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MOLECULAR INTERACTION STUDY OF AMINO ACIDS IN AQUEOUS GLUCOSE SOLUTION AT DIFFERENT TEMPERATURES

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ABSTRACT

The data for density (ρ), viscosity (η) and ultrasonic velocity (u) of solutions of amino acids (aspartic acid, L-glutamic acid) in different concentration range with aqueous glucose were determined at different temperatures. These studies are being increasingly used as tools for investigation of the properties of pure components and the nature of intermolecular interactions between the constituents of liquid mixture. Using these data various interaction parameters viz. adiabatic compressibility (βs), molar hydration number (n_H), apparent molar compressibility (φ_k), limiting apparent molal compressibility, apparent molar volume(φ_v) and their corresponding constants (S_k, Sv) have been calculated. The viscosity A and B coefficients have been determined using Jones-Dole equation. Volumetric parameters indicate the interactions of saccharide with amino acids. The results were interpreted in terms of solute-solute and solute-solvent interactions in the solutions.

KEYWORDS: Apparent Molar Volume, Adiabatic Compressibility, Viscosity A and B-coefficient, Molecular Interaction

The physicochemical properties of proteins are of considerable interest as it is an essential nutrient for humans and other animal species. Amino acids are basic and important compounds of protein molecules involve in the physiological processes of living cells. Amino acids are the simplest biomolecule and the building blocks of more complex peptides and proteins in all living organisms (Bhat and Ahluwalia,1985; Ogawa and Suji,1987; Wadi and Goyal,1992). Since amino acid molecules incorporate some of the structural features found in larger and more complex protein molecules, they serve as model compounds for the study of protein interactions in aqueous solution and are found to provide valuable information that leads to a better understanding of biological macromolecules (Parfenyuk *et.al.*, 2004; Ali *et.al.*, 2006; Miller *et.al.*,1980). The direct study of protein-water interactions is difficult as proteins are complex macromolecules. Further, amino acids when dissolved in water convert into zwitterionic form due to the ionization of their carboxyl and amino groups (Perrin and Armarego, 1980; Srivastava *et.al.*, 2010). In physiological media such as blood, membranes, cellular fluids, etc., where water is involved, the zwitterionic character of these compounds has an important biological functions.

Saccharides are well known to stabilize proteins and lipid bilayers in vitro during dehydration. Hydration of proteins is an important factor responsible for maintaining their native structures in aqueous solutions. The specific interactions of water with various functional groups on the protein, as well as other solvent related effects, contribute to the formation of the stable folded structure of proteins in solutions (CRC handbook,1995-1996). Studies have been carried out on amino acids and water mixtures probably due to the complex nature of their interactions (Stokes and Mills,1965). The study of saccharide-amino acids interactions in aqueous solutions (Gupta and Srivastava, 2019) play pivotal role in the pharmaceutical industries, biosynthesis, biological and food industrial processes. The simple saccharides have received considerable attention for their ability to protect biological molecules and structures against the stresses induced by freezing and drying process and during subsequent storage.

In recent years ultrasonic investigation provides an extensive applications in probing into the thermodynamic and physicochemical behaviour of the liquid mixtures (Saksena 2009; Saksena 2010). The ultrasonic studies in liquids are of great importance in order to understand the nature and extent of the patterns of molecular aggregation that exist in liquid mixtures, resulting from intermolecular interactions. The measurements have been adequately employed in understanding the nature of molecular systems and physicochemical behaviour in liquid mixtures (Srivastava, 2010; Srivastava *et.al.*, 2014). During the last two decades, the ultrasonic study of the carbohydrates in aqueous medium, has gained much importance in assessing the nature of molecular interaction present in the mixture. The study of the saccharides has become a

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subject of increasing interest because of the multidimensional, physical, biochemical and industrially useful properties of these compounds (Tiwari and Goldberg, 1991) in addition to their importance to the food processing industry, pharmaceutical and chemical industries. The acoustical parameters provide qualitative information about the physical nature and strength of the molecular interactions in the liquid mixtures. These considerations led us to undertake the study of amino acids i.e. aspartic acid, glutamic acid and aqueous D-glucose solutions at different temperatures (303.15, 308.15, 313.15, 318.15, 323.15) K by measuring density, viscosity and ultrasonic velocity.

MATERIALS AND METHODS

Aspartic acid and L-glutamic acid were recrystallized from ethanol and water mixtures and dried over phosphorous pentaoxide in a desiccators for 72 h before use. Glucose (Merck) was used after drying over anhydrous $CaCl_2$ in a vacuum desiccators for 48 h at room temperature. Deionized and doubly distilled water was used for the preparation of the solutions. Solutions were freshly prepared and kept in airtight bottles to minimize the absorption of atmospheric moisture. All the chemicals used were purified by standard procedure, discussed by Perrin and Armarego (1980). The solutions were prepared freshly by mass using an electronic balance (model GR-202R, Japan) with a precision of ± (0.01 mg) in doubly distilled deionized and degassed water. A double stem calibrated pyknometer and Ubbelohde type suspended level viscometer has been used to determine the density and viscosity of solvent and solutions. The capillary with graduated marks had a uniform bore and could be closed by a well-fitting glass cap. The marks on the capillary were calibrated using triple distilled water. The reproducibility of density measurements was within ± 0.01 kg m^{-3}. The ultrasonic velocity of pure components and their mixtures were measured by fixed frequency interferometer of 2 MHz (Model F-05, Mittal Enterprises). The calibration of ultrasonic interferometer was done by measuring the velocity in AR grade benzene and carbon tetrachloride. Standard value of ultrasonic velocity for benzene and carbon tetrachloride were calculated from the literature (Lide, 1995) at different temperature. The maximum estimated error in ultrasonic velocity measurements has been found to be ± 0.1m/s. The temperature of the test liquids during the measurements was maintained by circulating water from an electronically controlled thermostatic water bath (JULABO, model ME-3, M/s Mittal Enterprises) covered with cotton jacket to avoid thermal dissipation. The viscosity was measured by Ubbelohde type suspended level viscometer with a water circulation jacket has been used to determine viscosity of solvent and solutions. At least four time flow measurements were performed for each composition and temperature, and the results were average. The viscometer was kept vertically in a transparent walled bath about 30 min to attain thermal equilibrium. The times of flow were recorded with a digital stopwatch with an accuracy of ±0.01 second. The temperature of the test liquids in every experiment of density, viscosity, ultrasonic velocity was maintained by circulating water through the jacket from an electronically controlled thermostatic water bath by keeping short distanced rubber tubing wrapped with cotton. A thermostatically controlled well-stirred water bath, whose temperature was controlled to ±0.01 K, was used for the density, viscosity, ultrasonic velocity measurements. Mixtures were prepared by weighing the liquids in specially designed ground glass stopped bottles, taking extreme precautions to minimize preferential evaporation. Fresh solutions in double distilled water have been prepared by the variation of stock solution of sugar and amino acids keeping the total volume constant in air tight stopper volumetric flasks.

THEORY AND CALCULATIONS

The acoustical and thermodynamical parameters have been calculated using the following standard relations.

Adiabatic compressibility $$\beta = 1/U^2\rho \quad (1)$$

The apparent molal compressibility has been calculated from the relation,

$$\varphi_k = \frac{1000}{m\rho_0}(\rho_0\beta - \rho\beta_0) + \left(\frac{\beta_0 M}{\rho_0}\right) \quad (2)$$

where β, ρ and β0, ρ0 are the adiabatic compressibility and density of solution and solvent respectively, m is the molal concentration of the solute, and M is the molecular mass of the solute.

$$\varphi k = \varphi^0_k + S_k m^{0.5} \quad (3)$$

where φ^0_k is the limiting apparent molal compressibility at infinite dilution and S_K is a constant. φk and S_K of equation(3) have been evaluated by least square method. The apparent molal volume φ_V has been calculated using the relation,

$$\varphi_v = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0} \quad (4)$$

The apparent molal volume φ_V has been found to differ with concentration according to Masson's empirical relation as

$$\varphi_v = \varphi^0_v + S_v m^{0.5} \quad (5)$$

where φ^0_V is the limiting apparent molal volume at infinite dilution, m is the molal concentration of the solute and S_V is a constant and these values were determined by least square method. The viscosity A and B coefficients of glucose with amino acids in aqueous solutions were calculated from the Jones-Dole equation (Jones *et al.*, 1929).

$$\eta / \eta_0 = 1 + AC^{0.5} + BC \quad (6)$$

where, η and η_0 are the viscosities of the solution and solvent respectively. A is determined by the ionic attraction theory of Falkenhagen-Vernon and therefore also called Falkenhagen coefficient, B or Jones-Dole coefficient is an empirical constant determined by ion solvent interactions. The molal hydration number has been computed using the equation,

$$\text{Molal hydration number} \quad n_H = \left(\frac{n_1}{n_2}\right)\left(1 - \frac{\beta}{\beta_0}\right) \quad (7)$$

$$\text{Molar refraction,} \quad R_m = (n^2-1/n^2+2) \times V_m \quad (8)$$

$$\text{Rao's molar function} \quad R = M(u^{1/3})/\rho \quad (9)$$

$$\text{Solvation no.} = M_2/(M_1(1- (\beta_s / \beta_0)(100-X)/X) \quad (10)$$

$$\text{Redlich- kister eqn.} \quad Y^E = X_1(1-X_1) \sum A_k (2X_1-1)^k \quad (11)$$

$$Y^E = Yexp - Yid \quad (12)$$

where k is the number of estimated parameters and A_K, the polynomial coefficients were obtained by fitting the equation to the experimental results by least-squares regression method and Y may be any calculated physical parameter. An excess property of a solution is defined as the difference between the actual mixture property and that which would be obtained for an ideal solution at the same temperature, pressure and composition. So the excess molar properties represent the deviation from ideal behavior of the mixtures. The excess functions are found to be very sensitive towards mutual interactions between the component molecules of the binary mixtures. The sign and the extent of deviation of the functions from ideality depend on the strength of interactions between unlike molecules. In order to study the non-ideality of the liquid mixtures excess parameters (Y^E) of all the acoustic parameter were computed where X_1 is the mole fraction of the amino acid n_1 = no. of moles of solvent, n_2 = no. of moles of solute, M_{eff} = $M_1W_1+M_2W_2$, where M is effective molecular weight, 'b' stands for cubic packing assumed to be 2 for liquids and 'K' is a dimensionless constant independent of temperature and nature of liquids and its value is 4.281×10^9, T is the absolute temperature and R is gas constant.

RESULTS AND DISCUSSION

The experimental data of density, ρ, viscosity, η and ultrasonic velocity, u, for aspartic acid and glucose as system (i); L-glutamic acid and glucose as system (ii) in aqueous solution at different temperatures (303.15, 308.15, 313.15, 318.15 and 323.15) K are reported in Table (1-3). The results were interpreted in terms of complex amino acid-water-saccharide interactions. Various physical and acoustical parameters like adiabatic compressibility (β), hydration number (nH), apparent molar compressibility (φk), apparent molar volume (φv), limiting apparent molal compressibility and their constants (S_K, S_V), viscosity A and B coefficient of Jones Dole equation (Jonnes and Dole,1929) were calculated from the density, viscosity and ultrasonic velocity data and the results are presented in Tables (4-8). All these parameters are discussed in terms of solute-solvent and solute-solute interactions occurring in the amino acids and saccharide solutions. The experimental data of density (table 1) reveal that the values increase with increase in molal concentrations of amino acids. This increasing behaviour suggests the moderate strong electrolytic nature in which the solute tends to attract the solvent molecules. The values of ultrasonic velocity (Table 3) increases with increase in the concentrations of amino acids in aqueous glucose solution showing that the molecular association is responsible for the increase in ultrasonic velocity in these mixtures. The increase in ultrasonic velocity in these solutions may be attributed to the cohesion brought about by the ionic hydration.

Table (4) comprises of the data calculated for hydration number n_H. The positive values of hydration number (Table 4) indicates an appreciable solvation behaviour of solutes (CRC handbook, 1995-1996). This behaviour supports the structure promoting nature of the solutes as well as the presence of a appreciable dipole-dipole interaction between solute and water molecules. This behaviour also suggests that the compressibility of the solution will be less than that of the solvent. As a result, solutes will gain mobility and have more probability of contacting solvent molecules. This may enhance the interaction between solute and solvent molecules. The increasing trend of hydration number shows the increase in solute-co-solute interaction with the increase in glucose concentration. This behaviour leads to the reduction in the electrostriction showing that glucose has a dehydration effect on the amino acids (Cerdeiriña *et. al.*, 1997). The values of adiabatic compressibility, βs, summarised in (Table 5), decrease with increase in

concentration of solute (amino acids) as well as increase in concentration of glucose in water. The decrease in adiabatic compressibility shows that there is enhanced molecular associations in these system on increase in solute content, as the new entities become compact and less compressible. It is attributed to the influence of the electrostatic field of ammonium ions and carboxylate ions on the surrounding solvent molecules (Iqbal *et. al.*,1987). The magnitudes of adiabatic compressibility values are larger in aspartic acid than L-glutamic acid. The larger adiabatic compressibility values show molecular interaction are greater in aspartic acid than that of other amino acid. Amino acid molecules of neutral solution exist in the dipolar form and thus have stronger interaction with the surrounding water molecules (Ronero *et. al.*, 1999). The increasing electrostrictive compression of water around the molecules results in decrease in the compressibility of solutions. The structural arrangement of molecule results in decreasing adiabatic compressibility by showing intermolecular interactions as reported in Table (5). Table (6) summarises the data for apparent molar compressibility for the interaction between the amino acids and glucose in aqueous medium. It is well known that solutes causing electrostriction lead to decrease in the compressibility of the solution. This is reflected by the values of φ_k of amino acids in aqueous glucose solutions. The apparent molar volume, φ_v and φ_k values (Tables 7, Table 6) with respect to the solute concentration in the systems studied indicates an existence of solute-solvent interaction (Gavin and Hedwig,1991). Appreciable positive values of φ_k for the systems clear that the ionic-hydrophilic interactions are dominating over the ionic-hydrophobic interactions. Therefore the mutual overlap of the hydration spheres of solute and co- solute molecules will lead to an increase in the magnitude of hydrogen bonding interactions between amino acid and –OH groups of saccharide molecules.

It is observed from Table (2) that the values of viscosity increases with increasing concentrations of amino acids in aqueous glucose solution. This increasing trend indicates the existence of molecular interaction occurring in these systems. In order to have more clear picture, viscosity B-coefficient has also been obtained. From Table 8, it is observed the values of A and B are negative and positive in all the studied systems. Hydrophilic solutes often show negative compressibility that is introduced by them in water structure which is also indicated by the smaller magnitude of A values. Coefficient B is known as measure of order or disorder introduced by the solute into the solvent. It is also a measure of solute-solvent interaction and the relative size of the solute and solvent molecules (Yan *et.al.*,2004; Ali *et.al.*, 2007; Stokes *et.al.*, 1965). The behaviour of B-coefficient in systems suggest the existence of strong ion-solvent interactions. The values of S_v provides information regarding solute-solvent interaction and S_K that of solute-solute interaction in the solution. The negative values of S_v provides the existence of solute-solvent interaction. This behaviour shows that the existence of ion-solute or solute-solute interaction in all the systems studied. The positive values of S_K parameter represents the solute-solute interaction in the studied solution.

Table 1: Densities (ρ) of systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	ρ x10^{-2}(kg m^{-3})				
0.000	9.974	9.944	9.944	9.974	9.944
0.006	9.978	9.927	9.921	9.899	9.875
0.007	9.982	9.930	9.925	9.904	9.885
0.008	9.997	9.987	9.933	9.908	9.885
0.011	10.011	9.992	9.937	9.932	9.890
0.018	10.032	10.003	9.948	9.934	9.900
0.042	10.084	10.010	9.989	9.981	9.942
System (ii)					
0.006	9.938	9.936	9.917	9.891	9.874
0.007	9.964	9.941	9.918	9.899	9.875
0.008	9.988	9.961	9.930	9.901	9.895
0.011	9.994	9.962	9.930	9.918	9.897
0.018	9.995	9.964	9.931	9.921	9.899
0.042	10.008	9.965	9.947	9.924	9.902

Table 2: Viscosities (η) of systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	η x10^{-2}(kgm^{-1}s^{-1})				
0	8.544	7.895	7.451	6.393	6.272
0.006	7.452	7.231	7.169	6.487	6.124
0.007	7.508	7.487	7.322	6.769	6.143
0.008	8.624	7.507	7.412	6.802	6.164
0.011	8.648	7.596	7.415	6.840	6.203
0.018	8.708	7.627	7.465	6.850	6.255
0.042	8.788	7.659	7.468	7.071	6.257
System (ii)					
0.006	7.362	7.247	7.148	6.487	6.138
0.007	7.404	7.262	7.173	6.643	6.163
0.008	7.527	7.382	7.249	6.813	6.267
0.011	7.588	7.437	7.273	6.832	6.271
0.018	8.304	7.500	7.498	7.050	6.319
0.042	8.373	7.534	7.516	7.071	6.364

Table 3: Ultrasonic velocities (u) of systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	u x10^{-2}(kg m^{-3})				
0	1116.5	1118.0	1080.0	1062.3	1012.5
0.006	1120.5	1106.3	1089.5	1064.3	1014.5
0.007	1157.0	1123.4	1106.3	1081.5	1034.0
0.008	1206.3	1205.3	1203.8	1135.0	1126.5
0.011	1240.3	1224.5	1222.0	1214.5	1210.3
0.018	1251.5	1247.0	1233.0	1227.5	1223.5
0.042	1449.3	1388.0	1282.5	1246.5	1236.5
System (ii)					
0.006	1123.0	1109.0	1091.8	1065.5	1016.3
0.007	1159.5	1150.8	1107.0	1085.0	1035.3
0.008	1207.3	1206.8	1203.8	1137.5	1128.8
0.011	1250.3	1234.5	1232.0	1224.5	1220.3
0.018	1261.5	1257.0	1243.0	1237.5	1233.5
0.042	1459.3	1398.0	1292.5	1256.5	1246.5

Table 4: Hydration number (n_H) of systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	n_H				
10.0	0.810	0.933	1.095	0.682	0.914
12.5	0.834	0.950	1.101	0.711	0.932
16.5	0.917	1.075	1.233	1.208	1.358
25.0	0.917	1.068	1.222	1.215	1.341
50.0	1.026	1.172	1.336	1.332	1.464
100.0	1.265	1.443	1.629	1.624	1.778
200.0	1.322	1.495	1.687	1.683	1.832

System (ii)					
10.0	0.875	1.009	1.169	1.214	1.372
12.5	0.895	1.020	1.176	1.219	1.371
16.5	0.969	1.088	1.244	1.288	1.484
25.0	1.034	1.148	1.308	1.351	1.549
50.0	1.115	1.231	1.396	1.433	1.635
100.0	1.402	1.549	1.734	1.772	2.010
200.0	1.472	1.617	1.807	1.855	2.080

Table 5: Adiabatic compressibility (β_s) of systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	β_s x10^{10}(kg^{-1}ms^2)				
10.0	4.923	5.018	5.041	6.015	6.074
12.5	4.838	4.934	4.964	5.913	5.971
16.5	4.784	4.809	4.838	4.991	5.149
25.0	4.757	4.791	4.820	4.937	5.138
50.0	4.664	4.714	4.737	4.846	5.041
100.0	4.594	4.634	4.664	4.772	4.965
200.0	4.515	4.556	4.570	4.675	4.866
System (ii)					
10.0	4.809	4.873	4.892	4.902	5.021
12.5	4.730	4.800	4.813	4.822	4.940
16.5	4.643	4.726	4.742	4.751	4.771
25.0	4.566	4.657	4.668	4.679	4.695
50.0	4.489	4.580	4.585	4.610	4.626
100.0	4.410	4.485	4.506	4.540	4.559
200.0	4.312	4.383	4.390	4.404	4.434

Table 6: ($\phi_{k)}$ of system at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	ϕ_k x10^5				
0.006	5.890	5.487	4.328	4.435	5.845
0.007	3.886	3.919	3.008	3.051	4.023
0.008	2.559	2.010	1.026	1.639	1.656
0.011	1.591	1.325	0.589	0.333	0.304
0.018	0.911	0.683	0.291	0.125	0.100
0.042	-0.008	-0.004	-0.005	-0.009	-0.004
System (ii)					
0.006	3.758	3.469	2.780	2.864	3.747
0.007	2.536	2.243	2.013	1.996	2.676
0.008	1.571	1.264	0.674	1.023	1.028
0.011	0.936	0.792	0.341	0.188	0.161
0.018	0.496	0.376	0.157	0.062	0.046
0.042	0.001	0.001	0.000	0.000	0.002

Table 7: (ϕ_v) of system at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
System (i)	ϕ_v x10^5				
0.006	0.879	1.049	0.738	0.674	0.962
0.007	0.666	0.808	0.566	0.495	0.654
0.008	0.409	0.030	0.405	0.390	0.580
0.011	0.229	0.027	0.316	0.128	0.435
0.018	0.095	0.025	0.200	0.135	0.285
0.042	0.019	0.097	0.092	0.048	0.126
System (ii)					
0.006	1.077	0.624	0.586	0.584	0.695
0.007	0.655	0.485	0.491	0.427	0.583
0.008	0.389	0.280	0.340	0.362	0.349
0.011	0.301	0.249	0.300	0.226	0.297
0.018	0.246	0.213	0.245	0.197	0.241
0.042	0.194	0.195	0.189	0.185	0.208

Table 8: Various constants for systems at different temperature (K)

C(mol.m^{-3})/T(K)	303.15	308.15	313.15	318.15	323.15
	Sv x 10^5(Masson's)				
System (i)	-4.184	-1.974	-2.393	-1.896	-4.440
System (ii)	1.748	0.201	-0.394	1.205	1.708
System (iii)	5.418	3.863	3.265	4.872	5.377
System (iv)	10.845	9.998	10.582	11.478	11.346
	ϕ^0_k x 10^{10}				
System (i)	8.286	9.401	11.771	14.273	15.754
System (ii)	12.621	12.947	13.170	15.139	16.984
System (iii)	9.139	8.877	8.999	9.002	9.552
System (iv)	7.240	7.334	7.558	7.733	7.834
	S_k x 10^9				
System (i)	0.793	1.162	2.107	-0.673	0.795
System (ii)	1.691	1.771	1.832	3.022	3.742
System (iii)	0.109	-0.066	-0.079	0.123	0.550
System (iv)	-0.223	-0.898	-0.871	-0.817	-0.853
	B x 10^4(Jones-Dole)				
System (i)	-6.095	-7.919	-30.320	-35.723	-37.711
System (ii)	-7.370	2.163	-13.554	-37.472	-23.402
System (iii)	-8.474	1.107	-14.689	-38.726	-24.586
System (iv)	-13.962	-4.119	-21.969	-18.742	-36.260
	A(Jones-Dole)Falkenhagen coefficient				
System (i)	-0.609	-0.792	-3.032	-3.572	-3.771
System (ii)	-0.737	0.216	-1.355	-3.747	-2.340
System (iii)	-0.847	0.111	-1.469	-3.873	-2.459
System (iv)	-1.396	-0.412	-2.197	-1.874	-3.626
B(Feaking constant)					
System (i)	0.103	0.103	0.103	0.103	0.104
System (ii)	0.102	0.103	0.103	0.103	0.103
System (iii)	0.102	0.102	0.103	0.103	0.103
System (iv)	0.101	0.101	0.101	0.101	0.102

CONCLUSION

Density, ρ, viscosity, η, and ultrasonic velocity, u, measurements have been carried out on amino acids; aspartic acid, L-glutamic acid in aqueous glucose solution at temperatures (303.15, 308.15, 313.15, 318.15 and 323.15) K solution. These measurements have been performed to evaluate some important parameters, viz, adiabatic compressibility (β), molar hydration number (n_H), apparent molar compressibility(φ_k), apparent molar volume(φ_v), limiting apparent molar volume, viscosity A and B-coefficients of Jones–Dole equation, variation of B with temperature. These parameters have been interpreted in terms of solute-solute and solute-solvent interactions and structure making or breaking ability of solutes in the given solution. The thermo-acoustic analysis in biological medium would be interesting to discuss nonlinear behaviour with respect to concentrations and temperatures. Ultrasonic studies may throw more light on the molecular interactions to know the behaviour of biologically active macromolecules in aqueous solution. It is the key to solve the critical problems with the role and interaction of biologically active compounds in living organisms.

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