water[32,35] at T=298.15 K

values vary linearly as a function of the molar concentration of AA in water and aqueous Na_3CB solutions. Obtained η values at pH 7.40 may be attributed due to the destruction of the solvent structure caused by the formation of zwitterions of AA and anions to the solvent cluster[42].

The η_r values have been used to calculate viscosity B-coefficients using the following equation[43]:

$$\eta_r = 1 + Bc \qquad \ldots(8)$$

where $c(\mathrm{mol \cdot dm^{-3}})$ is the molar concentration (calculated from molality data), the viscosity B-coefficients provide valuable information concerning the solvation of ions and their impact on the solvent structure in the surrounding environment of the solute particles[44]. Viscosity B-coefficients of AA in water and aqueous Na_3CB solutions of pH 7.40 have been summarized in Table 6. Viscosity B-coefficients for both the AA are positive at studied pH and different temperatures, indicating that solute-solvent interactions are strong among these systems. For glycine, viscosity B-coefficients increases with an increase in the concentration of solvent/co-solvent (water/ aqueous Na_3CB) and temperature. Still, in the case of L-alanine, viscosity B-coefficients decrease with an increase in temperature whereas increases as the concentration of aqueous Na_3CB increases. Viscosity B-coefficients decrease with an increase in temperature for L-alanine, indicating that the magnitude of the hydrophobic nature of AA increases due to the presence of a hydrophobic group in L-alanine. From Table 6, it has also been noticed that the magnitude of viscosity B-coefficients in water and aqueous Na_3CB solutions increases with an increase in the molar mass and size of the alkyl side chains of AA and follows the order: L-alanine > glycine.

Solvation number S_n represents the quantity of atoms/molecules which remain connected with the ion during its movement through the solution. S_n is progressively significant because all particles experience constant thermal movement[45]. Usually, S_n decides the effective size of the particle and is calculated by using the following equation:

$$S_n = B/\phi_v^o \qquad \ldots(9)$$

where $B(\mathrm{dm^3 \cdot mol^{-1}})$ represents the viscosity B-coefficients, and $\phi_v^o(\mathrm{cm^3 \cdot mol^{-1}})$ represents the partial molar volume. The S_n values for the un-solvated ions range from (0 to 2.5), whereas for solvated ions, it is greater than 2.5[46]. In the present system, S_n values (Table 7) are found to be greater than 2.5 in water, and different concentrations of aqueous Na_3CB solutions of pH 7.40 show the presence of solvated solutes with a primary solvation sphere. Moreover, with an increase in temperature, the S_n for glycine increases in water, and different concentrations of aqueous Na_3CB solutions of pH 7.40 represent more solvation at a higher temperature. In contrast, the reverse is true for L-alanine in water and different concentrations of aqueous Na_3CB solutions of pH 7.40. This may be due to variation in the size of amino acids. Small will be the size of amino acid ions; more will be the solvation, as indicated by glycine, whereas a decrease in solvation number of L-alanine with an increase in temperature may be due to the presence of size of L-alanine ions and also due to the fact of the hydrophobic group.

Viscosity B-coefficients data for the current ternary system was used to calculate corresponding transfer values as follows and are reported in Table S4:

$$\Delta_{tr}B = \text{viscosity B-coefficients (in aqueous } Na_3CB \text{ solutions) - viscosity B-coefficients (in water)} \qquad \ldots(10)$$

Table 5 — Viscosities, η along with concentration, c of glycine and L-alanine in different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50 and 1.00) $mol \cdot kg^{-1}$ of pH 7.40 at T = (298.15 to 318.15) K

c/ (mol·dm^{-3})	η/ (mPa s)	c/ (mol· dm^{-3})	η/ (mPa s)	c/ (mol·dm^{-3})	η/ (mPa s)	c/ (mol·dm^{-3})	η/ (mPa s)
		Glycine in 0.10 mol kg^{-1} aqueous Na_3CB of pH= 7.40					
η_o/(mPa s)= 0.973		η_o/(mPa s)= 0.788		η_o/(mPa s)= 0.756		η_o/(mPa s)= 0.652	
0.1024	0.983	0.1021	0.795	0.1020	0.764	0.1017	0.660
0.2040	0.995	0.2034	0.806	0.2032	0.774	0.2026	0.700
0.3027	1.005	0.3018	0.814	0.3015	0.782	0.3006	0.676
0.3923	1.015	0.3912	0.822	0.3908	0.790	0.3895	0.684
0.4963	1.031	0.4948	0.835	0.4943	0.800	0.4926	0.693
0.5871	1.042	0.5853	0.844	0.5848	0.811	0.5827	0.701
0.6804	1.054	0.6784	0.854	0.6777	0.821	0.6753	0.710
0.7797	1.067	0.7775	0.865	0.7767	0.832	0.7738	0.719
0.8709	1.080	0.8684	0.876	0.8675	0.841	0.8643	0.727
0.9707	1.092	0.9679	0.887	0.9670	0.851	0.9633	0.736
		L-alanine in 0.10 mol kg^{-1}aqueous Na_3CB of pH= 7.40					
η_o/ mPa · s= 0.968		η_o/ mPa · s = 0.781		η_o/ mPa · s = 0.751		η_o/ mPa · s = 0.650	
0.0955	0.988	0.0952	0.796	0.0951	0.765	0.0948	0.662
0.1970	1.010	0.1964	0.814	0.1962	0.780	0.1956	0.675
0.2971	1.035	0.2962	0.834	0.2959	0.798	0.2950	0.690
0.3939	1.060	0.3926	0.854	0.3922	0.816	0.3910	0.704
0.4925	1.086	0.4908	0.870	0.4903	0.833	0.4889	0.720
0.5842	1.109	0.5822	0.887	0.5816	0.850	0.5799	0.736
1.6761	1.134	0.6737	0.907	0.6730	0.869	0.6710	0.748
0.7681	1.159	0.7653	0.926	0.7646	0.888	0.7623	0.766
0.8634	1.186	0.8602	0.948	0.8594	0.907	0.8569	0.778
0.9475	1.212	0.9439	0.970	0.9430	0.927	0.9402	0.792
		Glycine in 0.50 mol kg^{-1}aqueous Na_3CB of pH= 7.40					
T=298.15 K		T=308.15 K		T=310.15 K		T= 318.15 K	
η_o/ mPa · s =1.446		η_o/ mPa · s =1.165		η_o/ mPa · s =1.103		η_o/ mPa · s = 0.952	
0.1094	1.478	0.1090	1.191	0.1089	1.127	0.1088	0.972
0.2172	1.480	0.2164	1.198	0.2162	1.139	0.2160	0.984
0.4297	1.530	0.4279	1.234	0.4275	1.180	0.4271	1.027
0.6352	1.579	0.6325	1.272	0.6319	1.207	0.6314	1.055
0.8028	1.610	0.7993	1.298	0.7986	1.225	0.7978	1.067
0.9228	1.636	0.9187	1.324	0.9179	1.256	0.9171	1.098
1.0331	1.661	1.0284	1.349	1.0275	1.277	1.0266	1.121
		L-alanine in 0.50 mol kg^{-1}aqueous Na_3CB of pH= 7.40					
η_o/ mPa · s =1.397		η_o/ mPa · s =1.130		η_o/ mPa · s =1.067		η_o/ mPa · s = 0.916	
0.1041	1.449	0.1037	1.167	0.1036	1.100	0.1033	0.943
0.2098	1.482	0.2091	1.190	0.2089	1.123	0.2083	0.961
0.3202	1.519	0.3190	1.220	0.3188	1.149	0.3179	0.981
0.4229	1.549	0.4213	1.249	0.4210	1.174	0.4198	1.004
0.5243	1.582	0.5224	1.272	0.5219	1.199	0.5205	1.020
0.6277	1.627	0.6254	1.307	0.6248	1.232	0.6231	1.045
0.7235	1.668	0.7208	1.334	0.7202	1.250	0.7182	1.061
0.8169	1.704	0.8140	1.366	0.8132	1.277	0.8110	1.081
0.9166	1.745	0.9133	1.394	0.9124	1.304	0.9099	1.107
1.0139	1.783	1.0102	1.419	1.0093	1.337	1.0065	1.130
		Glycine in 1.00 mol kg^{-1}aqueous Na_3CB of pH= 7.40					
T=298.15 K		T=308.15 K		T=310.15 K		T= 318.15 K	
η_o/ mPa · s = 2.569		η_o/ mPa · s = 2.015		η_o/ mPa · s = 1.920		η_o/ mPa · s =1.598	
0.1167	2.645	0.1163	2.070	0.1162	1.972	0.1157	1.648
0.2226	2.691	0.2218	2.117	0.2217	2.016	0.2207	1.685

(*Contd.*)

Table 5 — Viscosities, ηalong with concentration, c of glycine and L-alanine in different concentrations of aqueous Na_3CB solutions (*i.e.* 0.10, 0.50 and 1.00) $\mathrm{mol \cdot kg^{-1}}$ of pH 7.40 at T = (298.15 to 318.15) K (*Contd.*)

c/ $(\mathrm{mol{\cdot}dm^{-3}})$	η/ (mPa s)	c/ $(\mathrm{mol{\cdot} dm^{-3}})$	η/ (mPa s)	c/ $(\mathrm{mol{\cdot}dm^{-3}})$	η/ (mPa s)	c/ $(\mathrm{mol{\cdot}dm^{-3}})$	η/ (mPa s)
Glycine in 0.10 mol kg^{-1} aqueous Na_3CB of pH= 7.40							
0.3485	2.732	0.3478	2.157	0.3471	2.055	0.3456	1.713
0.4584	2.776	0.4569	2.192	0.4565	2.088	0.4545	1.748
0.5670	2.833	0.5651	2.232	0.5646	2.126	0.5622	1.780
0.6773	2.876	0.6750	2.270	0.6744	2.163	0.6716	1.816
0.7819	2.907	0.7792	2.301	0.7786	2.192	0.7753	1.850
0.8881	2.955	0.8851	2.356	0.8844	2.245	0.8806	1.885
1.0008	3.005	0.9974	2.396	0.9966	2.283	0.9923	1.920
1.0937	3.039	1.0900	2.427	1.0891	2.313	1.0845	1.953
L-alanine in 1.00 mol kg^{-1}aqueous Na_3CB of pH= 7.40							
η_o/ mPa · s =2.568		η_o/ mPa · s =2.015		η_o/ mPa · s =1.924		η_o/ mPa · s =1.597	
0.1135	2.675	0.1130	2.062	0.1129	1.969	0.1125	1.632
0.2304	2.747	0.2294	2.134	0.2292	2.038	0.2284	1.688
0.3335	2.822	0.3320	2.190	0.3317	2.091	0.3306	1.728
0.4515	2.879	0.4496	2.240	0.4492	2.139	0.4477	1.762
0.5553	2.956	0.5530	2.314	0.5525	2.209	0.5506	1.832
0.6665	3.064	0.6637	2.383	0.6631	2.274	0.6608	1.880
0.7709	3.133	0.7677	2.425	0.7670	2.310	0.7644	1.910
0.8761	3.192	0.8724	2.489	0.8716	2.373	0.8686	1.962
0.9943	3.277	0.9901	2.553	0.9893	2.434	0.9859	2.026
1.0849	3.340	1.0803	2.616	1.0794	2.497	1.0757	2.069

The standard uncertainties are as follows :$u(m)$ = ±2 × 10^{-4} mol kg^{-1}, $u(\eta)$= ±0.30%, $u(c)$= ±2×10^{-4} mol dm^{-3}

Table 6 — Viscosity B-coefficient and temperature coefficients, dB/dT of glycine and L-alanine in water and different concentrations of Na_3CB solutions (*i.e.*, 0.10, 0.50, and 1.00) $\mathrm{mol \cdot kg^{-1}}$of pH 7.40 at T = (298.15to 318.15) K

Viscosity B-coefficients/ $(\mathrm{dm^3 \cdot mol^{-1}})$				$dB/dT/(\mathrm{dm^3 \cdot mol^{-1} \cdot K^{-1}})$
T=298.15 K	T=308.15 K	T=310.15 K	T= 318.15 K	
Glycine in water				
0.125±0.003	0.137±0.005	0.138±0.005	0.157±0.007	0.0016±0.0003
Glycine in 0.10 mol kg^{-1}aqueous Na_3CB				
0.122±0.012	0.126±0.004	0.127±0.004	0.130±0.004	0.0004±0.00001
Glycine in 0.50 mol kg^{-1}aqueous Na_3CB				
0.142±0.022	0.147±0.017	0.150±0.017	0.168±0.021	0.0013±0.0004
Glycine in 1.00 mol kg^{-1}aqueous Na_3CB				
0.171±0.011	0.189±0.007	0.189±0.007	0.205±0.008	0.0017±0.0001
L-alanine in water				
0.248±0.006	0.234±0.011	0.231±0.014	0.224±0.012	-0.0012±0.0001
L-alanine in 0.10 mol kg^{-1}aqueous Na_3CB				
0.257±0.005	0.245±0.007	0.237±0.006	0.227±0.006	-0.0015±0.0002
L-alanine in 0.50 mol kg^{-1}aqueous Na_3CB				
0.268±0.014	0.252±0.011	0.245±0.034	0.226±0.012	-0.0021±0.0002
L-alanine in 1.00 mol kg^{-1}aqueous Na_3CB				
0.279±0.015	0.270±0.013	0.269±0.013	0.265±0.016	-0.0007±0.0001

± are the standard errors.

The $\Delta_{tr} B$ values for AA are positive in different concentrations of aqueous Na_3CB solutions of pH 7.40 (except for glycine in 0.10 $\mathrm{mol \cdot kg^{-1}}$ Na_3CB). Positive $\Delta_{tr} B$ values for the studied system may be attributed due to the formation of a more structured medium in the presence of co-solute, whereas negative $\Delta_{tr}\mathrm{B}$ values for glycine in 0.10 $\mathrm{mol \cdot kg^{-1}}$ Na_3CB at pH 7.40 may be due to the presence of aggregation between the zwitterionic centers of AA (*i.e.*$-\mathrm{NH_3^+}$ and $\mathrm{COO^-}$) and ions of Na_3CB (*i.e.*, Na^+,

Table 7 — Solvation number, S_n of glycine and L-alanine in water, and different concentrations of aqueous Na_3CB solutions (*i.e.*, 0.10, 0.50, and 1.00) $\mathrm{mol \cdot kg^{-1}}$ of pH 7.40 at T=(298.15 to 318.15) K

S_n			
T=298.15 K	T=308.15 K	T=310.15 K	T= 318.15 K
Glycine in water			
2.89	3.14	3.15	3.56
Glycine in 0.10 mol kg^{-1} aqueous Na_3CB			
2.75	2.82	2.83	2.88
Glycine in 0.50 mol kg^{-1} aqueous Na_3CB			
3.07	3.17	3.23	3.60
Glycine in 1.00 mol kg^{-1} aqueous Na_3CB			
3.61	3.96	3.96	4.27
L-alanine in water			
4.26	3.84	3.79	3.66
L-alanine in 0.10 mol kg^{-1} aqueous Na_3CB			
4.21	3.98	3.85	3.67
L-alanine in 0.50 mol kg^{-1} aqueous Na_3CB			
4.36	4.09	3.97	3.65
L-alanine in 1.00 mol kg^{-1} aqueous Na_3CB			
4.35	4.20	4.18	4.10

$(\mathrm{H_2Cit^-, HCit^{2-}})$. Δ_{tr}B values almost decrease with an increase in temperature (except for L-alanine in 1.00 $\mathrm{mol \cdot kg^{-1}}$) for both the studied AA suggesting the weakening of ion-solvent interactions in these systems. These results again reveal that Δ_{tr}B values are mainly temperature and concentration-dependent, which are also consistent with the results obtained from $\Delta_{tr}\,\phi_v$ values. The first derivative of viscosity B-coefficients with temperature, *i.e.*, dB/dT, indicates the effect of solute on the structure of the solvent molecule[44]. The negative sign of dB/dT demonstrates the structure-making effect, while a positive sign of dB/dT demonstrates the structure-breaking effect of solute on a solvent structure. From Table 6, it can be seen that the positive dB/dT values for glycine in water and in aqueous Na_3CB of pH 7.40 indicate that glycine act as a structure breaker. In the case of L-alanine, negative dB/dT values at pH 7.40 show that L-alanine acts as a structure maker in these systems.

The activation free energy of solute, $\Delta\mu_1^{o\#}$ in the presence of pure and mixed solvents can be calculated according to Eyring's model[47] by using the following equation:

$$\eta_0 = \left({}^{hN_A}/_{V_1^0} \right) exp\left({}^{\Delta\mu_1^{0\#}}/_{RT} \right) \qquad \ldots(11)$$

where $\eta_o\,(\mathrm{mPa \cdot s})$ is the viscosity of water of pH 7.40 or (0.10, 0.50, and 1.00) $\mathrm{mol \cdot kg^{-1}}$ aqueous Na_3CB solutions, $h(\mathrm{J \cdot s})$ is the Planck's constant, $N_A\,(\mathrm{mol^{-1}})$ is Avogadro's number, $R(\mathrm{J \cdot mol^{-1} \cdot K^{-1}})$ is the universal gas constant, T(K) is the absolute temperature, respectively. $V_1^o\,(\mathrm{cm^3 \cdot mol^{-1}})$ is the average molar volume of (0.10, 0.50, 1.00) $\mathrm{mol \cdot kg^{-1}}$ aqueous Na_3CB solutions at T= (298.15 to 318.15) K of pH 7.40 have been calculated from density data. $\Delta\mu_1^{o\#}$ and V_1^o values of AA in aqueous Na_3CB solutions of pH 7.40 and at different temperatures are given in Table S5.

The activation free energy for the viscous flow, $\Delta\mu_2^{o\#}$ of AA in water and aqueous Na_3CB solutions is related to viscosity B-coefficients as suggested by Feakins et al.[48,49] and is as follows:

$$B = (V_1^o - \phi_v^o/1000) + V_1^o(\Delta\mu_2^{o\#} - \Delta\mu_1^{o\#}/1000RT) \qquad \ldots(12)$$

Eqn (12) can be rearranged as

$$\Delta\mu_2^{0\#} = \Delta\mu_1^{0\#} + \left({}^{RT}/_{V_1^0} \right)[1000B - (V_1^0 - \phi_v^0)] \qquad \ldots(13)$$

where $\phi_v^o\,(\mathrm{cm^3 \cdot mol^{-1}})$ is the partial molar volume of AA in water and aqueous Na_3CB solutions of pH 7.40, the $\Delta\mu_2^{o\#}$ values for the studied system are given in Table 8. The $\Delta\mu_2^{o\#}$ values are positive and are much greater than $\Delta\mu_1^{o\#}$ for the studied system suggesting that interactions between solute and co-solute molecules in the ground state are more robust than in the transition state. This might be ascribed because of the formation of a transition state followed by the rupture and distortion of intermolecular forces in the solvent structure. It can be seen that $\Delta\mu_1^{o\#}$ and $\Delta\mu_2^{o\#}$ values of AA in water are higher than in (0.10, 0.50, 1.00) $\mathrm{mol \cdot kg^{-1}}$ aqueous Na_3CB solutions of pH 7.40 may be due to the reason that in water the solute molecules require more energy from the ground state to transition state whereas in aqueous Na_3CB solution the solute molecules exist in the ordered form. The $\Delta\mu_2^o$ values for the studied system follow the order as L-alanine > glycine, which reflects that the solute having an alkyl group requires more energy in transferring from a ground state to a transition state.

Following transition state theory[49], each solvent molecule in one mole of the solution should pass through the transition state and interact more or less with the solute molecules. Thus, Gibbs free energy of transfer of solute (*i.e.*, AA), $\Delta G_2^o(1 \rightarrow 1')$ is the first

Table 8 — Activation free energies, $\Delta\mu_2^{0\#}$ for the viscous flow of glycine and L-alanine in water and different concentrations of Na_3CB solutions (*i.e.*, 0.10, 0.50, and 1.00) $\mathrm{mol \cdot kg^{-1}}$ of pH 7.40 at T = (298.15 to 318.15) K

$\Delta\mu_2^{0\#}$/ (kJ· mol^{-1})			
T=298.15 K	T=308.15 K	T=310.15 K	T= 318.15 K
Glycine in water			
29.79±0.41	31.90±0.71	32.13±0.71	33.66±1.02
Glycine in 0.10 mol kg^{-1}aqueousNa$_3$CB			
29.08±1.59	30.05±0.55	30.28±0.55	30.84±0.56
Glycine in 0.50 mol kg^{-1}aqueous Na$_3$CB			
31.02±2.67	32.08±2.12	32.50±2.13	35.21±2.70
Glycine in 1.00 mol kg^{-1}aqueous Na$_3$CB			
33.94±1.21	36.40±0.79	36.49±0.80	38.77±0.93
L-alanine in water			
49.23±0.83	48.29±1.56	48.01±2.00	47.79±1.75
L-alanine in 0.10 mol kg^{-1}aqueous Na$_3$CB			
49.44±0.67	48.82±0.96	47.92±0.83	47.28±0.85
L-alanine in 0.50 mol kg^{-1}aqueous Na$_3$CB			
47.98±1.69	49.95±1.37	46.20±1.26	44.46±1.54
L-alanine in 1.00 mol kg^{-1}aqueous Na$_3$CB			
47.62±1.65	47.45±1.47	47.51±1.48	47.67±1.86

± are the standard errors.

contribution to $\Delta\mu_1^{o\#}$ from a ground state to transition state solvents (*i.e.*, aqueous Na$_3$CB) and $\Delta G_2^o(2\rightarrow2')$ is the second contribution to $\Delta\mu_2^{o\#}$. The $\Delta G_2^o(1\rightarrow1')$ values of AA values in water and aqueous Na$_3$CB solution of pH 7.40 at different temperatures are given in Table 9. The obtained $\Delta G_2^o(1\rightarrow1')$values for AA in water and aqueous Na$_3$CB solutions of pH 7.40 at all studied temperatures form a ternary system that is more structured due to the presence of solute-solvent and ion/dipolar-ion-ion interactions[50].

Activation parameters entropy, $\Delta S^{o\#}$, and enthalpy, $\Delta H^{o\#}$ for the viscous flow of AA in water and aqueous Na$_3$CB solutions of pH 7.40 and at different temperatures have been compiled in Table 10 and were calculated by using the following equation:

$$\Delta S^{0\#} = -\,{d\left(\Delta\mu_2^{0\#}\right)}\Big/{dT} \qquad \dots (14)$$

Table 9 — Thermodynamic activation parameters, $\Delta G_2^o(1-1')$ of glycine and L

$\Delta G_2^o(1-1')$/ (kJ·mol^{-1})			
T=298.15 K	T=308.15 K	T=310.15 K	T= 318.15 K
Glycine in water			
20.62	23.01	23.30	25.03
Glycine in 0.10 mol kg^{-1}aqueous Na$_3$CB			
19.62	20.80	21.08	22.01
Glycine in 0.50 mol kg^{-1}aqueous Na$_3$CB			
20.34	21.58	22.09	24.91
Glycine in 1.00 mol kg^{-1}aqueous Na$_3$CB			
21.61	24.26	24.40	26.83
L-alanine in water			
40.08	39.37	39.18	39.07
L-alanine in 0.10 mol kg^{-1}aqueous Na$_3$CB			
40.00	39.61	38.75	38.24
L-alanine in 0.50 mol kg^{-1}aqueous Na$_3$CB			
37.39	36.53	35.86	34.26
L-alanine in 1.00 mol kg^{-1}aqueous Na$_3$CB			
35.28	35.32	35.41	35.74

Table 10 — Activation free energy of enthalpy $\Delta H^{o\#}$ and entropy, $\Delta S^{o\#}$parameters

$\Delta H^{o\#}$ (kJ·mol^{-1})	$\Delta S^{o\#}$ (kJ·mol^{-1})	$\Delta H^{o\#}$ (kJ·mol^{-1})	$\Delta S^{o\#}$ (kJ·mol^{-1})
Glycine in water		L-alanine in water	
-27.76±2.25	-0.19±0.01	71.20±4.63	0.07±0.02
Glycine in 0.10 mol kg^{-1}aqueous Na$_3$CB		L-alanine in 0.10 mol kg^{-1}aqueous Na$_3$CB	
2.64±1.88	-0.09±0.01	82.56±7.60	0.11±0.03
Glycine in 0.50 mol kg^{-1}aqueous Na$_3$CB		L-alanine in 0.50 mol kg^{-1}aqueous Na$_3$CB	
-30.39±15.81	-2.04±0.05	103.55±48.18	0.18±0.16
Glycine in 1.00 mol kg^{-1}aqueous Na$_3$CB		L-alanine in 1.00 mol kg^{-1} aqueous Na$_3$CB	
-37.31±4.81	-0.24±0.02	46.96±2.63	0.02±0.01

± are the standard errors.

The values of $\Delta S^{o\#}$ and$\Delta H^{o\#}$ describe the change in solute-solute interactions and explain the variation in solvent-solvent interactions. Further, the difference in solute-solvent interactions led to breaking or forming solute-solvent bonds in the transition state, which is related to a decrease or an increase in order. The obtained positive values of $\Delta S^{o\#}$ and $\Delta H^{o\#}$ indicate that the transition state is disorganized due to the breaking of solute-solvent bonds as compared to the ground state, which further supports the structure enforcement of the –CH$_2$ group, whereas the reverse is valid for the negative values of $\Delta S^{o\#}$ and $\Delta H^{o\#}$ for the studied system. It may be anticipated from $\Delta S^{o\#}$ and $\Delta H^{o\#}$ values that solute-solvent bonds are relatively stronger than solvent-solvent bonds, which further indicate a less favourable transition state in the presence of solute, whereas the ground states are in the ordered region.

FTIR studies

FTIR (Fourier Transform Infrared Spectroscopy) studies provide important information regarding different functional groups' structural and molecular interactions. FTIR spectra were recorded for (0.10 and 1.00) $\text{mol} \cdot \text{kg}^{-1}$glycine/L-alanine in water and in (0.10, 0.50, 1.00) $\text{mol} \cdot \text{kg}^{-1}$ aqueous Na_3CB solutions of pH 7.40 in the wavenumber region from 4000 cm^{-1} to 450 cm^{-1}. IR spectra show major absorptions around 3325-3341 cm^{-1} (-OH stretch of Na_3CB/water and -NH stretch of AA appear to be merged), 1625-1690 cm^{-1} (C=O, strong stretch), 1395-1576 cm^{-1} (–NH bend), 1350-1480 cm^{-1} (C-H bend), 1300-1000 cm^{-1} (C-O stretch) and 1080-1360 cm^{-1} (C-N stretch). Graphical representations of FTIR spectra of (0.10 and 1.00) $\text{mol} \cdot \text{kg}^{-1}$glycine/L-alanine in water and (0.10, 0.50, 1.00) $\text{mol} \cdot \text{kg}^{-1}$ aqueous Na_3CB solutions of pH 7.40 have been specified as Figs (S1-S3, 5) (For more clarification, inset picture of each spectrum has been given along with Figures). A band around 1570 cm^{-1} and 1270 cm^{-1}for glycine/L-alanine in (0.50 and 1.00) $\text{mol} \cdot \text{kg}^{-1}$ aqueous Na_3CB solutions represent N-H bending and a C-O stretch, respectively. A band near 1110 cm^{-1} was observed for glycine/L-alanine in 1.00 $\text{mol} \cdot \text{kg}^{-1}$ aqueous Na_3CB solutions, which further shows a C-O stretch only at a higher concentration of co-solute (*i.e.,* Na_3CB). A characteristic broad band between –OH of Na_3CB/water and –NH of AA has been observed around 3325 cm^{-1}, exhibiting intermolecular hydrogen bonding in these systems. These absorptions depend on the concentration of the solution, which further describes the involvement of intermolecular hydrogen bonding in these systems.

Reported values of asymmetrical/symmetrical stretching band of carboxylate (COO^-) vibrations appear around 1800-1900 cm^{-1}, but in the present system strong (COO^-) stretching frequency appear near 1600 cm^{-1} in (0.10, 0.50, 1.00) $\text{mol} \cdot \text{kg}^{-1}$$Na_3CB$ solutions. The decrease in the appearance of this frequency could be attributed to the presence of a conjugated carbonyl group of citrate buffer with that of an ammonium group of amino acids. The FTIR spectroscopic results thus obtained are in good agreement with volumetric studies and viscometric studies. Moreover; alteration produced in the wave number with the increase in the concentration of co-

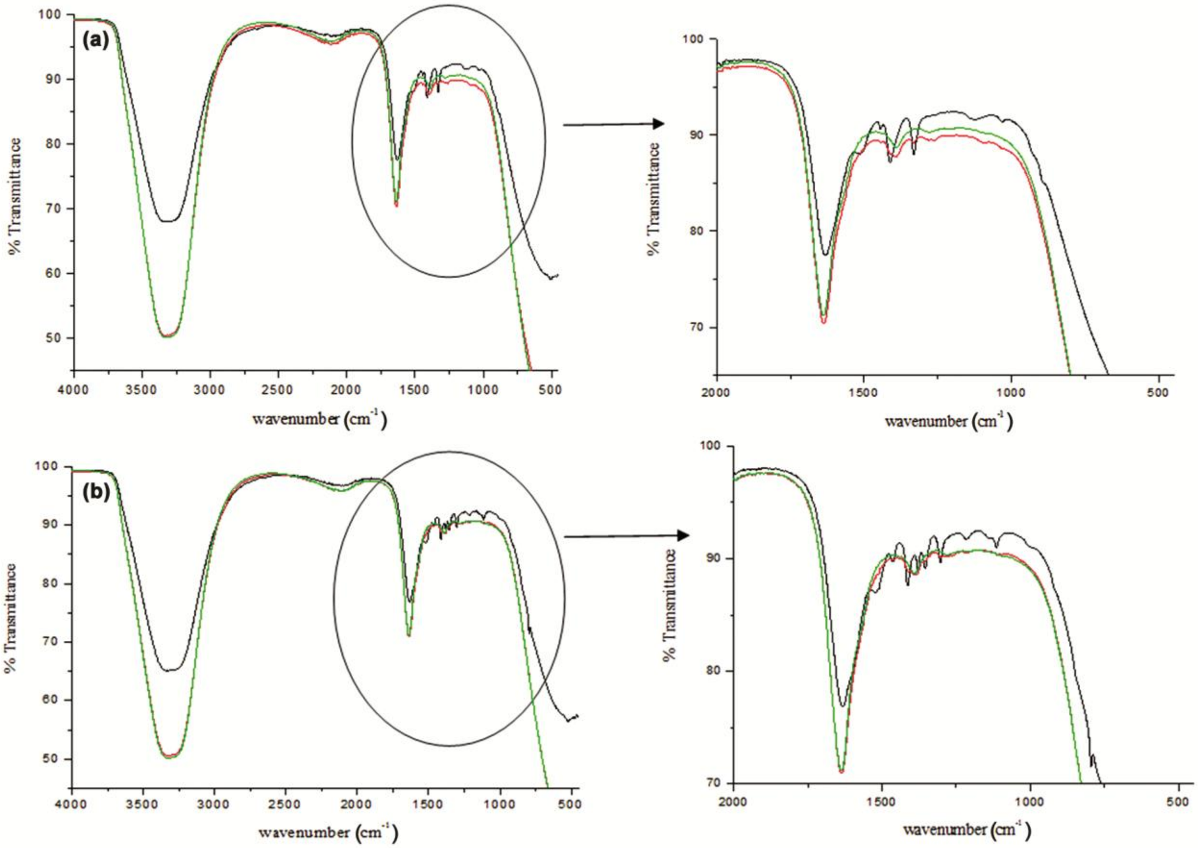

Fig. 5 —Plots of FTIR spectra (a) glycine (— 0.10 mol·kg^{-1} and — 1.00 mol·kg^{-1}) in —0.10 mol·kg^{-1} Na_3CB solutions and (b) L-alanine (—0.10 mol·kg^{-1} and — 1.00 mol·kg^{-1}) in —0.10 mol·kg^{-1} Na_3CB solutions

solute reveals the presence of strong solute-solvent interactions.

Conclusions

Volumetric, viscometric and FTIR studies of glycine and L-alanine in aqueous Na$_3$CB of pH 7.40, indicate the presence of ion-dipolar/ion-ion interactions in these systems. Positive dB/dT values for glycine show that glycine is more inclined to be a structure breaker whereas negative dB/dT values suggest that L-alanine act as a structure maker. The conclusion drawn from dB/dT values is consistent with $(\partial^2\phi_v^o/\partial T^2)_P$. Positive values of $\Delta\mu_2^{o\#}$, $\Delta G_2^o(1\rightarrow 1')$, and $\Delta H^{o\#}$ indicate that the formation of the transition state is less favoured than the ground state. FTIR results show a strong correlation with ϕ_v^o and viscosity B-coefficients, indicating the presence of strong solute-solvent interactions interms of H-bonding in these systems.

Supplementary Data

Supplementary data is available in the website http://nopr.niscair.res.in/handle/123456789/ 58776.

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