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Ultrasonic Study of Amino Acids in Aqueous Urea Mixture at 298K

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Ultrasonic velocity (U), density (ρ) and viscocity (η) have been measured for three amino acids *viz.*, glycine, L-alanine and L-serine in the aqueous urea (0.1 m) at 298K. Using the experimental values, the adiabatic compressibility (β), change in adiabatic compressibility (Δβ), relative change in adiabatic compressibility ($\Delta\beta/\beta^o$), apparent molal compressibility (φ_K) apparent molal volume (φ_V), limiting apparent molal compressibility (φ_K^o), limiting apparent molal volume (φ_V^o) and their constant (S_K, S_V) and viscosity B-coefficient were calculated for all the ternary systems. The results are interpreted in the light of intermolecular interaction in terms of the structure-making or structure-breaking effect of these amino acids in the mixture.

Key Words: Ultrasonic study, Amino acids, Water-urea mixture.

INTRODUCTION

Ultrasonic investigation in aqueous solutions of electrolytes and non-electrolytes with amino acids provides useful information in understanding the behaviour of liquid systems, because intramolecular and intermolecular association, complex formation and related structural changes affect the compressibility of the system which inturn produces corresponding variations in the ultrasonic velocity. During the last two decades considerable study has been carried out to investigate hydration of proteins through volumetric and ultrasonic measurements, since these properties are sensitive to the degree and nature of hydration[1-7]. The ultrasonic study of amino acid mixtures has gained much importance in assessing the nature of molecular interactions present in the mixtures. Sound velocity measurements in amino acid and carbohydrates furnish knowledge about solute-solvent and solute-solute interactions which, in turn, help to understand several biochemical process such as protein hydration. A better understanding of this type of interaction may be obtained from dipolar ions. Since, the amino acids and Zwitter ions in aqueous solutions[8-10] their volume and compressibility properties should reflect structural interactions with water molecules. For a better understanding of the hydration of amino acids, basic physico-chemical properties are needed.

Amino acids in aqueous solutions are ionized and can acts as acids or bases. Knowledge of the acid-base properties of amino acids is extremely important in understanding the physical and biological properties of proteins. The investigations of volumetric and thermodynamic properties of amino acids and peptides in aqueous and mixed aqueous solvents has been the area of interest of a number of researchers[11-16]. Since volumetric, compressibility and viscosity studies are lacking in aqueous mixtures of amino acids, an attempt has been made to understand the behaviour of glycine, L-alanine and L-serine in aqueous urea mixture of 0.1 m concentration at 298K through ultrasonic velocity measurements.

However, the ultrasound velocity data as such do not provide significant information about the native and relative strength of various types of intermolecular or interionic interactions between the components. Hence their derived parameters such as adiabatic compressibility (β), change in adiabatic compressibility ($\Delta\beta$), relative change in adiabatic compressibility ($\Delta\beta/\beta^o$), apparent molal compressibility (φ_K), apparent molal volume (φ_V), limiting apparent molal compressibility (φ_K^o), limiting apparent molal volume (φ_V^o) and their constants (S_K, S_V) and viscosity B-coefficient have been obtained to get more informations on such interactions.

EXPERIMENTAL

Fresh conductivity water has been used for preparing 0.1 m aqueous urea solution was taken as solvent for the preparation of amino acids solutions of different molality. All the chemicals used in this present research work are analytical (AR) reagent grade and spectroscopic (SR) reagent grade of minimum assay of 99.9 %. The density, viscosity and velocity of the amino acids-urea-water solutions were measured at 298K. The density was determined using a specific gravity bottle by relative measurement method. The weight of the sample was measured using electronic digital balance with an accuracy of ± 0.1 mg (Model: Shimadzu AX200). An Ostwald's viscometer (10 mL capacity) is used for the viscosity measurements and efflux time was determined using a digital chronometer to within ± 0.01 s. An ultrasonic interferometer having the frequency 3 MHz with an overall accuracy of ± 2 m s^{-1} has been used for velocity measurements. An electronically digital operated constant temperature bath (RAAGA Industries) has been used to circulate water through the double walled measuring cell made up of steel containing the experimental solution at the desired temperature. The accuracy in the temperature measurement is ± 0.1 K.

Theory and calculations

Using the measured data, some acoustical parameters have been calculated using the standard relations.

Adiabatic compressibility $$\beta = \frac{1}{U^2\rho} \quad (1)$$

The change in adiabatic compressibility was calculated by the relation

$$\Delta\beta = \beta - \beta^o \quad (2)$$

where, β and β^o are the adiabatic compressibility of the solution and solvent, respectively.

The relative change in adiabatic compressibility was determined by

$$\Delta\beta/\beta^o \quad (3)$$

The apparent molal compressibility has been calculated from relation,

$$\varphi_K = \frac{1000}{m\rho_o}(\rho_o\beta - \rho\beta^o) + \left(\frac{\beta^o M}{\rho_o}\right) \quad (4)$$

where, ρ and ρ_o are the density of solution and solvent, respectively, m is the molal concentration of the solute and M the molecular mass of the solute. φ_K is the function of m as obtained by Gucker from Debye Huckel theory[17] and is given by

$$\varphi_K = \varphi_K^o + S_K m^{1/2} \quad (5)$$

where, φ_K^o is the limiting apparent molal compressibility at infinite dilution and S_K is a constant, φ_K^o and S_K were obtained by least square method.

The apparent molal volume φ_V has been calculated using the relation

$$\varphi_V = \frac{1000}{m\rho_o}(\rho_o - \rho) + \left(\frac{M}{\rho_o}\right) \quad (6)$$

The apparent molal volume φ_V has been found to differ with concentration according to Masson[18] empirical relation as

$$\varphi_V = \varphi_V^o + S_V m^{1/2} \quad (7)$$

where, φ_V^o is the limiting apparent molal volume at infinite dilution and S_V is a constant and these values were determined by least square method.

The entire viscosity data have been analyzed in the light of Jones-Dole semi empirical equation[19]

$$\frac{\eta}{\eta_o} = 1 + Am^{1/2} + Bm \quad (8)$$

where, η and η_o are the viscosities of the solution and solvent, respectively A is known as the Falkenhagen[20] coefficient which characterizes the ionic interaction and B is the Jones-Dole or viscosity B-coefficient which depends on the size of the solute and the nature of solute-solvent interactions.

RESULTS AND DISCUSSION

The experimental values of density, viscosity and ultrasonic velocity for different molality of each of the three amino acids *viz.*, glycine, L-alanine and L-serine in aqueous urea mixture (0.1 m) are shown in Table-1. The values of adiabatic compressibility, change and relative change in adiabatic compressibility, apparent molal compressibility, apparent molal volume, limiting apparent molal compressibility, limiting apparent molal volume and their constants S_K and S_V and viscosity B-coefficient are given in Tables 2 and 3.

TABLE-1
VALUES OF DENSITY (ρ), VISCOSITY (η) AND ULTRASONIC VELOCITY (U) AT 298K

Molality $m/(\text{mol kg}^{-1})$	Density $\rho/(\text{kg m}^{-3})$	Viscosity $\eta/(\times 10^{-3}\text{ N sm}^{-2})$	Ultrasonic velocity $U/(\text{m s}^{-1})$
Glycine in urea + water mixture			
0.0	997.26	0.9080	1476.88
0.2	1002.30	0.9133	1486.20
0.4	1004.13	0.9252	1505.20
0.6	1004.87	0.9357	1511.88
0.8	1005.30	0.9631	1519.60
1.0	1006.40	0.9826	1520.60
L-Alanine in urea + water mixture			
0.0	997.26	0.9080	1476.88
0.2	1002.80	0.9239	1484.88
0.4	1004.38	0.9373	1497.40
0.6	1005.18	0.9519	1501.22
0.8	1005.78	0.9637	1506.42
1.0	1006.75	0.9869	1510.42
L-Serine in urea + water mixture			
0.0	997.26	0.9080	1476.88
0.2	1002.97	0.9253	1482.70
0.4	1005.03	0.9503	1490.45
0.6	1005.20	0.9618	1494.32
0.8	1005.98	0.9702	1502.86
1.0	1006.90	0.9991	1509.93

In all the amino acid system the values of density and ultrasonic velocity increases with increase in molal concentration of amino acids. Generally the values of ultrasonic velocities are smaller in L-alanine than other two amino acids. Molecular association is thus responsible for the observed increase in ultrasonic velocity in these mixtures. The factors apparently responsible for such a behaviour may be the presence of interactions caused by the proton transfer reactions of amino acids in aqueous urea mixture,

TABLE-2
VALUES OF ADIABATIC COMPRESSIBILITY (β), CHANGE IN ADIABATIC COMPRESSIBILITY (Δβ), RELATIVE CHANGE IN ADIABATIC COMPRESSIBILITY (Δβ/β°), APPARENT MOLAL COMPRESSIBILITY (φ_K) AND APPARENT MOLAL VOLUME (φ_V) AT 298K

Molality m/ (mol kg^{-1})	Adiabatic compressibility β/(×10^{-10} m^2 N^{-1})	Change in adiabatic compressibility -Δβ/(×10^{-12} m^2 N^{-1})	Relative change in adiabatic compressibility -(Δβ/β°)/(×10^{-3})	Apparent molal compressibility -φ_K/(×10^{-8} m^2 N^{-1})	Apparent molal volume -φ_V/(m^3 mol^{-1})
Glycine in urea + water mixture					
0.0	4.5950	–	–	–	–
0.2	4.5183	7.67	1.67	4.9902	24.19380
0.4	4.3956	19.93	4.34	5.7712	17.14640
0.6	4.3527	24.13	5.25	4.6026	12.64280
0.8	4.3077	28.73	6.25	4.0505	10.00220
1.0	4.2973	29.76	6.48	3.3938	9.08970
L-Alanine in urea + water mixture					
0.0	4.5950	–	–	–	–
0.2	4.5232	7.17	1.56	4.8587	27.68660
0.4	4.4404	15.45	3.63	4.6792	17.75900
0.6	4.4144	18.06	3.93	3.6144	13.14687
0.8	4.3813	21.37	4.65	3.1572	10.58980
1.0	4.3539	24.11	5.25	2.8435	9.42670
L-Serine in urea + water mixture					
0.0	4.5950	–	–	–	–
0.2	4.5353	5.97	1.30	4.2944	28.52290
0.4	4.4792	11.58	2.52	3.7849	19.37250
0.6	4.4551	13.99	3.04	2.9356	13.16720
0.8	4.4012	19.38	4.22	2.9191	10.82450
1.0	4.3561	23.89	5.20	2.8278	9.56110

TABLE-3
VALUES OF LIMITING APPARENT MOLAL COMPRESSIBILITY (φ_K^o) AND LIMITING APPARENT MOLAL VOLUME (φ_V^o), THEIR CONSTANTS S_K AND S_V AND VISCOSITY COEFFICIENT OF AMINO ACIDS IN UREA + WATER MIXTURE AT 298K

Amino acids	Limiting apparent molal compressibility -φ_K^o/(×10^{-8} m^2 N^{-1})	Constant S_K/(×10^{-8} N^{-1} m^{-1} mol^{-1})	Limiting apparent molal volume -φ_V^o/(m^3 mol^{-1})	Constant S_V/(m^3 lt$^{-1/2}$ mol$^{-3/2}$)	Viscosity	
					A/(dm$^{3/2}$ mol$^{-½}$)	B/(dm^3 mol^{-1})
Glycine	7.08	3.36	36.98	29.57	-0.0480	0.1261
L-Alanine	6.84	4.02	40.31	32.80	0.0018	0.1798
L-Serine	5.49	2.85	42.49	34.95	0.0082	0.1877

hydrophilic nature of L-serine and hydrophobic nature of glycine and L-alanine[21,22]. The increase in ultrasonic velocity in these solutions may be attributed to the cohesion brought about by the ionic hydration. It is known that aqueous urea mixture of glycine, L-alanine and L-serine contain in addition to the uncharged molecules NH_2CH_2COOH, an electrically neutral molecule, *viz.*, $^+NH_3CH_2COO^-$ dipolarions (Zwitter ions). When the amino acids are dissolved in urea + water mixtures, the cations NH_3^+ and anions COO^- are formed. The water molecules are attached to the ions strongly by the electrostatic forces, which introduce a greater cohesion in the solution. Thus the cohesion increases with the increase of amino acid concentration in the solution. The increased association, observed in these solutions, may also be due to water structure enhancement brought about by the increase in electrostriction in the presence of urea. The electostriction effect which brings about the shrinkage in the volume of solvent, caused by the Zwitter ionic portion of the amino acid, is increased in mixed solvents as compared to that in pure water. This effect is similar to the results of previous workers[23,24].

Table-2 shows the values of adiabatic compressibility with molal concentration of amino acids at 298K. The decrease in adiabatic compressibility, observed in aqueous urea mixture with amino acids in the present study generally confirms that conclusions drawn from the velocity data. The adiabatic compressibility values are larger in L-serine compared to glycine and L-alanine which shows molecular association/interaction in greater in L-serine than that of other two amino acids. Amino acid molecules in the neutral solution exist in the dipolar form and thus have stronger interaction with the surrounding water molecules. The increasing electrostrictive compression of water around the molecules results in a large decrease in the compressibility of solutions. Table-2 shows the values of change in adiabatic compressibility and relative change in adiabatic compressibility of amino acids in aqueous urea mixture. The negative values of $\Delta\beta$ decreases with increasing molalities of all systems. These values of $\Delta\beta$ are higher in L-serine and glycine and L-alanine. Same trend has been observed in relative change in adiabatic compressibility values. The behaviour lend further support to the contention of having apparently ideal systems caused by solute-solvent interactions.

The following observations have been made on φ_K of the three amino acids in aqueous urea mixture. The values of φ_K are all negative over the entire range of molality and the same increases with increase in concentration of solute in all the three systems. The maximum φ_K is recorded for Glycine with the rise of solute content. From the above observations clearly suggest that the negative values of φ_K indicate electrostriction, hydrophilic and hydrophobic interactions occuring in these systems. The increases values of φ_K in all the three systems reveals that less strengthening the solute-solvent interaction.

The limiting apparent molal compressibility due to Masson[18] φ_K^o and the related constant S_K for all the amino acids have been computed using least square method. φ_K^o provides information regarding solute-solvent interactions S_K, that of solute-solute interaction in the solution. One can notice from Table-3 that φ_K^o values are negative in all the systems studied. Appreciable negative values of φ_K^o for all systrems reinforce our earlier view that existence of solute-solvent interaction in the present systems. The variation of φ_K^o for amino acids in negative magnitude is in the order: L-serine > L-alanine > glycine. The values of S_K exhibits positive, this indicates the existence of ion-ion/solute-solute interaction and suggests strucutre making/breaking effect of the amino acids. It is well known that solutes causing electrostriction lead to decrease in the compressibility of the solution. This is reflected by the negative values of φ_K of the amino acids.

The following observations have been made on φ_V (Table-2) of the three amino acids in urea + water mixture. The values of φ_V are all negative over the entire range of molality. The value of φ_V are increases with the increase in concentration of solute in all the three systems. The maximum value of φ_V obtained in all three systems is in the order: glycine > L-alanine > L-serine. This observations clearly suggest that the negative values of φ_V in all systems indicate the presence of solute-solvent interaction. The increase in φ_V is due to strong ion-ion interaction and *vice-versa*. The negative values of φ_V indicate electrostrictive solvation of ions[25]. From the magnitude of φ_V, it can be concluded that, strong molecular association is found in L-serine than the other two and hence L-serine is a more effective structure maker than others.

It is clear from the Table-3 that the values of φ_V^o in all the systems are negative. This enhances/reduces the electrostriction of water molecules. Decrease in φ_V^o value is due to the reduction in electrostriction at the terminal groups of amino acids, which increases the interaction between these polar ends and ions. The negative values of φ_V^o indicate smaller solute-solvent interactions present in these systems. The magnitude of φ_V^o is in the order L-serine < L-alanine < glycine. It is evident from the above table that S_V exhibits positive values in all the systems. The positive values of S_V predicts strong solute-solute interaction in the systems.

From Table-1, it is observed that the values of viscosity increases with increase in solute concentration. This increasing trend indicates the existence of molecular interaction occuring in these systems. In order to shed more light on this, the role of viscosity B-coefficient has been obtained.

From Table-3, it is observed that the values of A are positive in all systems studied except in glycine and B-coefficient are positive. Sin A is a measure of ionic interaction[26] it is evident that there is a weak ion-ion interaction in the amino acids studied, which is indicated by the smaller magnitude of A values. B-coefficient is also known as measure of order or disorder introduced by the solute in to the solvent. It is also a measure of solute-solvent interaction and the relative size of the solute and solvent molecules. The behaviour of B-coefficient in all the amino acids suggest the existence of strong ion-solvent interaction. The magnitude of B values is in order of L-serine > L-alanine > glycine. This conclusion is in excellent agreement with that drawn from S_V and φ_V^o data and the larger values of B indicates structure making capacity of the solute.

Conclusion

In the light of the above observations, it may be concluded that the existence of intermolecular interaction of electrostriction, hydrophilic and hydrophobic nature exist in the system studied. The existence of ion-solvent/solute-solvent interactions resulting in attractive forces promote the structure making tendency while ion-ion interaction/solute-solute interaction resulting in dipole-dipole, dipole-induced dipole and electrostrictive forces enhance the structure breaking properties of aminoacids. The existence of molecular interaction is in the order of L-serine > L-alanine > glycine. The suggests L-serine is a strong structure maker than the other two amino acids. But glycine is a structure breaker in urea + water mixture. Therefore, glycine being polar in nature is involved in hydrogen binding with the solvent components resulting in the breaking of the solvent structure. Similar structure making/breaking effects of solute-solvent interaction have been discussed in detail of Waris[21] which supports our present investigation.

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