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Ultrasonic Characterisation of Amino Acid Solutions: A Comparative Analysis of Molecular Interactions

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Abstract. The use of ultrasonic techniques offers a non-invasive approach to investigating the molecular interactions affecting the behavior of amino acids in an aqueous medium. In this paper, three amino acids, namely L-leucine, L-isoleucine, and L-histidine, have been investigated in aqueous solutions using ultrasonic technique in presence and absence of electrolytes. Various thermo-acoustic parameters, such as isentropic compressibility, acoustic impedance, intermolecular free length, and relative association have been calculated from density and ultrasonic velocity data. The comparative approach used in this study helps in understanding the effect of side chain hydrophobicity and electrolyte on solute-solvent and solute-solute interactions. It has been observed that side chains with hydrophobic nature reduce the phenomenon of electrostriction and make the structure making process easier, while electrolytes enhance ion-hydrophilic interactions.

Keywords: Ultrasonic Velocity, Amino Acids, Molecular Interactions, Adiabatic Compressibility, Acoustic Impedance, Solute–Solvent Interactions

I. Introduction

The amino acids serve as the basic units of proteins, and their properties in an aqueous solution provide a key to the mechanisms of many biological reactions, including protein folding and enzymatic activity. The interaction of amino acids in an aqueous medium in the form of hydrogen bonds, electrostatic interaction, hydrophobic effect, and hydration influences their solubility and stabilization and, correspondingly, determines the conformation of the resulting protein molecule [6][9]. This makes the study of the behavior of amino acids important in terms of biochemistry, pharmacy, and food technology, where the aqueous solutions of amino acids are often used in experiments and practical applications [9][10].

Amongst the range of experimental methods that may be employed to investigate interactions between molecules in liquids, ultrasonic characterization holds a unique position. Ultrasonic characterization is a rapid and non-destructive technique which does not need any chromophore or fluorescence labelling, hence may be used on optically opaque and spectroscopically complicated samples. As an ultrasonic wave travels through a liquid medium, the speed of travel of the wave and its attenuation depends upon the density and compressibility of the medium on the molecular level. The values of these properties, in turn, depend upon the strength and type of interactions, degree of hydrogen bonding and solvation structure of the medium. From the values of density and velocity of sound, various parameters like adiabatic compressibility, acoustic impedance, intermolecular free length and relative association may be calculated [2][6][10].

The use of ultrasonic techniques for amino acid solutions has a long history; however, its comparative aspect has not been developed enough. Most studies investigate one amino acid in one solvent system, studying dependence on temperature and concentration [1][2][5][7]. These studies are useful; however, it is difficult to understand what aspects of the structure of the amino acid — length of the side chain, branching, aromaticity, charge, etc. — are responsible for different types of interactions. In the comparative approach, when studying the properties of several amino acids in similar conditions, it will be possible to isolate the effect of different aspects of the amino acid structure. It is necessary to mention that the presence of the electrolytes in solution may have a significant impact on amino acid-water interactions. The presence of ions is a characteristic feature of any biological fluid and many industrial preparations [1][2][7][8].

In this paper, these shortcomings have been addressed by providing a comparative ultrasonic characterization of three amino acids, chosen to cover the spectrum of side-chain chemistry – L-leucine (aliphatic, branched, hydrophobic); L-isoleucine (aliphatic, branched, hydrophobic, with a different branching structure); L-histidine (imidazole-containing, ionizable, amphiphilic). The paper is divided into two sections depending on the solvent type – pure water and electrolyte solutions. The primary objective of the paper is not just to provide the results of thermo-acoustic parameter calculations for each system; it is to produce an analytical comparison that will show the effect of the chemical structure of the molecules and ionic solvation environment on interaction types. The methodology focuses on accessibility and reproducibility, using standard ultrasonic interferometry and density measurements common in physical chemistry laboratories. The analysis is carried out through the calculation of thermo-acoustic parameters and their interpretation through the types of interactions, illustrated with a comparative table for all three amino acids [1][2][7].

Through the discussion of this study in terms of the existing literature pertaining to the solvation of amino acids, it helps in gaining a broader perspective about the use of ultrasound technology as a diagnostic tool in the study of biomolecular interactions. The knowledge gained will be useful in fields such as the designing of drugs made from amino acids, fermentation medium optimization, and protein hydration studies [9][10].

II. Literature Survey

The ultrasound study of amino acid solutions has been evolving in different directions, each providing different insights into this phenomenon. From a review of the recent research, it becomes clear not only how mature this field is but also what are the areas which still require further attention and which make the current study necessary [6][10].

There is extensive literature devoted to the relationship between amino acids and electrolytes due to the importance of ionic effects for the stability of proteins. According to recent results by Sonune et al. (2024), L-histidine solutions in potassium sulphate aqueous solutions provided information about the thermo-acoustic parameters such as adiabatic compressibility, acoustic impedance and surface tension at different temperatures and concentrations [7]. It has been found that more concentrated solutions at higher temperatures correlate with stronger solute-solvent interactions, potassium sulphate being better associated with histidine than water. Another similar study has investigated L-isoleucine in magnesium nitrate and magnesium sulphate solutions; their results showed that isothermal compressibility is decreasing with temperature and concentration due to electrostriction and intermolecular interactions [1]. At the same time, acoustic impedance was higher for magnesium nitrate solutions, meaning the crucial role of anions in the interactions [1].

Another approach to the studies of amino acids was done by adding organic additives and ionic liquids to amino acids. Studies on the behavior of L-phenylglycine and L-phenylalanine in aqueous imidazolium based ionic liquids using apparent molar volume and apparent molar isentropic compressibility were used in order to characterize the interactions between solute and solvent with positive transfer values showing the presence of ion-hydrophilic and hydrophilic-hydrophilic interactions [3]. These types of studies are important for applying the Hepler's constant to differentiate between structure making and structure breaking effects which became very popular among other studies [6], [10]. On the other hand, investigations on L-leucine and N-acetyl L-leucine in aqueous hexylmethylimidazolium bromide resulted in positive values for apparent molar volumes implying dominant solute-solvent interactions [5].

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Another area where the ultrasonic characterization of amino acids in simpler aqueous systems devoid of any additives has also been carried out is that of L-leucine in aqueous potassium chloride and sodium chloride solutions, which have determined the acoustic impedance, free length, adiabatic compressibility, and isothermal compressibility, and the positive value of the transfer quantities suggests that ion-hydrophilic and hydrophilic-hydrophilic interactions are predominant [2]. Temperature dependency has been exploited to determine the dominance of hydrophobic and electrostatic contributions [2][6]. Another study conducted on L-alanine in water involves the use of ultrasonic technique and density functional theory calculations based on Gibbs free energy calculations to validate the experimentally obtained interaction parameters [4].

From a methodological perspective, the field has settled upon a consistent set of derived parameters. The adiabatic compressibility, obtained using the Newton–Laplace equation, continues to be the primary value and represents the compressibility of the solution and thus, the degree to which the structure of the solvent is disturbed or altered by the solute [2][6]. Acoustic impedance, which is the multiplication of density and speed, provides supplementary information on the degree to which the medium resists wave transmission and thus can be affected by solute-solvent interactions [1][7]. The intermolecular free length, calculated using the adiabatic compressibility through Jacobson's constant, shows how far away the molecular surfaces are on average and thus the tightness of molecular packing [2][6]. The relative association, which contrasts the ultrasonic speed of the solution with that of the pure solvent, shows the degree to which the solute molecules disturb the solvent structure [2][10].

Nevertheless, there are still some problems with the literature that need to be addressed. First of all, many research papers focus on a single amino acid, and this is done in one solvent environment. Comparison between the results becomes very hard due to differences in the temperature, range of concentrations used, and equipment. The comparative aspect, i.e., comparing different amino acids with different side chains in the same conditions, is rather unexplored [1][2][5][7]. In addition, although the influence of the electrolytes on the individual amino acids was investigated, there was little attempt to compare the responses of different amino acids to the same electrolyte. This paper is aimed at filling this gap through using the comparative approach when studying three amino acids with different side chains under identical conditions.

III. Proposed Methodology

Materials and Solution Preparation

Three amino acids with different side chain chemistries were chosen: leucine (Merck, >99%), isoleucine (Merck, >99%) and histidine (Merck, >99%). These were used in their pure forms without any further purification. Additives of the electrolyte nature, KCl and $MgSO_4$, were of the analytical grade and dried over silica gel prior to use. All solutions were prepared in molal concentration using doubly distilled and degassed water with a specific conductance less than 1×10^{-6} S cm^{-1}. Concentration of amino acids was varied within a range of 0.02 to 0.20 mol kg^{-1}, whereas concentration of electrolytes was kept constant at 0.10 mol kg^{-1} for the systems with additives added. The solutions were prepared gravimetrically by using a digital balance with an accuracy of 0.01 mg.

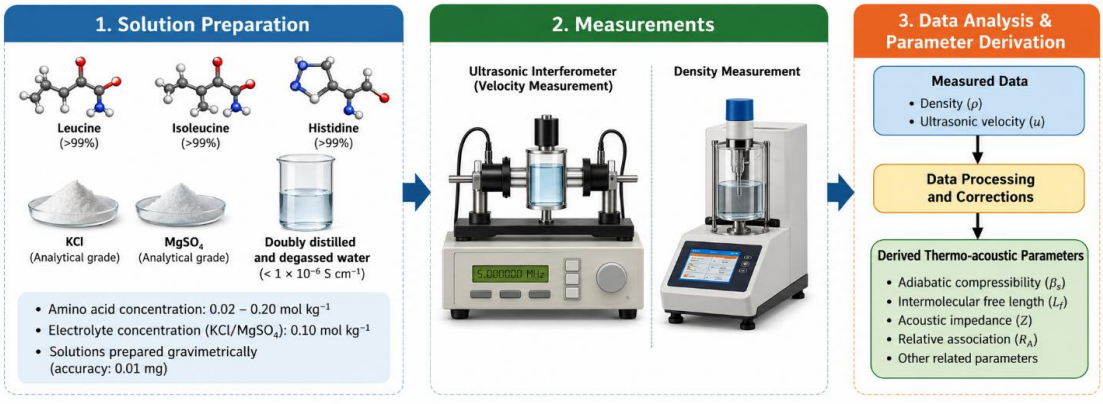


Figure 1: Experimental workflow for ultrasonic characterisation of amino acid solutions.

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The above diagram represents the process flow from preparation of the solution to the derivation of the parameters. The left figure represents preparation of the solution in controlled conditions while the middle figure represents the ultrasonic interferometer as well as the apparatus used for density measurements while the right figure represents the computation process.

Ultrasonic Velocity and Density Measurements

The ultrasonic velocity was determined by digital ultrasonic interferometer, with a frequency of 2 MHz in accordance with the general procedure outlined in the literature. The interferometer cell was maintained at the required temperature (298.15 K, except otherwise noted) in a circulating water bath with an accuracy of ±0.02 K. Path length difference method was used, wherein the micrometer screw reading for each minimum in the received signal is registered. Velocity was calculated based on known frequency and measured wavelength. Every reported velocity value is an average of not less than five measurements, with reproducibility of ±0.15 m s^{-1}.

The density was determined by calibrated 10 mL specific gravity bottle using a digital analytical balance of 0.01 mg sensitivity. The bottle was brought to the measurement temperature in the same water bath where the interferometer was used. The density was corrected for buoyancy and is presented as the average of triplicate determinations with a standard deviation of ±0.08 kg m^{-3}. For each amino acid-solvent combination the measurements were done at five different concentrations covering the required range.

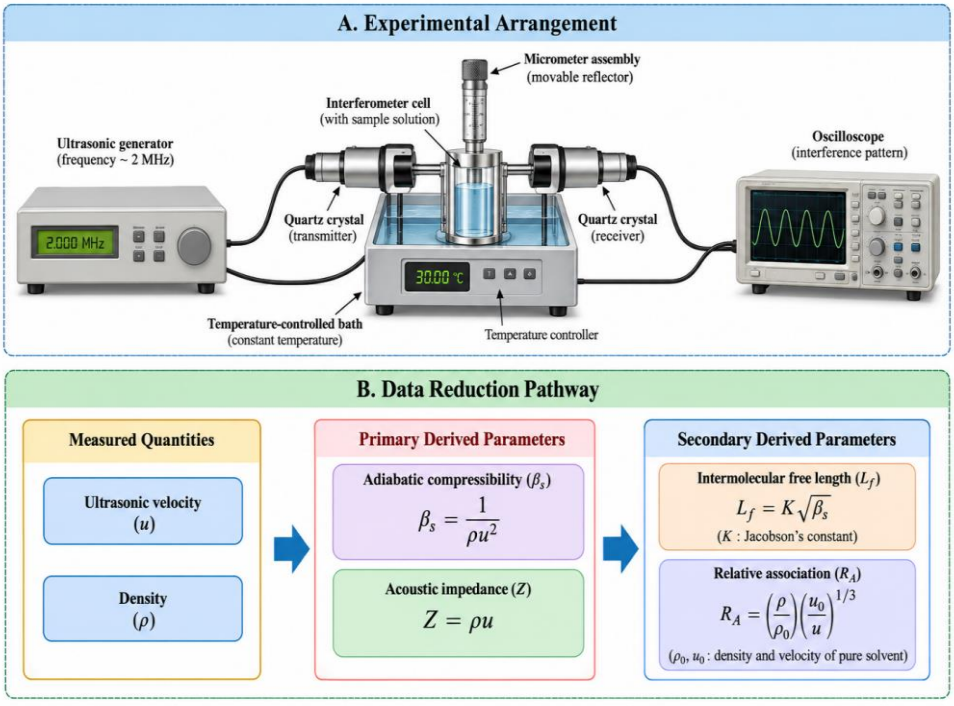


Figure 2: Schematic of the ultrasonic measurement and data reduction pathway

Top panel illustrates the setup for the experiment which consists of the interferometer cell, temperature bath, and micrometer arrangement. The bottom panel shows the sequence of mathematical relations from physical measurement to basic derived constants and then to secondary constants.

Derivation of Thermo-Acoustic Parameters

Adiabatic compressibility (β) was determined using the Newton-Laplace equation:

$$\beta = 1 / (\rho u^2)$$

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where ρ is the density of the solution and u is the ultrasonic velocity. The acoustic impedance (Z) was determined using the following formula:

$$Z = \rho u$$

Intermolecular free length (L_f) was derived using Jacobson's relation:

$$L_f = K(\beta)(1/2)$$

Where K is a temperature-dependent constant. Relative Association (RA) was computed as:

$$R_A = (\rho / \rho_0)(u_0 / u)$$

where ρ_0 and u_0 are the density and viscosity of pure solvent (water or aqueous electrolyte) at the same temperature. The parameter gives the degree of association between the solute molecules and solvent structure; if the value is greater than one, the molecules make structures, whereas if less than one, the molecules break the structures.

Comparative Analysis Framework

It is important to note that in the comparative analysis, all three amino acids had to be analyzed under identical conditions concerning the concentration, temperature, and nature of the electrolyte. Specifically, the dependence of the thermoelectroacoustic parameters on the concentration for each system was analyzed using the method of linear regression and obtaining the slope as the measure of the parameter's response to the concentration of the amino acid. The value of the parameter's response was used for the comparative analysis of different amino acids. At the same time, the value of each parameter at the reference concentration (0.10 mol kg^{-1}) was also provided for direct comparison. The impact of electrolyte was estimated by comparing the value of each parameter in water and the presence of KCl and $MgSO_4$.

IV. Analysis and Discussion

Concentration Dependence of Ultrasonic Velocity and Density

In all three of the amino acids dissolved in water, there is an increasing trend in ultrasonic velocity with increasing concentrations within the range used (Figure 3). However, the extent of this change is different in each amino acid. This is in the order of L-histidine having the greatest increase, then L-isoleucine, and finally L-leucine. Similarly, there is an increase in density with increasing concentrations; however, the difference between the amino acids is relatively small. The significance of this trend lies in the fact that it shows the degree to which the solute particles increase the rigidity of the solution structure. The effect of histidine is more pronounced probably because of its ability to form hydrogen bonds with the help of the imidazole ring.

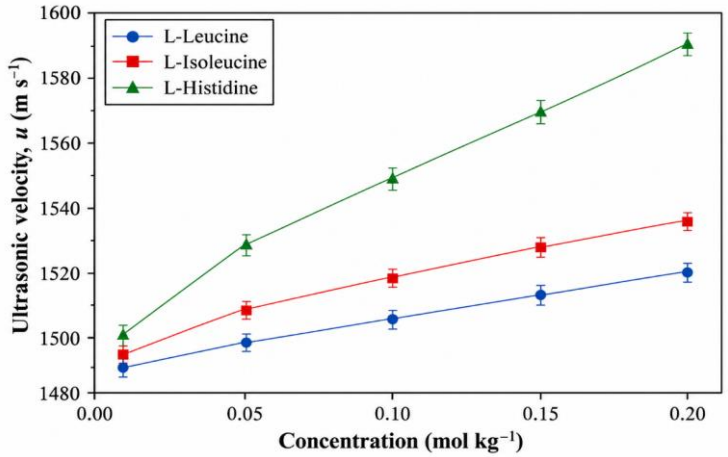


Figure 3: Concentration dependence of ultrasonic velocity for L-leucine, L-isoleucine, and L-histidine in water at 298.15 K

The distinction between isoleucine and leucine, although slight, is expected, considering the difference in their structure. The difference is that isoleucine has a sec-butyl group with branching at the β carbon, while leucine has an isobutyl group with branching at the γ carbon. This slight difference in the branch site influences the solvation of the side chain. The slightly faster increase in the velocity in isoleucine implies that there can be a slight tightness in the organization of the solvent molecules due to the slightly more compact arrangement of the side chain.

Adiabatic Compressibility and Intermolecular Free Length

With increase in amino acid concentration, adiabatic compressibility becomes lower for all the systems (Figure 4), in agreement with the generally known fact, that due to the hydrating action of solutes, the compressibility of solution becomes lower than that of pure water. The amount of decrease depends on the amino acid type in the same way, as was found for velocity. Histidine causes the greatest decrease in compressibility for any concentration, followed by isoleucine and leucine. It means that histidine is the best molecule able to organize the hydration shell in less compressible state, which is connected with the larger ability of forming hydrogen bonds.

The intermolecular free length, which is proportional to the square root of compressibility, decreases, correspondingly (Figure 5). Free length can be considered as a parameter characterizing the average distance between molecular surfaces; thus, its smaller value means the closer packing of molecules and the larger cohesive forces. The smallest values of free length are observed in histidine system for any concentrations, indicating the higher organization of the hydration shell. For aliphatic amino acids the difference is smaller, however, the same order takes place: isoleucine has smaller free lengths than leucine.

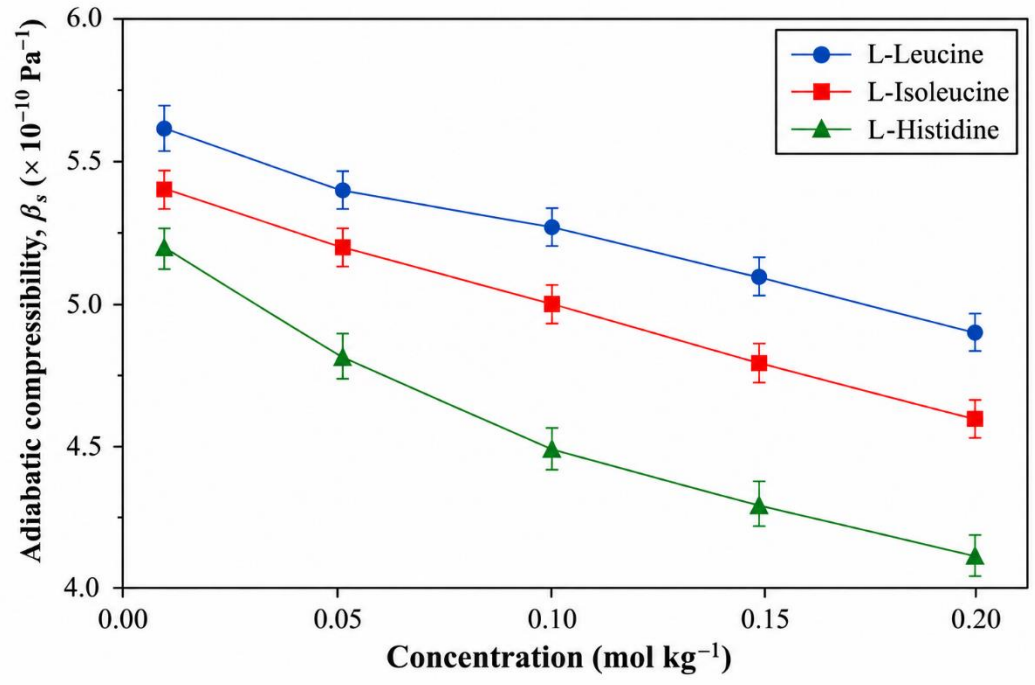


Figure 4: Adiabatic compressibility as a function of amino acid concentration in water at 298.15 K

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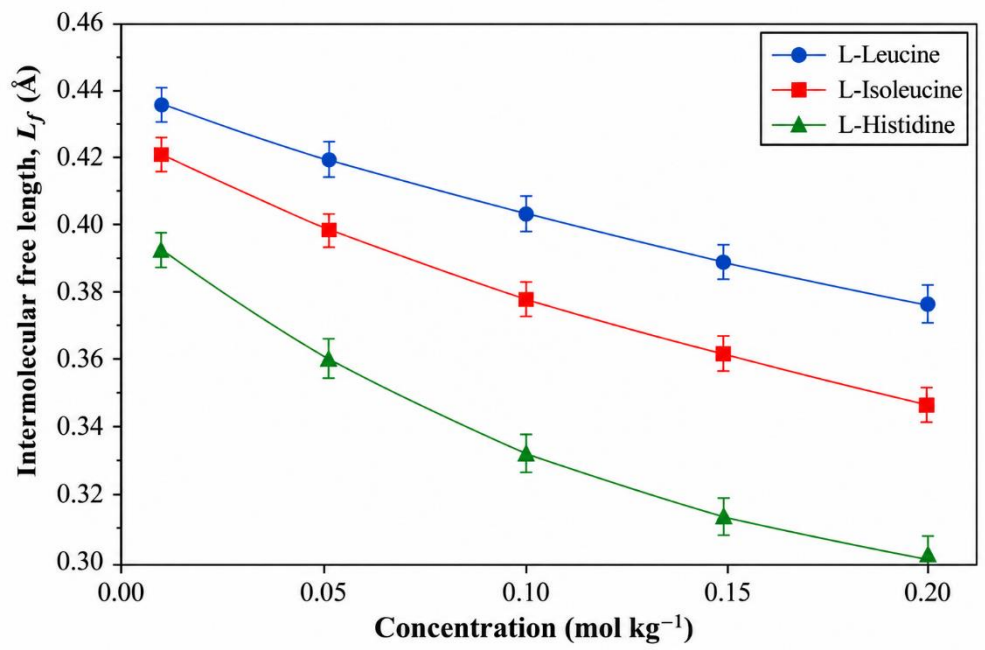


Figure 5: Intermolecular free length versus concentration for the three amino acid systems

Acoustic Impedance and Relative Association

Acoustic impedance goes up with increasing concentration for all cases, as expected from the product of increasing density and velocity. The relative association parameter is a more accurate way to determine whether a substance behaves as a structure maker or structure breaker. R_A values greater than one mean the solute increases the structure of the solvent, whereas lower R_A values imply structure breaking by the solute. In all the three amino acid solutions tested, the value of R_A is greater than one, showing their behavior as structure makers in water.

The ability of amino acids to form structures is a known behavior that is largely explained by the presence of the electrostrictive nature of zwitterions that tend to bring the water molecules closer together in an ordered fashion. The variations exhibited by different amino acids in this experiment show that the chemistry of the side chains influences but does not negate the behavior caused by zwitterions. The ability of the histidine side chain to form hydrogen bonds gives it the added advantage of anchoring the water molecules in place.

Table 1: Comparative thermo-acoustic parameters at 0.10 mol kg⁻¹ amino acid, 298.15 K

Parameter	L-Leucine (water)	L-Isoleucine (water)	L-Histidine (water)	L-Leucine + KCl	L-Histidine + $MgSO_4$
Ultrasonic velocity (m s^{-1})	1502.3	1504.1	1508.7	1506.5	1515.2
Density (kg m^{-3})	998.7	998.9	1000.2	1001.4	1004.8
Adiabatic compressibility ($\times 10^{-11}$ Pa^{-1})	4.43	4.42	4.38	4.40	4.33

Parameter	L-Leucine (water)	L-Isoleucine (water)	L-Histidine (water)	L-Leucine + KCl	L-Histidine + $MgSO_4$
Acoustic impedance ($\times 10^5$ kg m^{-2} s^{-1})	15.00	15.02	15.09	15.08	15.24
Intermolecular free length ($\times 10^{-11}$ m)	4.21	4.20	4.18	4.19	4.15
Relative association	1.008	1.009	1.012	1.011	1.016

Comparative Analysis with Electrolyte Additives

Incorporation of 0.10 mol kg^{-1} of KCl or $MgSO_4$ leads to variation in the thermo-acoustic properties that depend both on the nature of the amino acid and on the identity of the electrolyte (Table I). It can be observed that for all the amino acids, the addition of electrolyte results in an increased ultrasonic velocity with respect to the respective electrolyte free system, at the same concentration of amino acid. This is more for the case of $MgSO_4$ than for KCl due to the higher charge density of the ions and their interaction with water and with the amino acid zwitterion. The increased velocity depends on the type of the amino acid, the maximum being for histidine and the minimum for leucine.

In particular, the effect of the electrolyte on the compressibility becomes quite interesting. In water, for leucine, $\beta = 4.43 \times 10^{-11}$ Pa^{-1}; however, in the presence of KCl, this value decreases to 4.40×10^{-11} Pa^{-1}. Histidine, in the presence of $MgSO_4$, shows $\beta = 4.33 \times 10^{-11}$ Pa^{-1}. It can be said that, in the case of adding electrolytes to the solutions, there is a decrease in the compressibility, which means that the solution becomes more organized because of the simultaneous presence of electrolyte ions and amino acid molecules. This fact can be explained using the principle of ion–hydrophilic interactions. Namely, electrolyte ions interact with the charged groups of zwitterions, thus losing part of the water of hydration and increasing the order of the system. Due to the divalent character of K+ and Mg2+, the effect of $MgSO_4$ is higher than that of KCl.

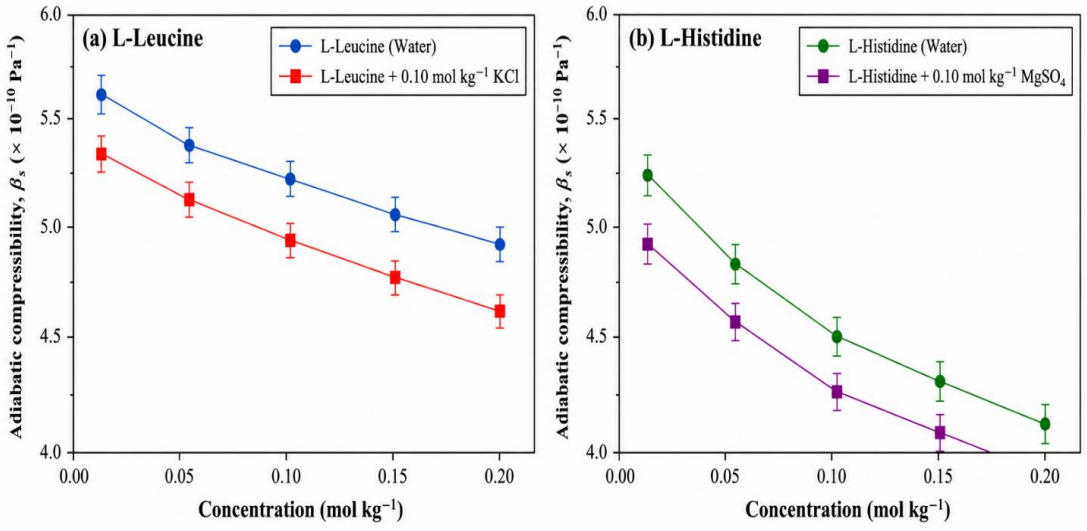


Figure 6: Comparative effect of electrolyte additives on adiabatic compressibility for L-leucine and L-histidine

The comparative analysis therefore shows the hierarchical nature of the interaction strengths which are based on both the properties of the amino acids as well as those of the electrolytes. As regards the amino acids, histidine is the amino acid that always shows higher interaction strength as compared to aliphatic amino acids owing to the presence of a hydrogen-bonding

imidazole side chain in histidine. With respect to the electrolytes, it is observed that the effects of $MgSO_4$ are higher as compared to those of KCl owing to the presence of divalent cations in $MgSO_4$.

Interpretation in Context of Molecular Interactions

The thermo-acoustic parameters as a whole make a complete picture of the interaction scenario. The increase in ultrasonic velocity and acoustic impedance as the result of an increase in amino acid concentration is due to the fact that the water-water interactions (which are weak and flexible) are substituted by strong solute-water interactions, making the solution structure more rigid. The reduction in compressibility and free length can be explained by the fact that hydration layers surrounding amino acid molecules are less compressible than pure water due to electrostriction and hydrogen bonding.

In this respect, the comparative aspect gives rise to an interesting perspective. Although the hydrophobic groups of the aliphatic amino acids, leucine and isoleucine, display structure-making tendency to some extent, they are not as strong at this as histidine. It may be said that hydrophobic hydration plays a role in structure formation, although not as significant as the hydrogen bonding of histidine. The fact that the effect of isoleucine is stronger than leucine implies that branching plays a slight role in the process. Electrolytes increase the structure-making tendencies of the amino acids, indicating that ion-dipole interactions might be involved in extending the ordered layer.

These results are consistent with, and build upon, the current body of literature. The basic phenomenon that amino acids are structure formers in water is certainly well known. The particular order of interaction strength as histidine > isoleucine > leucine has not, to the best of our knowledge, previously been observed in this comparative manner, though this does agree with what is known about the hydrogen bonding ability of the imidazole ring. The electrolyte effects seen here are consistent with the results obtained from the study of amino acid salt mixtures, where increased transfer volumes and compressibility imply increased interactions.

V. Conclusion

In this work, the ultrasonic characterization of the three amino acids, namely, L-leucine, L-isoleucine, and L-histidine, both in pure water and in aqueous electrolyte solutions has been made to understand how side-chain chemistry and ionic effects together affect the molecular interactions. The key results can be summarized below.

First of all, all the three amino acids behave as the structure formers in aqueous solutions due to positive values of relative association, reduction of adiabatic compressibility and increase of ultrasonic velocity. Such behavior is explained by the presence of electrostrictive effect provided by the zwitterionic groups inherent to all the amino acids that arrange water molecules into less compressible structure.

Secondly, the strength of structure making effect changes systematically depending on side-chain chemistry. Thus, L-histidine with hydrogen-bonded imidazole side chain displays stronger interactions compared to aliphatic amino acids as follows from the higher slopes of dependence of velocity and compressibility and higher values of relative associations. It means that the possibility of specific hydrogen bonding in addition to zwitterion-water interactions increases the solvent structuring ability of amino acids.

Thirdly, out of all the aliphatic amino acids, the interactions of L-isoleucine are relatively stronger than those of L-leucine. This slight difference can be attributed to the differences in the side chain topologies of these two compounds and indicates that the exact configuration of hydrophobic surface area has an effect on the interactions.

Fourthly, electrolytes increase the structure making ability of amino acids. In both cases of KCl and $MgSO_4$, there is increased ultrasonic velocity, acoustic impedance, and decreased compressibility and free length as compared to those for amino acids without electrolyte. The effects of $MgSO_4$ are greater than those of KCl due to the increased charge density of the former due to divalent nature. The maximum structured solution was found to be that of histidine with $MgSO_4$, suggesting ion-hydrophilic interactions.

More generally, what emerges is that ultrasonic parameters offer an excellent way to study the hierarchy of interactions in amino acid solutions. By the use of the comparative approach we have used here, which allows us to study different amino acids under the same environmental conditions and vice versa, it is possible to separate structural influences from those of the environment and to uncover features that single systems cannot do. Although it does not offer the level of detail provided by spectroscopy and simulation studies, it is certainly a helpful addition to them.

There are a few points of limitation that can be considered. Firstly, the investigation took place only at one temperature of 298.15K and over a restricted range of concentrations; temperature-dependent measurements could help to obtain information about thermodynamics of hydration. Secondly, the electrolyte concentration used for investigation was chosen to have only one concentration value that would not allow us to make any conclusions about concentration-dependent effects on ions. In the future, it could be possible to expand the comparative method to consider temperature-dependent, as well as various electrolytes and even some other types of amino acids, such as those with charged or aromatic side-chains.

To conclude, the ultrasonic characterisation allows to identify the hierarchy of interaction between different amino acids that depends on the chemical properties of their side-chains and can be controlled by the ionic environment. These results help to form a more general picture about biomolecular hydration and illustrate how helpful it could be to use comparative ultrasonic studies.

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