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# Volumetric and Ultrasonic Studies of Some Amino Acids in Aqueous Sodium Butyrate Solution

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**Abstract:** Ultrasonic velocity (U), density ( $\rho$) and viscosity ($\eta$) measurements were carried out for three amino acids namely L-arginine, L-lysine and L-histidine in aqueous sodium butyrate solution as a function of composition at 303, 308 and 313K. Using these experimental values, the acoustical parameters such as adiabatic compressibility ($\beta$), apparent molal compressibility ($\varphi_K$), apparent molal volume ($\varphi_V$), limiting apparent molal compressibility ($\varphi^0_K$), limiting apparent molal volume ($\varphi^0_K$) and the constants ($S_K$, $S_V$) and viscosity B-coefficients were calculated for all the systems. The results are interpreted in the light of structure-making or structure-breaking effects of these amino acids in the mixture.

**Key words:** Adiabatic compressibility · Apparent molal compressibility · Viscosity-B coefficient · Apparent molal volume

## INTRODUCTION

Ultrasonic study on the amino acids with aqueous solution of electrolytes and non-electrolytes provides useful information in understanding the behaviour of liquid systems, intramolecular and intermolecular associations, complex formation and related structural changes. For the past two decades, a considerable study has been carried out to investigate the hydration of proteins through volumetric and ultrasonic measurements, since these properties are sensitive to the degree and nature of hydration [1-6]. Due to the complex molecular structure of proteins, direct study is somewhat difficult. Therefore, the useful approach is to study simpler model compounds, such as amino acids which are building blocks of proteins. Most of the studies on amino acids have been carried out in pure and mixed aqueous solution [7-9].

Amino acids, the monomer units of protein molecule play an important role in all biological species. Since amino acids are zwitterions in aqueous solution [10], their volume and compressibility properties should reflect structural interactions with water molecules as in the case of electrolytes. Water and sodium butyrate mixtures have proved to be most interesting owing to the strong hydrogen bond interactions of sodium butyrate with water. Since volumetric, compressibility and viscosity studies are lacking in aqueous mixtures of amino acids L-arginine, L-lysine and L-histidine, an attempt has been made to understand their behaviour in aqueous sodium butyrate solution at 0.4 molality and varying temperatures of 303, 308 and 313K through ultrasonic velocity measurements. However, the ultrasound velocity data do not provide significant information about the nature and relative strength of various types of intermolecular or inter ionic interactions between the components. Hence their derived parameters such as adiabatic compressibility ($\beta$), apparent molal compressibility ($\varphi_K$), apparent molal volume ($\varphi_V$), limiting apparent molal compressibility ($\varphi^0_K$), limiting apparent molal volume ($\varphi^0_V$) and the constants ($S_K$, $S_V$) and viscosity B-coefficient have been obtained to shed more light on such interactions.

## MATERIALS AND METHODS

All the chemicals used in this present research work are of analytical (AR) reagent grade and spectroscopic (SR) reagent grade of minimum assay of 99.9% obtained from Aldrich, E-Merck and Sdfine. Fresh conductivity water is used throughout the investigation. An electronically 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

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temperature. The accuracy in the temperature measurement is ± 0.1K. Since, density is one of the prime parameters characterising many physical properties of a liquid medium, the density of amino acid solutions are sdetermined using a specific gravity bottle by relative measurement method. A specific gravity bottle with 5mL capacity is cleaned well and dried and filled with reference liquid (conductivity water) and then suspended in a temperature controlled water bath. The temperature of the bath can be maintained at any desired value. The bottle with water is allowed to attain the temperature at which density is to be measured and the weight is determined with an accuracy of ± 0.1mg (Model: SHIMADZU AX-200). An Ostwald's viscometer which is 10mL capacity is used for the viscosity measurement of amino acid solutions. The viscometer is calibrated with fresh conductivity water immersed in the water bath which is kept at the experimental temperature. The time of flow of water and the time flow of solution is measured with digital stop clock having an accuracy of 0.01s (Model: RACER - HS-10W). By knowing the flow time of reference liquid (water), the viscosity of the mixture can be determined. The measured viscosity values are accurate to ± 0.001 Ns $m^{-2}$. An ultrasonic interferometer (Model: F-81) supplied by M/s. Mittal Enterprises, New Delhi, having the frequency 3MHz with an overall accuracy of ±2 $ms^{-1}$ has been used for velocity measurement.

**Theory and Calculations:**

Adiabatic compressibility (β) is given by:

$$\beta = \frac{1}{U^2 \rho} \tag{1}$$

Apparent molal compressibility ($\varphi_K$) is given by:

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

Where β, ρ and $\beta_0$, $\rho_0$ are the adiabatic compressibility and density of solution and solvent respectively, m is the molal concentration of the solute and M the molecular mass of the solute. $\varphi_K$ is the function of 'm' as obtained by Gucker [11] from Debye- Huckel theory [12] and is given by:

$$f_K = f_K^0 + S_K m^{1/2} \tag{3}$$

Where $\varphi^0_K$ is the limiting apparent molal compressibility at infinite dilution and $S_K$ is a constant. $\varphi^0_K$ and $S_K$ were obtained by least-squares method.

Apparent molal volume ($\varphi_V$) is obtained by:

$$f_V = \frac{1000}{m\rho_0}(\rho_0 - \rho) + \left(\frac{M}{\rho_0}\right) \tag{4}$$

The apparent molal volume has been found to differ with concentration according to Masson's empirical relation [13] as:

$$f_V = f_V^0 + S_V m^{1/2} \tag{5}$$

Where $\varphi^0_V$ the limiting apparent molal volume at infinite solution and $S_V$ is a constant and these values were determined by least- squares method.

The entire viscosity data have been analysed in the light of Jones-Dole semi empirical equation [14],

$$\frac{\eta}{\eta_0} = 1 + Am^{1/2} + Bm \tag{6}$$

The same may be expressed as,

$$\frac{\left(\eta/\eta_0\right) - 1}{m^{1/2}} = A + Bm^{1/2} \tag{7}$$

Where η and $\eta_0$ are the viscosities of the solution and solvent respectively and 'm' is the molal concentration. A and B are constants which are definite for a solute-solvent system. A is known as the Falkenhagen [15] coefficient which characterises the ionic interaction and B is the Jone's-Dole or viscosity B-coefficient which depends on the size of the solute and nature of solute-solvent interactions.

## RESULTS

The experimental values of density, viscosity and ultrasonic velocity for different molal composition of each of the amino acids viz, L-arginine, L-lysine and L-histidine in aqueous sodium butyrate solution at different temperatures, are shown in Table 1.The values of adiabatic compressibility are tabulated in Table 2. The apparent molal compressibility and the apparent molal

Table 1: Density (ρ), viscosity (η) and velocity (U) of amino acids in Aqueous Sodium Butyrate solution

| | Density ρ/(kg/m$^3$) | | | Viscosity η/(×10$^{-3}$Nsm$^{-2}$) | | | Velocity U/(ms$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temperature (K) | | | | | | | | |
| Molality m/ (mol.Kg$^{-1}$) | 303 | 308 | 313 | 303 | 308 | 313 | 303 | 308 | 313 |
| | System - 1: L–arginine + aqueous sodium butyrate solution | | | | | | | | |
| 0.0 | 1019.75 | 1016.31 | 1012.38 | 0.9630 | 0.8556 | 0.7662 | 1555.56 | 1567.20 | 1573.50 |
| 0.1 | 1025.11 | 1022.53 | 1016.72 | 1.0000 | 0.8885 | 0.7939 | 1573.95 | 1582.18 | 1590.78 |
| 0.2 | 1030.12 | 1027.52 | 1022.05 | 1.0506 | 0.9288 | 0.8314 | 1576.80 | 1584.78 | 1592.10 |
| 0.3 | 1032.21 | 1029.83 | 1023.53 | 1.1032 | 0.9742 | 0.8707 | 1578.20 | 1585.30 | 1597.30 |
| 0.4 | 1038.76 | 1034.77 | 1030.83 | 1.1536 | 1.0156 | 0.9063 | 1586.98 | 1600.56 | 1615.30 |
| 0.5 | 1041.60 | 1038.99 | 1035.33 | 1.2063 | 1.0649 | 0.9475 | 1597.56 | 1603.98 | 1618.32 |
| | System - 2: L–lysine + aqueous sodium butyrate solution | | | | | | | | |
| 0.0 | 1019.75 | 1016.31 | 1012.38 | 0.9630 | 0.8556 | 0.7662 | 1555.56 | 1567.20 | 1573.50 |
| 0.1 | 1021.86 | 1018.88 | 1016.05 | 0.9955 | 0.8882 | 0.7964 | 1569.42 | 1576.62 | 1588.32 |
| 0.2 | 1026.90 | 1022.80 | 1018.58 | 1.0446 | 0.9250 | 0.8270 | 1571.00 | 1578.6 | 1591.80 |
| 0.3 | 1029.30 | 1026.27 | 1022.75 | 1.0856 | 0.9631 | 0.8650 | 1582.8 | 1602.80 | 1615.36 |
| 0.4 | 1032.86 | 1029.22 | 1024.71 | 1.1350 | 1.0038 | 0.8926 | 1593.00 | 1605.96 | 1618.24 |
| 0.5 | 1034.16 | 1031.75 | 1028.36 | 1.1839 | 1.0423 | 0.9298 | 1601.55 | 1608.00 | 1620.20 |
| | System - 3: L–histidine + aqueous sodium butyrate solution | | | | | | | | |
| 0.00 | 1019.75 | 1016.31 | 1012.38 | 0.9630 | 0.8556 | 0.7662 | 1555.56 | 1567.20 | 1573.50 |
| 0.02 | 1019.93 | 1016.71 | 1014.67 | 0.9647 | 0.8582 | 0.7703 | 1567.83 | 1575.97 | 1584.16 |
| 0.04 | 1020.20 | 1017.99 | 1015.03 | 0.9718 | 0.8652 | 0.7723 | 1573.65 | 1580.49 | 1589.23 |
| 0.06 | 1023.46 | 1019.54 | 1016.82 | 0.9824 | 0.8706 | 0.7792 | 1579.28 | 1592.71 | 1604.18 |
| 0.08 | 1025.13 | 1022.53 | 1018.87 | 0.9963 | 0.8809 | 0.7884 | 1591.54 | 1603.82 | 1616.55 |
| 0.10 | 1027.08 | 1023.63 | 1020.13 | 0.9986 | 0.8839 | 0.7958 | 1600.95 | 1608.25 | 1621.28 |

Table 2: Adiabatic compressibility (β) of amino acids in Aqueous Sodium Butyrate solution

| | Adiabatic Compressibility β/(×10$^{-10}$m$^2$N$^{-1}$) | | |
|---|---|---|---|
| | Temperature (K) | | |
| Molality m/(mol. Kg$^{-1}$) | 303 | 308 | 313 |
| System - 1: L–arginine + aqueous sodium butyrate solution | | | |
| 0.0 | 4.0525 | 4.0061 | 3.9895 |
| 0.1 | 3.9377 | 3.9067 | 3.8866 |
| 0.2 | 3.9044 | 3.8750 | 3.8599 |
| 0.3 | 3.8896 | 3.8735 | 3.8293 |
| 0.4 | 3.8224 | 3.7723 | 3.7179 |
| 0.5 | 3.7617 | 3.7410 | 3.6880 |
| System - 2: L–lysine + aqueous sodium butyrate solution | | | |
| 0.0 | 4.0525 | 4.0061 | 3.9895 |
| 0.1 | 3.9731 | 3.9484 | 3.9012 |
| 0.2 | 3.9456 | 3.9234 | 3.8746 |
| 0.3 | 3.8799 | 3.7929 | 3.7470 |
| 0.4 | 3.8152 | 3.7672 | 3.7266 |
| 0.5 | 3.7699 | 3.7484 | 3.7043 |

Table 2: Comtinued

| | | | |
|---|---|---|---|
| System - 3: L–histidine + aqueous sodium butyrate solution | | | |
| 0.00 | 4.0525 | 4.0061 | 3.9895 |
| 0.02 | 3.9887 | 3.9601 | 3.9271 |
| 0.04 | 3.9582 | 3.9325 | 3.9007 |
| 0.06 | 3.9175 | 3.8665 | 3.8216 |
| 0.08 | 3.8511 | 3.8020 | 3.7558 |
| 0.10 | 3.7987 | 3.7770 | 3.7293 |

Table 3: Values of apparent molal compressibility ($\varphi_k$) and apparent molal volume ($\varphi_v$) of

| | $-\varphi_k$ /(×10$^{-8}$ m$^2$ N$^{-1}$) | | | $-\varphi_v$ /(×m$^3$ mol$^{-1}$) | | |
|---|---|---|---|---|---|---|
| Molality m/(mol.Kg$^{-1}$) | 303 | 308 | 313 | 303 | 308 | 313 |
| | System-I: L–arginine + Aqueous Sodium Butyrate solution | | | | | |
| 0.1 | 13.609 | 11.602 | 11.999 | 52.561 | 61.201 | 42.869 |
| 0.2 | 9.4649 | 8.7639 | 8.3851 | 50.845 | 55.150 | 47.758 |
| 0.3 | 7.0803 | 6.1962 | 6.6768 | 40.728 | 44.343 | 36.711 |
| 0.4 | 7.6408 | 7.6637 | 8.6073 | 46.603 | 45.408 | 45.560 |
| 0.5 | 7.5523 | 7.0899 | 7.8385 | 42.852 | 44.632 | 45.337 |
| | System-I: L–Lysine + Aqueous Sodium Butyrate solution | | | | | |
| 0.1 | 8.7784 | 6.7829 | 10.2757 | 20.691 | 25.287 | 36.251 |
| 0.2 | 6.7653 | 5.4136 | 5.4568 | 35.057 | 31.929 | 30.620 |
| 0.3 | 7.0182 | 8.4149 | 8.9516 | 31.216 | 32.666 | 34.143 |
| 0.4 | 7.2346 | 7.2443 | 7.7870 | 32.139 | 31.755 | 30.447 |
| 0.5 | 6.7969 | 6.3710 | 6.9632 | 29.437 | 30.384 | 31.568 |
| | System-I: L–Histidine + Aqueous Sodium Butyrate solution | | | | | |
| 0.02 | 32.257 | 23.788 | 35.711 | 8.335 | 19.678 | 29.099 |
| 0.04 | 24.022 | 20.055 | 24.810 | 11.031 | 41.325 | 65.439 |
| 0.06 | 24.956 | 25.387 | 30.899 | 60.635 | 52.968 | 73.094 |
| 0.08 | 27.847 | 28.576 | 26.294 | 65.947 | 76.501 | 80.132 |
| 0.10 | 28.292 | 25.795 | 29.073 | 71.880 | 72.024 | 76.552 |

Table 4: Values of limiting apparent molal compressibility ($\varphi_k^0$), limiting apparent molal volume ($\varphi_v^0$) and the constants $S_k$ and $S_v$ of the Amino Acids in Aqueous Sodium Butyrate solution

| | Limiting apparent molal compressibility $-\varphi_k^0$ /(×10$^{-8}$ m$^2$ N$^{-1}$) | | | Constant $S_k$/(×10$^{-8}$ N$^{-1}$ m$^{-1}$ mol$^{-1}$) | | | Limiting apparent molal volume $-\varphi_v^0$ /(×m$^3$mol$^{-1}$) | | | Constant $S_v$/(×10$^{-8}$ N$^{-1}$ m$^{-1}$ mol$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino Acids | 303 | 308 | 313 | 303 | 308 | 313 | 303 | 308 | 313 | 303 | 308 | 313 |
| L-arginine | -17.24 | -14.23 | -13.63 | 15.42 | 11.25 | 9.30 | -60.17 | -74.57 | -42.53 | 25.39 | 46.07 | -2.10 |
| L-lysine | -9.41 | -6.19 | -10.50 | 3.95 | -1.23 | 4.93 | -20.07 | -23.97 | -38.18 | -18.16 | -12.11 | 10.53 |
| L-histidine | -30.88 | -17.98 | -36.86 | 14.38 | -28.40 | 31.68 | -58.06 | -26.52 | -119.22 | -428.70 | -333.33 | 158.45 |

Table 5: Values of A and B parameters of Jones – Doles equation of Amino Acids in Aqueous Sodium Butyrate Solution

| | A/(× dm$^{-3/2}$m$^{1/2}$) | | | B/ (×dm$^3$ mol$^{-1}$) | | |
|---|---|---|---|---|---|---|
| Amino Acids | 303 | 308 | 313 | 303 | 308 | 313 |
| L-arginine | -0.06880 | -0.06242 | -0.06252 | 0.6053 | 0.5731 | 0.50210 |
| L-lysine | -0.06474 | -0.03514 | -0.02166 | 0.55039 | 0.48631 | 0.45576 |
| L-histidine | -0.08465 | -0.04872 | -0.050196 | 0.67741 | 0.50752 | 0.52473 |

volume for the three amino acid system are represented in Table 3. The limiting apparent molal compressibility, limiting apparent molal volume and the constants $S_K$ and $S_V$ are given Table 4 and viscosity B-coefficients are given in Table 5.

## DISCUSSION

In all the amino acid systems (Table 1), the density increases with increase in molal concentration of amino acids. Such an increasing density values with increasing concentration of solutes suggest a solute–solvent interaction exists between the amino acids and aqueous sodium butyrate solution. Increase of density with concentration indicates the increase in solvent-solvent and solute-solvent interactions. In other words, the increase in density may be interpreted to the structure-maker of the solvent due to the added solute. It may also be true that solvent-solvent interactions bring about a bonding, probably hydrogen bonding between them. The change in structure of solvent or solutions as a result of hydrogen bond formation or dissociation or hydrophobic (structure-breaking) or hydrophilic (structure-forming) character of solute [16].

The ultrasonic velocity (U) for the three amino acids (Table 1) increases with increase in the molal concentration of the solute as well as rise in temperature. The increase in ultrasonic velocity (U) in these solutions may be attributed to the cohesion brought about by the ionic hydration. When the amino acids are dissolved in aqueous sodium butyrate, the water molecules are attracted to the ions strongly by the electrostatic forces [17], which introduce a greater cohesion in the solution. Thus, cohesion increases with increase of amino acid concentration in the solutions. The electrostriction effect, brings about the shrinkage in the volume of solvent caused by the zwitterionic portion of the amino acid. Such a similar effect was reported by earlier workers [17,18]. The factors apparently responsible for such behaviour may be the presence of interactions caused by the proton transfer reactions of amino acids [19] and hydrophilic nature of aqueous sodium butyrate [20].

Table 2 shows the variation of adiabatic compressibility ($\beta$) with molal concentration of amino acids. The values of adiabatic compressibility in all the amino acids show the decreasing trend. Amino acid molecules in the neutral solution exist in dipolar form and then have stronger interaction with the surrounding water molecules. The increasing electrostrictive compression of water around the molecules results in a larger decrease in the compressibility of the solutions.

The values of apparent molal compressibility ($\varphi_K$) are all negative over the entire range of molality (Table 3). Also, a non-linear variation between $\varphi_K$ and solute has been observed throughout the entire concentration range. Themaximum negative value of $\varphi_K$ recorded for L-histidine,andminimumforL-lysine irrespective of molality and temperature indicates electrostriction and hydrophilic interactions occurring in these systems,clearlyindicating solute-solvent interaction. The perusal of Table 3 represent the values of apparent molal volume $\varphi_V$, which are all negative over the entire range of molality. No systematic variation has been found in $\varphi_V$ values with increase in molality of the solute as well as rise of temperature. The maximum value of $\varphi_V$ obtained in L-histidine system and minimum in L-lysine system, which clearly shows the L-lysine is a more effective structure maker comparing others. The observations clearly suggest that the negative values of $\varphi_V$ in all systems indicate presence of solute-solvent interaction. The negative values of $\varphi_V$ indicates electrostrictive solvation of ions [21]. From the magnitude of $\varphi_V$, it can be concluded that the strong molecular association is found in L-lysine system than the other two amino acidsandhence L-lysine is a more effective structure maker in aqueous sodium butyrate solution.

The limiting apparent molal compressibility ($\varphi^0_K$) and the related constant $S_K$ for the amino acids have been tabulated in Table 4. The limiting apparent molal compressibility ($\varphi^0_K$) provides information regarding solute-solvent interaction and the related constant $S_K$ that of solute-solute interaction in the solution. From Table 4, it can be observed that all $\varphi^0_K$ values are negative in all the systems. Appreciable negative values of $\varphi^0_K$ for all systems reinforce the view that the existence of solute-solvent interaction in the present system. The values of $S_K$ exhibit both negative and positive and it is non-linear with increase in temperature in all amino acids. This behaviour indicates the existence of ion-ion / solute-solute interaction with increase in temperature. It is well known that solutes causing electrostriction lead to decrease in the compressibility of the solution, which is reflected by the negative values of $\varphi_K$ of the amino acids. It is clear from the Table 4, the values of limiting apparent molal volume ($\varphi^0_K$) in all the systems are negative and non-linear with rise in temperature. This enhances the electrostriction of water molecules. The negative values of $\varphi^0_K$ indicate smaller solute-solvent interactions present in these systems. An increase in $\varphi^0_K$ values is due to the reduction of electrostriction at the terminal groups of amino acids, which in turn increases the interaction between these polar ends and ions, indicating L-lysine

having minimum $\varphi^0_K$ value compared to other two amino acids. It is evident from the Table 4, that the constant $S_V$ exhibits both negative and positive values in all the systems suggesting the presence of ion-ion interactions. The negative values of $S_V$ indicate the presence of weak ion-ion interactions whereas the positive values of $S_V$ predict strong solute-solute interaction in the systems.

Viscosity is an important parameter in understanding the structure as well as molecular interactions occurring in the solutions. Viscosity variation is attributed to the structural changes. The structural changes influence the viscosity to a certain extent as compared to density and compressibility. From Table 5, it is observed that the values of viscosity increases with increase in solute concentration. In order to shed more light on this, the role of viscosity B-coefficient has also been obtained. From Table 5, it is observed that values of A are negative and B-coefficient are positive in all systems. Since A is a measure of ionic interaction [22], it is evident that there is a weak ion-ion interaction in the amino acids. B-coefficient which is known for measure of order or disorder introduced by the solute in the solvent. It is also a measure of solute-solvent interaction. The behaviour of B-coefficient in all the amino acids suggests the existence of strong solute-solvent interaction. The magnitude of B-values is maximum in L.histidine system and minimum for L-lysine system, which clearly shows that the L-lysine is a effective structure maker in aqueous sodium butyrate solution.

## CONCLUSION

In the light of the above discussion, it may be concluded that intermolecular interaction of electrostriction and hydrophilic nature exist in the systems studied. The existence of ion-solvent or solute-solvent interactions resulting in attractive forces which promote the structure-making tendency, while ion-ion or solute-solute interaction resulting dipole-dipole, dipole-induced dipole and electrostrictive forces enhance the structure-breaking properties of amino acids. In the present study, the molecular interaction follows the order: L-lysine 〉 L-arginine 〉 L-histidine. This suggests that the amino acid L-lysine is a strong structure-maker in aqueous sodium butyrate solution over the other two amino acids. Further there is much scope for further studies in these systems by varying pH of the solution and temperature which may reveal more about hydrogen bonding interaction as well as other interaction existing between solute- solvent molecules. Hence it is evident that the ultrasonic velocity measurement in the given medium serves as a powerful probe in characterising the physico-chemical properties of that medium.

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