



Ultrasonic investigation of amino acids in aqueous electrolytes medium by ultrasonic method

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ABSTRACT

The ultrasonic velocity(u), density(ρ) and viscosity (η) have been measured for mixed aqueous systems (water +NaCl+ serine) & (water+ $MgCl_2$ + serine) at 303.15,308.15 &313.15K at 2MHz. From experimental data thermodynamic parameters such as adiabatic compressibility(β_a),acoustic impedance (z), intermolecular free length (L_f), relative association (R_A) have been estimated using the standard relations. The results have been analyzed on the basis of variations of thermodynamic parameters. These parameters were used to study the ion-solvent interaction present in each solution.

Keywords: ultrasonic velocity(u), density(ρ), viscosity (η),acoustical parameters.

INTRODUCTION

Amino acids and peptides are used as probe molecules to understand the complex nature of proteins. There is information on the zwitter-ionic nature of amino acids in water in the literature[1-4].The properties of proteins such as their structure, solubility, denaturation, activity of enzymes, etc, are greatly influenced by electrolyte[5,6]. An electrolyte, when dissolved in water perturbs the arrangement of water molecules with the strong electric field of its ions. This property of electrolyte known as structure maker or breaker has been widely used to understand the effect of electrolytes on the structure and function of both proteins and nucleic acids[7]. In the past, thermodynamic properties of amino acids in dilute electrolyte solutions have been studied in order to understand the complex nature of proteins using amino acid-ion interactions[8, 9].There are instances where high concentrations of electrolytes can affect the function and structures of proteins[10,11]. Ion-ion and ion-amino acid interactions dominate in such situations. The thermodynamics of interaction of concentrated NaCl in dilute amino acids have been reported in the literature[12-14]. How the ion-ion and ion-amino acid interactions together with ion-water and amino acid-water ones are altered in concentrated electrolyte and concentrated amino acid solutions is the object of current investigations. Information is available on activity coefficients, enthalpies and heat capacities of aqueous amino acids in electrolytes[15-18]. To our knowledge no systematic efforts have been made to the ion-ion and ion amino acid interactions in concentrated electrolyte solutions.

MATERIALS AND METHODS

All the chemicals used were of AR grade and dried over anhydrous CaCl_2 in desiccators before use. All solutions were prepared in deionized and distilled water (degassed by boiling), having specific conductivity $\sim 10^{-6} \text{ S cm}^{-1}$. The stock solutions of 1M concentration were prepared by weighing the serine on a digital balance with an accuracy of $\pm 1 \times 10^{-4} \text{ g}$. Solutions of NaCl & MgCl_2 were made by mass on the mole fraction scale. Uncertainties in solution concentrations were estimated at $\pm 1 \times 10^{-5} \text{ mol kg}^{-1}$ in calculations.

The solutions were kept in the special air tight bottles and were used within 12 h after preparation to minimize decomposition due to bacterial contamination. Ultrasonic velocity was measured with a single crystal interferometer (F- 81, Mittal Enterprises, New Delhi) at 2MHz. The interferometer was calibrated against the ultrasonic velocity of water used at $T = 303.15\text{K}$. The present experimental value is 1508.80 ms^{-1} which is in good agreement with literature value 1509.55 ms^{-1} . Accuracy in the velocity measurement was $\pm 1.0 \text{ ms}^{-1}$. The density measurements were performed with recalibrated specific gravity bottle with an accuracy of $\pm 2 \times 10^{-2} \text{ kg m}^{-3}$. An average of triple measurements was taken into account. Sufficient care was taken to avoid any air bubble entrapment. Viscosity was measured with recalibrated Ostwald type viscometer. The flow of time was measured with a digital stop watch capable of registering time accurate to $\pm 0.1 \text{ s}$. An average of three or four sets of flow of times for each solution was taken for the purpose of calculation of viscosity. The accuracy of the viscosity measurements was $\pm 0.5 \%$. Accuracy in experimental temperature was maintained at $\pm 0.1\text{K}$ by means of thermostatic water bath.

RESULTS AND DISCUSSION

From the measured values ultrasonic velocity (u), density (ρ) and viscosity (η) various acoustical parameters such as the adiabatic compressibility (β_a), acoustic impedance (z), intermolecular free length (L_f) and relative association (R_A) were calculated by using the following relations [19-22].

$$\begin{aligned} \text{Ultrasonic velocity} & \quad (u) = n \times \lambda \quad \text{_____} (1) \\ \text{Adiabatic compressibility} & \quad (\beta_a) = 1/u^2 \rho \quad \text{_____} (2) \\ \text{Acoustic impedance} & \quad (z) = u \cdot \rho \quad \text{_____} (3) \\ \text{Intermolecular free length} & \quad (L_f) = K / u \cdot \rho^{1/2} \quad \text{_____} (4) \\ \text{Relative association} & \quad (R_A) = (\rho / \rho_0) \cdot (u_0 / u)^{1/3} \quad \text{_____} (5) \end{aligned}$$

Where, K is the temperature dependant Jacobson constant [10].

The values of $K \times 10^4$ are taken as 207.556×10^{-8} , 209.431×10^{-8} and 211.306×10^{-8} at 303.15, 308.15 and 313.15K. T is the absolute temperature, ρ_0 , ρ and u_0 , u are the density and ultrasonic velocity of solvent and solution respectively.

The experimentally measured values of ultrasonic velocity (u), density (ρ) and viscosity (η) of the solutions and calculated values of acoustical parameters such as adiabatic compressibility (β_a), acoustic impedance (z), intermolecular free length (L_f) and relative association (R_A) are reported in table 1 for the systems (water + NaCl + serine) and table 2 for the system (water + MgCl_2 + serine) respectively, and the graph plotted for ultrasonic velocity (u), adiabatic compressibility (β_a), acoustic impedance (z), intermolecular free length (L_f) and relative association (R_A) at different temperatures and various concentrations at 2 MHz frequency for the systems (water + NaCl + serine) are shown in fig. 1 to fig. 5 and for the systems (water + MgCl_2 + serine) are shown in fig. 6 to fig. 10 respectively.

Table-1 Variation of thermodynamic parameters at different mole fractions (x) and different temperatures for the system (Water + NaCl + Serine) at 2MHz.**303.15K**

m mol kg ⁻¹	u ms ⁻¹	ρ Kg m ⁻³	η Nm ⁻² s	$\beta \times 10^{-10}$ m ² N ⁻¹	$z \times 10^{-6}$ Nm ⁻²	L_f A ⁰	R_A
0.000	1551.80	1058.11	1.06195	3.92502	1.64180	0.41120	1.05285
0.008	1564.88	1064.54	1.16268	3.83071	1.66816	0.40623	1.057 85
0.017	1593.18	1078.71	1.37615	3.65472	1.71745	0.39679	1.06339
0.026	1601.16	1081.52	1.55944	3.60831	1.73085	0.39426	1.06458
0.034	1618.12	1087.01	1.61528	3.51368	1.75890	0.38905	1.06674
0.043	1651.64	1094.01	1.87772	3.35085	1.80689	0.37994	1.06630

308.15K

m mol kg ⁻¹	u ms ⁻¹	ρ Kg m ⁻³	η Nm ⁻² s	$\beta \times 10^{-10}$ m ² N ⁻¹	$z \times 10^{-6}$ Nm ⁻²	L_f A ⁰	R_A
0.000	1560.51	1055.11	0.98731	3.89242	164634	0.41319	1.05255
0.008	1574.20	1063.22	1.07613	3.79621	167697	0.40805	1.06534
0.017	1603.15	1068.12	1.09777	3.64314	171219	0.39974	1.06552
0.026	1613.80	1080.25	1.18695	3.55532	174290	0.39489	1.07749
0.034	1628.30	1086.42	1.42941	3.47906	176833	0.39029	1.08348
0.043	1664.20	1090.24	1.45927	3.31264	181398	0.38117	1.08747

313.15K

m mol kg ⁻¹	u ms ⁻¹	ρ Kg m ⁻³	η Nm ² s	$\beta \times 10^{-10}$ m ² N ⁻¹	$z \times 10^{-6}$ Nm ⁻²	L_f A ⁰	R_A
0.000	1583.60	1053.38	0.95536	3.78693	1.66753	0.41060	1.04796
0.008	1587.80	1061.78	0.94504	3.73852	1.68466	0.40797	1.05499
0.017	1609.83	1064.25	1.08785	3.62665	1.71286	0.40182	1.05313
0.026	1615.76	1079.66	1.16157	3.54591	1.74341	0.39556	1.06667
0.034	1648.04	1083.70	1.27930	3.39967	1.78482	0.38904	1.06360
0.043	1695.10	1088.39	1.36861	3.19889	1.84427	0.37738	1.05854

Where m, mole fraction; ρ , density of the solution ; η , viscosity of solution; u, ultrasonic velocity; β_a , adiabatic compressibility; z, acoustic impedance; L_f , intermolecular free length; R_A , relative association.

Table2. variation of thermodynamic parameters at different mole fractions (x) and different temperatures for the system (Water + MgCl₂ + Serine) at 2MHz.**303.15K**

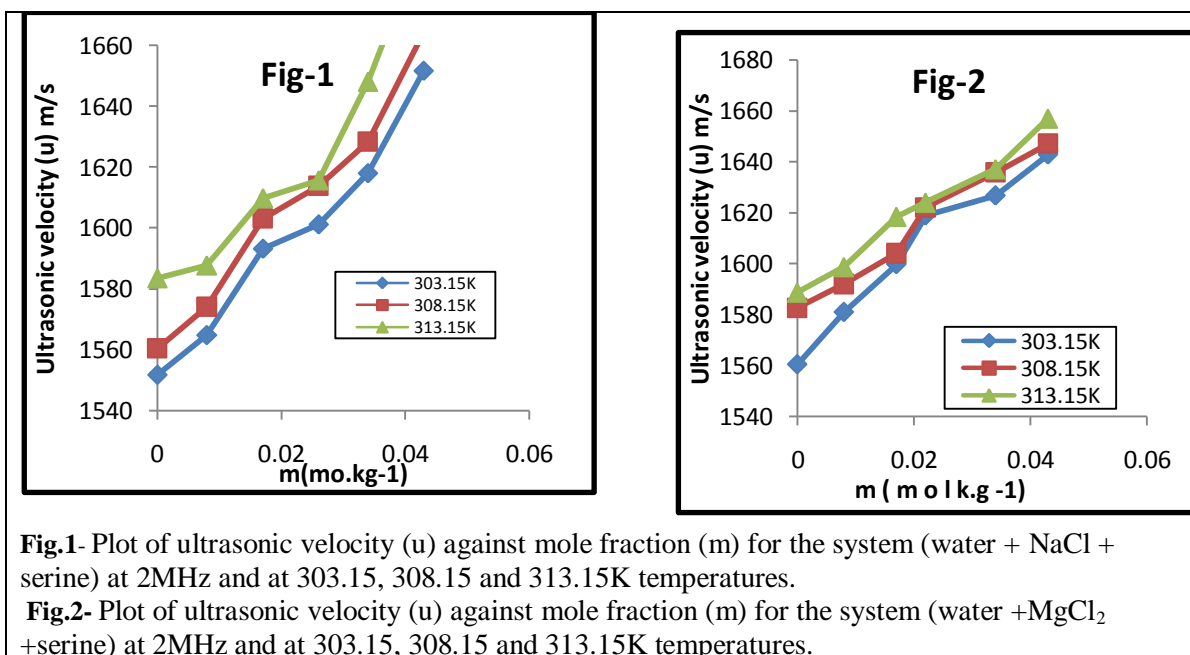
m mol kg ⁻¹	u ms ⁻¹	ρ Kg m ⁻³	η Nm ⁻² s	$\beta \times 10^{-10}$ m ² N ⁻¹	$z \times 10^{-6}$ Nm ⁻²	L_f A ⁰	R_A
0.000	1560.51	1058.38	1.06103	3.88131	1.65101	0.40891	1.05089
0.008	1581.00	1064.78	1.17284	3.74601	1.68851	0.40172	1.05622
0.017	1599.76	1081.34	1.38746	3.61468	1.72934	0.39817	1.06489
0.026	1618.88	1096.66	1.56448	3.48142	1.74234	0.39461	1.07540
0.034	1627.20	1100.24	1.38931	3.43346	1.78992	0.38459	1.07749
0.043	1642.80	1116.85	1.89523	3.32024	1.83336	0.38384	1.08969

308.15K

m mol kg^{-1}	u ms^{-1}	ρ Kg m^{-3}	η Nm^{-2}s	$\beta \times 10^{-10}$ m^2N^{-1}	$z \times 10^{-6}$ Nm^{-2}	L_f A^0	R_A
0.000	1582.70	1055.38	0.98733	3.78403	1.66753	0.40740	1.04899
0.008	1592.10	1065.58	1.15562	3.70431	1.68466	0.40308	1.05684
0.017	1604.10	1080.71	1.25545	3.59823	1.71286	0.39727	1.06904
0.026	1622.16	1094.12	1.48615	3.48337	1.74341	0.39087	1.07599
0.034	1635.80	1102.73	1.44376	3.39125	1.78482	0.38567	1.08374
0.043	1647.16	1113.96	1.60581	3.31165	1.84427	0.38112	1.09204

313.15K

m mol kg^{-1}	u ms^{-1}	ρ Kg m^{-3}	η Nm^{-2}s	$\beta \times 10^{-10}$ m^2N^{-1}	$z \times 10^{-6}$ Nm^{-2}	L_f A^0	R_A
0.000	1588.77	1053.14	0.95533	3.76224	1.67297	0.40927	1.04683
0.008	1598.76	1068.21	1.03814	3.66328	1.70748	0.40384	1.05952
0.017	1618.48	1078.95	1.11385	3.34133	1.74472	0.39707	1.06509
0.026	1630.10	1090.51	1.10846	3.45261	1.77681	0.39206	1.07438
0.034	1637.15	1100.25	1.37749	3.39185	1.80087	0.38860	1.08268
0.043	1657.00	1112.12	1.45346	3.27533	1.84258	0.38186	1.09011



Ultrasonic Velocity (u): The ultrasonic velocity (u), for amino acid electrolytes solutions at 2MHz frequency, for different temperatures and various concentrations (m) have been determined using Eq. (1) and experimental values of u have been presented in Tables 1 for the systems (water + NaCl + serine) and Table 2 for the system (water + MgCl₂ + serine). From Tables it is seen that ultrasonic velocity increases with increase in concentration of solutes serine. The variation of ultrasonic velocity in a solution depends on the intermolecular free length (L_f). Intermolecular free length is a predominant factor, as it determines the sound velocity in fluid state. Presence of an ion alters the intermolecular free length. Therefore, ultrasonic velocity of a solution will be different from that of the solvent. According to model proposed

by Eyring and Kincaid[23, 24] the increase in ultrasonic velocity with the decrease in intermolecular free length (L_f) and vice versa,

Ultrasonic velocity (u) is related to intermolecular free length. As the free length decreases due to the increase in concentrations, the ultrasonic velocity has to increase and vice-versa[23]. The experimental results support the above statement in both the cases. Consequently ultrasonic velocity of system increases depending on the structural properties of solutes. The solute that increases the ultrasonic velocity is of structure maker type (SM). The variations in ultrasonic velocity with molar concentrations of NaCl and MgCl₂ in 1M serine are given in tables 1 and 2. From table 1 and fig1 (water + NaCl + serine) and table 2 and fig.2 (water+ MgCl₂ + serine), it is seen that ultrasonic velocity in aqueous NaCl solution increases with increase in concentration of solutes serine. The value of ultrasonic velocity of serine in aqueous NaCl is less as compared to the value of serine in aqueous MgCl₂. The ultrasonic velocity in aqueous NaCl is maximum at temperature 313.15K and minimum at 303.15K. Such an increase in ultrasonic velocity clearly shows that molecular association is being taking place in these liquid systems. The same results are observed for aqueous MgCl₂. The order of variation of ultrasonic velocity with increase in concentrations and temperatures is observed as follows-

$$u_{\text{water}} < u_{\text{NaCl}} < u_{\text{MgCl}_2}$$

Such type of variation in the sound velocity is attributed to different types of interactions taking place in the solutions. These interactions are as follows.

- Ionic group of serine i.e. zwitter ionic centers of serine with Na⁺, Cl⁻ and Mg⁺⁺ ions.
- NH₂ group (hydrophilic) of serine through H-bonding) CH₃-CH-OH group (hydrophobic) of serine, non polar molecules.
- COO⁻ and NH₃ or NH₂ of serine and ions of solvents.

These different types of interactions affect the solute solvent interactions. The factors apparently responsible for such behavior may be the presence of interaction caused by the proton transfer reactions of amino acid and hydrophilic nature of aqueous NaCl and MgCl₂.with variation in concentration and temperature. The ultrasonic velocity increases with molar concentration of solute as well as rise in temperature. The increase in ultrasonic velocity in water, in aqueous NaCl and in aqueous MgCl₂ may be attributed to the overall increase in cohesion brought about by the solute- solute, solute-solvent and solvent-solvent interactions[25].

Density: Density assure of solvent-solvent and ion-solvent interactions. Increase of density with concentration indicates the lesser magnitude of solute-solvent and solvent-solvent interactions. Increase in density with concentration is due to the shrinkage in the volume which in turn is due to the presence of solute molecules. In other words, the increase in density may be interpreted to the structure-maker of the solvent due the added solute. Similarly, the decrease in density with concentration indicates structure-breaker of the solvent. It may be also true that solvent-solvent interactions bring about a bonding, probably H-bonding between them. So, size of the resultant molecule increases and hence there will be decrease in density. Density of the solution in both the systems increases with increase in concentration of serine .However, from Table-1(water + NaCl +serine) and Table- 2(water+ MgCl₂ + serine), density of the solution is less in aqueous NaCl for serine as compared to aqueous MgCl₂ for serine .The order of variation of density with increase in temperatures is observed as follows;

$$\rho_{\text{MgCl}_2} > \rho_{\text{NaCl}} > \rho_{\text{water}}$$

Viscosity: viscosity is an important parameter in understanding the structure as well as molecular interactions occurring in the solutions. The viscosities are determined for these systems at various concentrations of donor-acceptor mixtures. Increase in viscosity with concentration in all the systems suggests that the extent of complexation increases with increase in concentration. Also from the Tables- 1 (water + NaCl +serine)&Table 2(water+ MgCl₂ + serine), it is observed that viscosity of the solutions shows a non-linear behavior in both the systems. The order of variation of viscosity with increase in concentrations in the three systems is observed as follows;

$$\eta_{\text{water}} < \eta_{\text{NaCl}} < \eta_{\text{MgCl}_2}$$

This supports the association of molecules of serine in aqueous MgCl₂ is more than serine in aqueous NaCl and pure water.

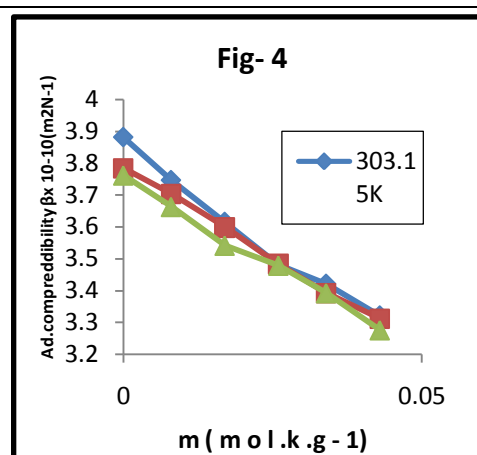
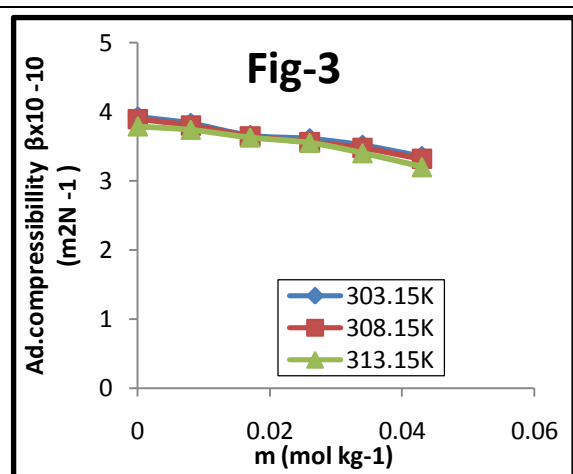


Fig.3- Plot of adiabatic compressibility (β_a) against mole fraction for the system (water + NaCl + serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

Fig.4- Plot of adiabatic compressibility (β_a) against mole fraction for the system (water + MgCl₂ + serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

Adiabatic Compressibility (β_a): The adiabatic compressibility is calculated using Eq. (4). The calculated experimental values of (β_a) are presented in tables 1 and 2. In the present case, adiabatic compressibility (β_a) decreases with increase in concentrations of serine as shown in fig 3(water + NaCl + serine) and fig 4(water + MgCl₂ + serine), for both the systems. This is because as the concentrations of solute increases, a larger portion of water molecules are electrostatic and the amount of bulk water decreases causing the compressibility to decrease[26]. When an amino acid is added to a solvent, its compressibility decreases and this decrease is due to the interaction between the ions and the water molecules. When an ion is added to a solvent, it attracts certain solvent molecules towards itself by wrenching the molecules from bulk of the solvent due to the forces of electrostriction. Because of this, the available solvent molecule for the next incoming ion gets decreased. This process is known as compression. Every solvent is having a limit for compression called the limiting compressibility value. The compressibility of a solvent is higher than that of a solution and it decreases with increase in concentrations. The values of adiabatic compressibility are maximum in water, moderate in aqueous NaCl and minimum in aqueous MgCl₂.

Acoustic Impedance (z): Acoustic impedance is calculated by using Eq. (5). From tables 1 and 2 and fig 5(water + NaCl + serine) and fig 6(water + MgCl₂ + serine), it was observed that the adiabatic compressibility (β_a) decreases with increase in concentrations, whereas, acoustic impedance (z) increases for the same concentrations. For a given concentration, (β_a) decreases and acoustic impedance (z) increases. So the composition is the specific composition, where the acoustic parameter becomes either maximum or minimum. This is the stage where complex formation is taking place in the system due to increased ion – solvent interaction. In (water + NaCl + serine) and (water + MgCl₂ + serine) systems, for a given concentration, the values of acoustic impedance (z) increases with increase in concentrations. It is in good agreement with the theoretical requirements because ultrasonic velocity increases with increase in concentrations of serine. The increase in acoustic impedance (z) with the increase in concentration of solution can be explained on the basis of hydrophobic interaction between solute and solvent molecules[27, 28] which increases the intermolecular distance, making relatively wider gap between the molecules. This also indicates significant interactions in the systems of serine in aqueous NaCl as a co-solvent. The values of acoustic impedance are maximum in aqueous MgCl₂ solution, moderate in water and minimum in NaCl.

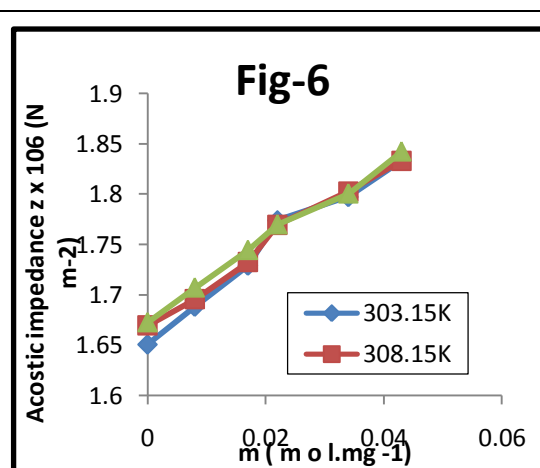
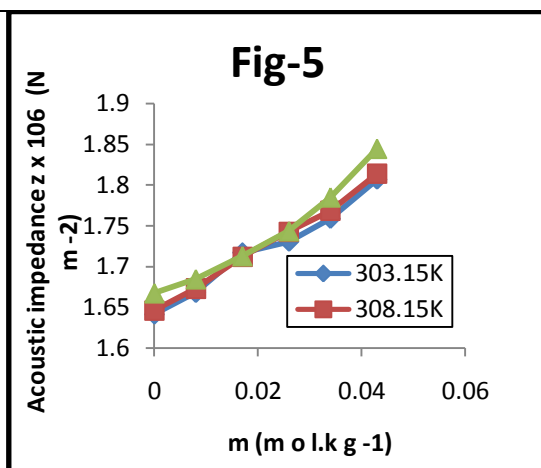


Fig.5- Plot of acoustic impedance (z) against mole fraction for the system (water +NaCl) +serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

Fig.6- Plot of acoustic impedance (z) against mole fraction for the system (water +MgCl₂ +serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

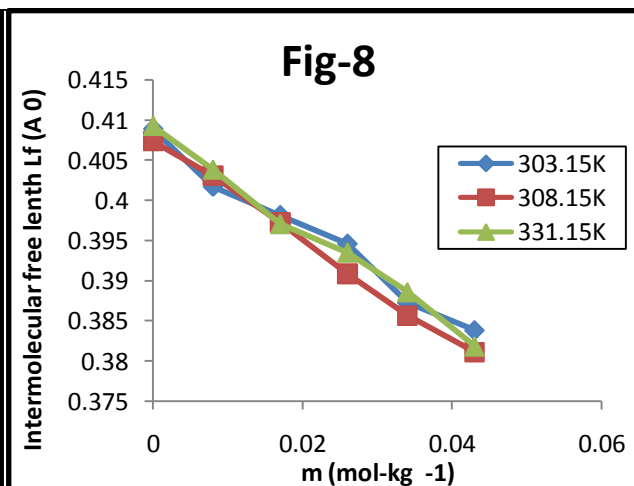
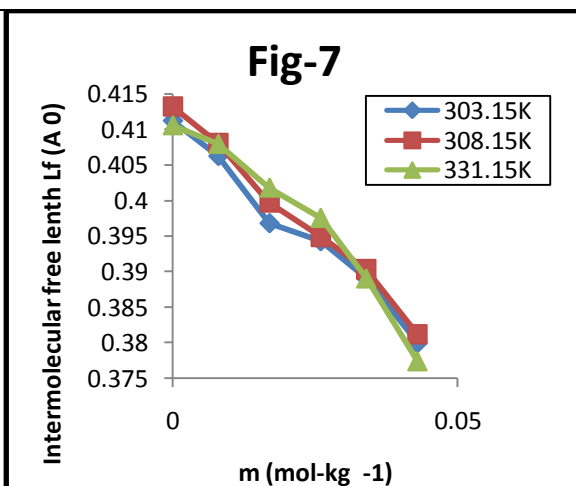
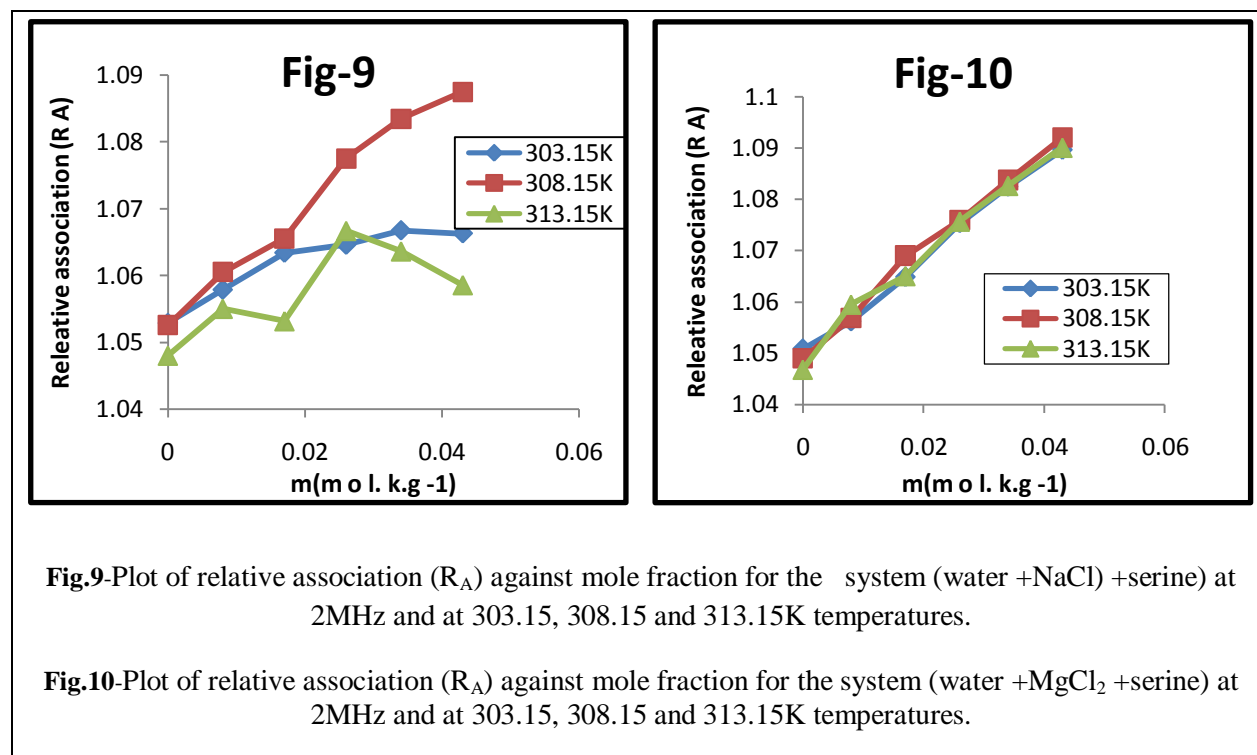


Fig.7-Plot of intermolecular free length (L_f) against mole fraction for the system (water +NaCl +serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

Fig.8-Plot of intermolecular free length (L_f) against mole fraction for the system (water +MgCl₂ +serine) at 2MHz and at 303.15, 308.15 and 313.15K temperatures.

Intermolecular Free Length (L_f): The values of intermolecular free length for (water+ NaCl+ serine) and (water+ MgCl₂ + serine) systems have been calculated using Eq.(6). Increase in concentrations leads to decrease in gap between two species which is referred by intermolecular free length (L_f). With the increase in concentrations, intermolecular free length (L_f) has to decrease. This ideal trend is clearly observed which is shown in fig .7(water + NaCl +serine), and fig 8 (water+ MgCl₂ + serine), Intermolecular free length (L_f) is a predominant factor in determining the variation of ultrasonic velocity in fluids and in their

solutions[29]. From tables-1 and 2 it has been observed that, in the present investigation, intermolecular free length decreases linearly on increasing concentrations of solutes[27]. The decrease in L_f with increase of solutes concentrations in solution indicates that there are significant interactions between solute and solvent suggesting the structure promoting behavior of solutes. In (water + NaCl + serine) & (water + MgCl₂ + serine) systems, ultrasonic velocity increases with concentrations of solutes indicates stronger the intermolecular forces in the solution. This gives increase in closed packed structure of aqueous amino acids i.e. enhancement of the closed structure. This provides the cohesion between amino acids and water molecules increases. The reduction in degree of dissociation among the liquid molecules of the mixture. Thus the inter molecular distance decreases with concentrations of solutes. The decrease in intermolecular free length may due to the gain of dipolar association, making up of hydrogen bonds in the molecules of the liquid mixtures. The values of intermolecular free length are maximum in water, moderate in aqueous MgCl₂ and minimum in aqueous NaCl.



Relative Association (R_A): The values of relative association (R_A) for (water + NaCl + serine) and (water + MgCl₂ + serine) systems were calculated using Eq. (7). The property which can be studied to understand the molecular interaction is the relative association (R_A). It is influenced by two factors: (i) Breaking up of the associated solvent molecules on addition of solute in it and (ii) The solvation of solute molecule²⁶. The former leads to the decrease and later to the increase of relative association. From tables 1 and 2 it is observed that, R_A increases with increase in the solute concentrations (serine) in the NaCl solution. The graphs for (R_A) versus mole fraction (m) of these systems were plotted as shown in fig 9 (water + NaCl + serine) and fig 10 (water + MgCl₂ + serine). The values of relative association (R_A) are minimum in water, moderate in aqueous MgCl₂ and maximum in aqueous NaCl.

APPLICATIONS

The studied parameters were useful to study the ion-solvent interaction present in each solution and also provides useful information about inter and intra molecular interactions of liquid systems.

CONCLUSIONS

Ultrasonic velocity, density and viscosity have been measured for serine in aqueous NaCl and MgCl₂ solution at 303.15, 308.15 and 313.15 K. The variation in ultrasonic velocity, density and viscosity and other related thermodynamic parameters such as adiabatic compressibility (β_a), acoustic impedance (z), intermolecular free length (L_f) and relative association (R_A), of serine at various concentrations and temperatures in both the NaCl – based and MgCl₂ – based systems, shows the non-linear increase or decrease behavior. The non linearity confirms the presence of solute-solvent, ion-ion, dipole-dipole, ion-solvent interactions. The observed molecular interaction, complex formation, hydrogen bond formation are responsible for the heteromolecular interaction in the liquid mixture. This provides useful information about inter and intra molecular interactions of liquid systems.

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