Acoustical behavior of some amino acids in aqueous disodium citrate solutions over temperature range (298.15-313.15) K

Muhammad Asghar Jamal[1*], Muhammad Kaleem Khosa[1], Majid Muneer[1]*, Fazal-ur-Rehman[2], Muhammad Jawad Saif[2], Haq Nawaz Bhatti[3], Sadaf Naz[1] and Jaweria Javed[1]

[1]Department of Chemistry, Government College University, Faisalabad, Pakistan
[2]Department of Applied Chemistry, Government College University, Faisalabad, Pakistan
[3]Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

Abstract: Sound velocity, U, and density, ρ, of some amino acids viz. glycine, methionine, phenylalanine and tryptophan were determined in 0.1, 0.2 and 0.3 mol kg^{-1}aqueous disodium citrate solutions as a function of concentration at 298-313.15K using DSA 5000M. The experimental data were further used to compute various acoustical parameters such as adiabatic compressibility, β, apparent molar compressibility, Φ_k, partial molar compressibility, Φ^0_k, transfer adiabatic compressibility, Φ^0_{ktr}, constant, S_k, and the hydration number, n_H. The transfer adiabatic compressibility shows the supremacy of hydrophilic-ionic interactions under hydrophobic–ionic interactions. The above-mentioned parameters are relevant for the efficiency of mediation in pharmacology and can be interpreted in terms of structure-making or structure-breaking ability of these amino acids in the solution.

Keywords: Disodium citrate, amino acids, adiabatic compressibility, hydration number.

INTRODUCTION

Ultrasonic velocity and density studies on amino acids with disodium citrate give valuable information for better understanding the behaviors of intramolecular and intermolecular associations, liquid systems, related structural changes and complex formation in biochemical reactions within living organisms. During the last few years, considerable work has been done to study the hydration of proteins using acoustical and volumetric parameters, therefore these types of interactions are sensitive to the nature and degree of hydration (Millero *et al*.,1978; Cabani *et al*., 1981; Hoiland., 1980; Leyendekkers, 1988; Kharakoz, 1989; Kharakoz, 1991). Many molecular interactions are present in proteins with a variety of different molecules. Proteins are complex in nature, so their direct study is difficult. Therefore, to understand the nature of interactions between citrate and proteins in aqueous solutions it is necessary to study the basic unit of proteins known as amino acids. Complex physiological processes such as immune function, oxygen transport and muscle contraction becomes the base of these interactions. In pharmaceutical grade, glycine is used in intravenous injections and analgesic drugs whereas methionine and tryptophan are essential amino acids used in supplements to cure steatohepatitis, anemia and depression. The solute and solvent macromolecules affect the molecular interactions (Hippel and Schleich, 1969). The hydrophobic interactions, having non-polar side-chain, can be explained by acoustical and volumetric measurements that give valuable information for explaining the proteins unfolding (Enea and Jolicoeur, 1982). Many amino acids and their derivative forms are considered as compensatory solutes for the stabilization of proteins and they enhance enzyme activity, which is the other reason to analyze such properties. Among the organic salts, citrates have great importance in many chemical and biochemical reactions and these salts are used on a large scale in cosmetic, food, pharmaceutical and chemical process. Sodium salts of citric acid are used as an alkalinizing agent, as an in *vitro* anticoagulant in blood stored for transfusion, as an intermediate in the tricarboxylic acid (TCA) cycle and the citric acid cycles, as a carrier of acetyl-CoA, as a building material for fatty acids, a critical component of bone, as an antioxidant in food as well as to improve the effects of other antioxidants and help to regulate the size of calcium crystals. It is also used as an acidity regulator and sequestrant.

The main work presented here involves understanding the physicochemical behavior of glycine, methionine, phenylalanine and tryptophan in 0.1, 0.2 and 0.3mol kg^{-1} aqueous disodium citrate solutions at different temperatures through sound velocity and density measurements. However, the sound velocity and density analyses do not give enough information about the relative and native strengths of many types of inter-ionic or intermolecular interactions between the components. Therefore, the parameters like adiabatic compressibility, β, apparent molar compressibility, Φ_k, partial molar compressibility, Φ^0_k, transfer adiabatic compressibility, Φ^0_{ktr}, constant, S_k, and the hydration number, n_H, were calculated to fall more light on these molecular interactions.

**Corresponding author:* e-mails: m.asgharjamal@gmail.com, majid.chemist@yahoo.com

MATERIALS AND METHODS

Glycine, methionine, phenylalanine and tryptophan were used after the process of recrystallization from methanol water mixture. These amino acids were dried in vacuum using P_2O_5 at room temperature for about 72 hrs. All the chemicals used were of high purity and purchased from (Merck, Aldrich Chem. Co), Germany. Analytical reagent grade disodium citrate was used after recrystallization and dried in vacuum in order to attain maximum purity. The various concentrations of 0.1, 0.2 and 0.3 mol Kg^{-1} in aqueous disodium citrate were prepared and further it was used as a solvent to make 0.05 to 0.19 mol Kg^{-1} solutions of glycine, methionine, phenylalanine and tryptophan using doubly distilled, degassed and deionized water. The weighing was done using Wiggen Hauser electronic balance having±0.001mg precision. By using density of the standard materials like air, H_2O etc. from the literature; calibration of density sound velocity meter (DSA 5000M) was done. The sound velocity and density of the given amino acids mixtures having different concentrations were measured at different temperatures. The sound velocity and density data were calculated with a precession of 0.5 m/s and $5x10^{-6}$ g/cm^3, respectively and carried out in duplicate to minimize errors. For statistical analysis of data, least square fit method was used.

RESULTS

Many acoustical and thermodynamic parameters were computed from the sound velocity, U, and density, ρ data such as adiabatic Compressibility, β,

$$\beta = 1 / \mu^2.\rho \qquad \text{(Eq. 1)}$$

The apparent molar compressibility was calculated (Φ_k) by using following relation (Kauzmann, 1959),

$$\Phi_k = 1000\,(\rho_o\beta - \rho\beta_o) / m\rho\rho_o + M\beta / \rho \qquad \text{(Eq. 2)}$$

Where β_o and β are the adiabatic compressibilities of solvent and solution respectively, M the molecular mass of the solute, m is the molality of the solution, and Φ_k is the function of m; obtained by Gucker from Debye Huckel theory (Zhao, 2005; Kannappan and Palani, 2007) and is given by

$$\Phi_k = \Phi^o_k + S_k.m \qquad \text{(Eq. 3)}$$

Where Φ^o_k, is the partial molar compressibility and S_k, is a constant that is obtained using least square fit method. The values of partial molar compressibility, Φ^o_k, transfer adiabatic compressibility, Φ^0_{ktr}, constant, S_k, and the hydration number, n_H, for the above mentioned amino acids are presented in table 1.

The transfer adiabatic compressibility, (Φ^0_{ktr}), as shown in Table 1, has been estimated by the following formulae.

$$\Phi^0_{ktr} = \Phi^0_{k(\text{disodium citrate soln.})} - \Phi^0_{k((\text{in water})} \qquad \text{(Eq. 4)}$$

The hydration number (n_H) was calculated by using the following standard equation 5 and values are shown in table 1.

$$n_H = n_1 / n_2\,[(1-\beta) / \beta_o] \qquad \text{(Eq. 5)}$$

where n_2 and n_1 are the number of moles of solute and solvent, respectively. βo and β are the adiabatic compressibilities of solvent and solution, respectively.

DISCUSSION

From fig. 1, it is found that the values of adiabatic compressibility, β, decrease by increasing the concentration of solutes in aqueous disodium citrate solution and rise with the temperature. This trend was observed for all the above mentioned amino acids in 0.1, 0.2 and 0.3mol Kg^{-1} aqueous solutions of disodium citrate over temperature range (298.15-313.15) K. A large decrease in the compressibility of solution occurs when the electrostrictive compression around the solute molecules increases due to water molecules. The decreasing trend of adiabatic compressibility shown in Fig.1 implies that there is enhanced molecular associations in these systems due to the increase in the solute content because the new entities become less compressible and compact. The compressibility appeared to decrease with increasing hydrogen bond strength between the solute and solvent molecules (Ragouramme and Srinivasa, 1998).

In the neutral solutions amino acid molecules exist in the form of zwitter ions and have very strong interaction with the surrounding water molecules. Larger decrease in the compressibility of the solution occurs due to increase inelectrostrictive compression of water around the molecules. Let the size of the ions and amino acids are pressure independent and the water is compressed to its maximum limit by the amino acids and the charge on the ions (Wawer *et al.,* 2008), we consider that the compressibility of a solution is due to the affect of pressure on the bulk (un-hydrated) water molecules. A huge portion of water is electrostricted when the concentration of the solute (amino acid) increases. The decrease compressibility decreases by decreasing the amount of bulkwater. It also tends to take more water molecules to hydrate themselves due to divalent citrate ion (Cit^{-2}) ion produced from the dissociation of Na_2HCit. The concentration dependence of amino acids (i.e. glycine, methionine, phenylalanine and tryptophan) becomes greater when the concentration of electrolyte and temperature decreased (table 1). The decrease in the number of water molecules that affect the ion pairs is due to the formation of physically bonded ion-pairs between the anion and the cation of the electrolyte and the charged groups of amino acids that lowers the electrostatic interactions between amino acids and water molecules (Hyder *et al*., 2006). Interactions between Cit^{-2} and

ammonium ions become stronger with the increase in concentration of Na_2Hcit and therefore the Cit^{-2} release more water molecules from amino acids to the bulk. The electrostriction effect causes the shrinkage in the volume of the solvent due to the presence of zwitterionic portion of the amino acids. Such a similar effect was also reported by the earlier workers (Banipal *et al*., 2006).

The data Table 1 reveals that Φ^0_{ktr} has positive values for glycine, methionine, phenylalanine and tryptophan in aqueous electrolytes solutions, decreases by increasing the salt concentration and increases by decreasing the temperature. Further, these values decrease with increasing the concentration of amino acids. The decreasing behavior of Φ^0_{ktr} shows weaker interactions between amino acids and co-solute and vice-versa. The co-sphere overlap model also helps to explain the transfer adiabatic compressibility. The overlap of amino acid ions and co-solute is because of the interactions between (1) the amino acid having hydrophobic groups and ions of co-solute and (2) amino acid having hydrophilic (-OH) sites and the ions of co-solutes (disodium citrate). The former types of interaction show positive behavior in values, whereas the latter types of interaction show negative behavior in values.

The hydration behavior of amino acid can be examined by taking the following molecular interactions:

The groups like $^+NH_3$ and COO^-, which are present at the terminal side of amino acids are surrounded by the water molecules in an electro restriction manner. The surrounding of the water molecules depends on the nature of interaction, which may be hydrophobic, hydrophilic or aminophilic.

Electro restriction of $^+NH_3$ is greater than COO^- group by a factor, which is near to 10.

The overlap of water molecules and the terminal ($^+NH_3$ and COO^-) groups of amino acid causes the change in volume.

Hydration number, n_H, plays an important role in the aqueous system of amino acids as shown in table 1. The significance of hydration in water, glycine and sodium citrate systems is due to the zwitter ionic behavior of amino acids in aqueous solutions and the existence of charged ions in the solutions. The formation of ion-pairs neutralized the charges on amino acid or amino molecules at higher electrolyte concentrations and the electrostatic interactions between water molecules and amino acid molecules are suppressed that's why the water molecules become more compressible. The positive value of hydration number shows the solvation of solutes which indicate the presence of dipole-dipole interaction between water and solute molecules. Further, decreasing behavior of n_H shows that glycine, methionine, phenylalanine and tryptophan have a dehydration effect.

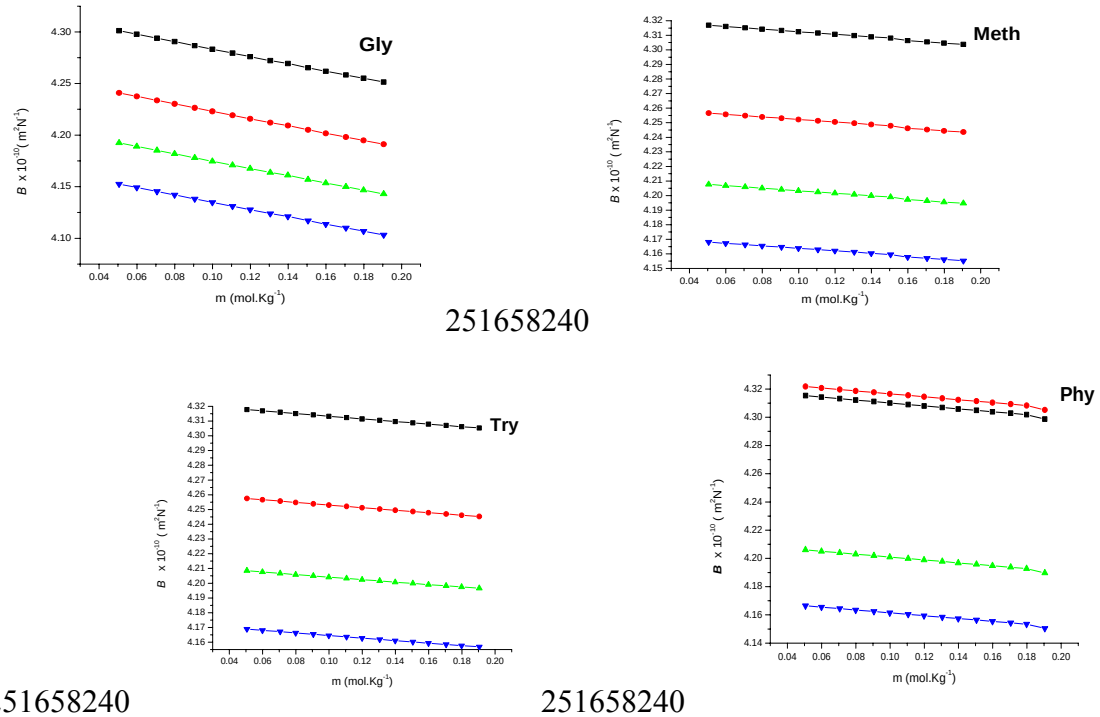

Fig. 1: Plots of adiabatic compressibility, β, versus molality, m, for amino acids in 0.1 mol.kg^{-1} aqueous disodium citrate solution at ■ 298.15 K, ● 303.15 K, ▲308.15 K and ▼313.15 K.

Table 1: Partial molar adiabatic compressibilities, Φ^0_k, and pair wise interaction coefficient for compressibility, S_k, transfer molar volumes, Φ^0_{ktr}, and hydration number, n_H, of glycine, methionine, phenylalanine and tryptophan in 0.1, 0.2 and 0.3mol Kg^{-1} aqueous disodium citrate at 298.15 to 313.15 K.

Amino Acids	Molality (mol/kg)	Temperature (K)	Φ^0_k (cm^3/mol)	S_k ($cm^3.kg/mol^2$)	Φ^0_{ktr} (cm^3/mol)	n_H
Glycine	0.1	298.15K	-0.212±0.000	0.031±0.002	0.067	1.32
		303.15K	-0.208±0.000	0.026±0.002	0.055	1.30
		308.15K	-0.201±0.000	0.032±0.002	0.058	1.29
		313.15K	-0.197±0.000	0.025±0.001	0.056	1.14
	0.2	298.15K	-0.196±0.000	0.018±0.002	0.084	1.37
		303.15K	-0.194±0.000	0.022±0.001	0.070	1.36
		308.15K	-0.192±0.000	0.022±0.001	0.068	1.33
		313.15K	-0.191±0.000	0.028±0.002	0.063	1.30
	0.3	298.15K	-0.190±0.000	0.024±0.001	0.089	1.50
		303.15K	-0.188±0.000	0.024±0.001	0.076	1.47
		308.15K	-0.187±0.000	0.027±0.001	0.073	1.45
		313.15K	-0.185±0.000	0.022±0.001	0.068	1.42
Methionine	0.1	298.15K	-0.228±0.001	0.263±0.082	0.099	5.22
		303.15K	-0.223±0.002	0.244±0.100	0.090	5.10
		308.15K	-0.221±0.001	0.316±0.070	0.087	5.07
		313.15K	-0.216±0.001	0.410±0.085	0.085	4.96
	0.2	298.15K	-0.225±0.001	0.387±0.054	0.102	5.40
		303.15K	-0.216±0.001	0.220±0.079	0.097	5.22
		308.15K	-0.213±0.001	0.224±0.060	0.095	5.17
		313.15K	-0.207±0.001	0.329±0.067	0.095	5.03
	0.3	298.15K	-0.197±0.001	0.558±0.060	0.130	4.92
		303.15K	-0.186±0.001	0.186±0.050	0.127	4.67
		308.15K	-0.183±0.001	0.253±0.083	0.125	4.62
		313.15K	-0.179±0.001	0.455±0.070	0.123	4.54
Phenylalanine	0.1	298.15K	-0.355±0.001	0.433±0.077	0.066	6.90
		303.15K	-0.346±0.001	0.250±0.077	0.065	6.79
		308.15K	-0.344±0.001	0.214±0.066	0.063	6.74
		313.15K	-0.341±0.001	0.464±0.068	0.060	6.69
	0.2	298.15K	-0.349±0.001	0.333±0.066	0.072	7.05
		303.15K	-0.344±0.001	0.331±0.044	0.067	7.02
		308.15K	-0.341±0.001	0. 311±0.080	0.066	6.94
		313.15K	-0.336±0.001	0.435±0.063	0.065	6.85
	0.3	298.15K	-0.346±0.001	0.424±0.075	0.075	7.38
		303.15K	-0.339±0.001	0.284±0.066	0.073	7.30
		308.15K	-0.336±0.001	0.264±0.061	0.071	7.22
		313.15K	-0.334±0.001	0.494±0.064	0.068	7.20
Tryptophan	0.1	298.15K	-0.310±0.001	0.825±0.097	0.025	5.18
		303.15K	-0.306±0.004	1.256±0.387	0.023	5.14
		308.15K	-0.303±0.003	2.124±0.327	0.021	5.13
		313.15K	-0.301±0.002	0.824±0.218	0.018	5.11
	0.2	298.15K	-0.302±0.001	0.88-±0.138	0.033	5.33
		303.15K	-0.295±0.002	1.178±0.204	0.035	5.23
		308.15K	-0.293±0.004	1.437±0.351	0.032	5.23
		313.15K	-0.289±0.002	0.546±0.158	0.030	5.16
	0.3	298.15K	-0.284±0.002	0.982±0.222	0.051	5.33
		303.15K	-0.282±0.004	0.853±0.354	0.047	5.32
		308.15K	-0.280±0.003	1.650±0.251	0.046	5.32
		313.15K	-0.275±0.002	1.521±0.153	0.044	5.26

CONCLUSIONS

The sound velocity and density data have been measured for glycine, methionine, phenylalanine and tryptophan in aqueous disodium citrate solution at T=(298.15 to 308.15) K and their molecular interactions have been studied with the help of these acoustical parameters. It can be concluded from the behavior of acoustical parameters that solute-solvent interactions decreased with addition of aqueous disodium citrate and also by increasing the temperature. The transfer adiabatic compressibility suggests that hydrophilic-ionic interactions show dominance over hydrophobic-ionic interactions. The co-sphere overlap model shows that the solute and co-solute interactions are more dominant.

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