Chapter I

Introduction

Biomolecules are molecules that occur naturally in living organisms. Biomolecules include macromolecules like proteins, carbohydrates, lipids and nucleic acids. It also includes small molecules like primary and secondary metabolites and natural products. Biomolecules, like proteins, are very large molecules, and besides water proteins form the largest portion of our body weight. In order to understand the role played by the biological molecules in the living organism, it is necessary to study the interactions of proteins with its surrounding environment. These interactions are mainly between the protein molecules and the solvent. Most of these interactions, such as, hydrogen bonding and electrostatic interactions have non-covalent nature. The studies of these interactions provide important insight into the conformational stability and folding/unfolding of globular proteins. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Upon unfolding, the interactions between protein groups within the core are disrupted and replaced with the interactions of these groups with the solvent, thus leading to change in protein solvation. The solubility and stability of proteins have generated a great interest for a long time. However, due to complications involved in dealing with these complex molecules, various low molecular weight model compounds are generally taken for investigations. Therefore, the physicochemical properties of amino acids, peptides and their derivatives which mimic some specific aspects of protein structure in aqueous solutions have been extensively studied in order to gain a better understanding of solute-solvent interactions and their role in the conformational stability of proteins. This chapter discusses aspects of carbohydrates, vitamins, drugs, etc. along with review of literature at length.

Chapter II

Experimental details

The various experimental techniques used to carry out the present study are explained in this chapter. In addition, it includes materials used in different systems, procedure for solution preparation, and theoretical background of physico-chemical properties (thermodynamics and transport properties). The densities of solutions have been measured with a vibrating-tube digital density meter (Model: DMA 5000M, Anton Paar, Austria). The speed of sound in the solutions have been measured using a single
crystal variable path multi frequency ultrasonic interferometer (Model: M-82S, Mittal Enterprises, India) having stainless steel sample cell (with digital micrometer) operating at a frequency of 4 MHz. A suspended level Ubbelohde’s viscometer is used for the measurement of viscosity. For speed of sound and viscosity results, the temperature of the sample solution was maintained to an accuracy of ±0.02 K using an electronic controlled thermostatic water bath (Model: TIC-4000N, Thermotech, India). The solutions were prepared by weighing on an electronic single pan four digit Mettler Toledo Balance (Model ML204) with an accuracy of ±0.1 mg. The densities, speeds of sound and viscosities have been measured as a function of different concentrations and temperature for some biomolecules and drugs. This experimental data of density, speed of sound and viscosity have been used to evaluate volumetric, acoustic (ultrasonic) and viscometric parameters to arrive at logical conclusion.

Chapter III
Solvation behaviour of some amino acids (L-serine, L-threonine and L-leucine) in water and in aqueous solution of an antibiotic drug (streptomycin sulfate) at different temperatures: volumetric, acoustic and viscometric approach

The experimental values of densities of L-serine, L-threonine and L-leucine in water and in (0.01 and 0.02) mol kg⁻¹ aqueous solutions of streptomycin sulfate, at T = (305.15, 310.15 and 315.15) K have been measured in this chapter. The apparent molar volume, \( V_\phi \), limiting apparent molar volume, \( V_0^{\phi} \), the slope, \( S_v \), partial molar volume of transfer, \( \Delta_v V_0^{\phi} \), partial molar expansivity, \( E_0^{\phi} \), and Hepler’s constant, \( (\partial^2 V_0^{\phi} / \partial T^2)_P \), have been calculated from density data. The apparent molar isentropic compression, \( K_{\phi,s} \), limiting apparent molar isentropic compression, \( K_0^{\phi,s} \), its slope, \( S_k \), and partial molar isentropic compression of transfer, \( \Delta_v K_0^{\phi,s} \), have been calculated from speed of sound data. The compression data is also used for calculating the number of water molecules hydrated, \( n_H \), to the amino acids. The viscosity data has been used to determine relative viscosity, \( \eta_r \), viscosity B-coefficients, temperature derivative of B-coefficients, \( dB/dT \), viscosity B-coefficients of transfer, \( \Delta_v B \), and activation parameters of viscous flow. Solvation number, \( S_n \), of amino acids has also been computed.

From the values of \( V_\phi \), it has been observed that the values for L-serine, L-threonine and L-leucine in water and in aqueous solutions of streptomycin sulfate increase with increase in temperature as well as increase in molality of amino acids. The linear
square fit of $V_\phi$ against molality, $m_A$, gives the values of $V_\phi^0$ and $S_\nu$. The values of $V_\phi^0$ are positive and greater than the values of $S_\nu$ indicating the presence of solute-solvent interactions. The values of partial molar volume of transfer ($\Delta_\nu V_\phi^0$) are found to be positive for all these amino acids which indicates that the ion-hydrophilic interactions between zwitterionic groups of amino acids and polar groups of streptomycin sulfate, and hydrophilic-hydrophilic interactions between polar groups of amino acids and polar groups of streptomycin sulfate, dominates over ion-hydrophobic and hydrophobic-hydrophobic interactions. The positive values of $E_\phi^0$ indicate the presence of solute-solvent interactions. The values of Hepler’s constant, $(\partial E_\phi^0 / \partial T)_p$, are found to be positive for L-serine and L-threonine which indicates that both these amino acids acts as structure maker while for L-leucine, the values of $(\partial E_\phi^0 / \partial T)_p$ are negative which indicates that it acts as structure breaker in aqueous streptomycin sulfate solutions.

In case of acoustic properties, the comparison of speed of sound of L-serine, L-threonine and L-leucine in aqueous medium at $T = (305.15, 310.15$ and $315.15)$ K are found to be in good agreement with the available literature values. The values of $K_{\phi,s}$ are found to be negative at all temperatures and concentrations of streptomycin sulfate, the magnitude of which decreases with increase in concentration of drug and temperature. The positive values of $\Delta_\nu K_{\phi,s}^0$ indicate the dominance of the charged end groups NH$_3^+$ and COO$^-$. The interactions between the streptomycin sulfate and the zwitterionic center of amino acids increase with increasing drug concentration due to decrease in electrostriction. The values of hydration number, $n_H$, calculated from compression data decrease with the increase in temperature for all amino acids under study, which again indicates an increase in solute-co-solute interactions which reduces the electrostriction effect of amino acids.

From viscometric data, it has been observed that the values of $B$-coefficients which have been calculated by least-squares fitting of relative viscosity against molarity are positive for these amino acids and increase with increase in concentration of solvent, implying the existence of strong solute-solvent interactions in presence of streptomycin sulfate. The $dB/dT$ values are negative for all these amino acids in water as well as in aqueous solutions of streptomycin sulfate indicate its structure-making behavior. The solvation number i.e., $B/V_\phi^0$ ratio lying in between (0 and 2.5) depicts unsolvated solutes, while a higher value indicates solvated solutes with primary
solvation sphere. In present case, the values are greater than 2.5 for all these amino acids which suggest that these solutes are solvated and the solvation increases with increase in concentration of streptomycin sulfate. Based upon transition state theory proposed by Feakins et al., the activation Gibbs free energy has been related to viscosity $B$-coefficients which gives rise to activation Gibbs free energy for viscous flow per mole of the solute, $\Delta \mu^0_2 \phi$, and per mole of the solvent, $\Delta \mu^0_1 \phi$. It has been found that the values of $\Delta \mu^0_2 \phi$ are large and positive than those of $\Delta \mu^0_1 \phi$ reflecting that interactions between studied amino acids and aqueous streptomycin sulfate solutions in the ground state are stronger than in the transition state. Thus, the solvation of the solutes in the transition state is less favored in terms of free energy.

**Chapter IV**

**Effect of saccharides (glucose/lactose) on the solution thermodynamics of a vitamin (thiamine hydrochloride) in aqueous solution at different temperatures**

In this chapter, the experimental values of densities, speeds of sound and viscosities of thiamine-HCl having molalities of (0.02, 0.04, 0.06, 0.08 and 0.10) mol kg$^{-1}$ in water and in (0.1, 0.2 and 0.3) mol kg$^{-1}$ aqueous solutions of glucose/lactose, at $T = (293.15, 298.15, 303.15, 308.15$ and $313.15)$ K have been measured. The comparison of experimental densities of thiamine-HCl in aqueous medium with literature data at $T = (298.15$ and $308.15)$ K shows that the values are in good agreement with the available literature values. The apparent molar volume calculated by using density data increase with increase in molality as well as with increase in temperature. The values of $\Delta n V^0_\phi$ are positive which indicates that the ion-hydrophilic interactions between ionic (-NH$_3^+/-N^+/Cl^-$) groups of thiamine-HCl and polar (-OH/-C=O/-O-) groups of glucose/lactose, and hydrophilic-hydrophilic interactions between polar (-N/-S/-OH) groups of thiamine-HCl and polar (-OH/-C=O/-O-) groups of glucose/lactose, dominate over ion-hydrophobic and hydrophobic-hydrophobic interactions.

Furthermore, the $\Delta n V^0_\phi$ values of thiamine-HCl in lactose solution are larger than corresponding values for glucose solution. The explanation lies in the fact that the glucose is monosaccharide whereas lactose is disaccharide. Due to the presence of two monosaccharide units (glucose and galactose) in lactose, it contains larger number of OH groups than in glucose solution which leads to the stronger ion-hydrophilic and hydrophilic-hydrophilic interactions of thiamine-HCl in aqueous...
lactose solution. Such interactions of thiamine-HCl in aqueous lactose solution cause the further release of the electrostrictive water molecules into the bulk water resulting in higher $\Delta \nu V_{0}^{\nu}$ values. The values of $E_{0}^{\nu}$ are positive which are a useful measure of solute-solvent interactions in the solution. The positive values of $(\partial E_{0}^{\nu} / \partial T)_{P}$ in the present study indicate that thiamine-HCl act as structure maker in aqueous solutions of both glucose and lactose.

From the measurement of acoustic parameters, it has been found that the $K_{0}^{\phi,s}$ values for thiamine-HCl in water and in presence of glucose/lactose are negative which increases with increasing concentration of glucose/lactose as well as temperature. The values of $\Delta \nu K_{0}^{\phi,s}$ are positive and increase with increase in concentration of glucose/lactose which confirms that interactions between ions of thiamine-HCl and polar OH groups of glucose/lactose increase due to structure-making tendency of the ions and decrease in electrostriction. As a result, the electrostricted water is much less compressible than bulk water giving rise to a large decrease in the compressibility with increase in the glucose/lactose concentration. The positive values of $V_{AB}$ and $K_{AB}$ predict the pair wise interaction between thiamine-HCl and glucose/lactose.

From viscosity data, the viscosity $B$-coefficients calculated by linear fit of relative viscosity against molarity are found to positive and increase with increase in molality ($m_{B}$) of glucose/lactose at all temperatures studied implying the existence of strong solute-solvent interactions in presence of co-solute. The viscosity $B$-coefficients of transfer increase with increase in concentration of glucose/lactose and is larger for thiamine-HCl in lactose than glucose. The values of solvation number are found to be greater than 2.5 which suggest that thiamine-HCl or its constituent ions remain solvated with primary solvation spheres in solution investigated here, and higher temperature favours solvation of thiamine-HCl or its constituent ions as already discussed on the basis of $V_{0}^{\nu}$ values. The values of $\Delta \mu_{2}^{0}$ are large and positive than those of $\Delta \mu_{1}^{0}$ reflecting that interactions between thiamine-HCl and aqueous glucose/lactose solutions in the ground state are stronger than in the transition state. Thus, the solvation of the solutes in the transition state is less favored in terms of free energy. The positive values of $\Delta \mu_{2}^{0}$ are also indicative of structure promoter/maker behavior of thiamine-HCl in aqueous glucose/lactose solutions.
Chapter V

Intermolecular interactions of amino acids (L-serine and L-valine) with the vitamin (thiamine-HCl) in aqueous solution analyzed by volumetric, acoustic and viscometric methods at different temperatures

This chapter deals with the study of volumetric, acoustic and viscometric properties of L-serine and L-valine having molalities (0.02, 0.04, 0.06, 0.08 and 0.10) mol kg\(^{-1}\) in water and in (0.05, 0.10 and 0.15) mol kg\(^{-1}\) aqueous solutions of vitamin namely thiamine-HCl at different \(T = (293.15, 298.15, 303.15, 308.15 \text{ and } 313.15) \text{ K}\). The experimental data of density has been used for calculation of apparent molar volume which increase with an increase in molality of amino acid as well as increase in thiamine-HCl concentration and temperature. The \(V^0_\phi\) values are found to be positive, and greater than the values of \(S_v\), which increase with an increase in the molality of thiamine-HCl, as well as with increase in temperature. The increase of temperature weakens the binding of the solvent molecules from the terminal zwitterions of amino acids, releasing solvent molecules into the bulk and accordingly leading to an expansion of volume. Further, at each temperature, the \(V^0_\phi\) values increase with size of alkyl group, i.e., increase in chain length of alkyl part from L-serine to L-valine.

The positive values of \(\Delta_\phi V^0_\phi\) for both L-serine and L-valine indicates that ion-ion interactions between zwitterionic groups (NH\(^3+\), COO\(^-\)) of amino acids and the ionic (-NH\(^3+\)/N\(^+\)/Cl\(^-\)) groups of thiamine-HCl molecule and ion-hydrophilic interactions between zwitterionic groups (NH\(^3+\), COO\(^-\)) of amino acids and polar groups (-N/-S/-OH) of thiamine-HCl dominate over ion-hydrophobic and hydrophobic-hydrophobic interactions. In addition, these transfer values are larger for L-serine as compared to L-valine (L-serine > L-valine) due to the presence of additional hydrophilic-hydrophilic interactions between polar groups (-OH) of amino acids and polar groups (-N/-S/-OH) of thiamine-HCl. The positive increase in \(E^0_\phi\) values means that amino acids occupy the interstitial space in solution resulting in structure making.

The values of \((\partial E^0_\phi / \partial T)\)\(_P\) for both L-serine and L-valine are positive indicating that both these amino acids behaves as structure maker in presence of thiamine-HCl. The coefficient of thermal expansion, \(\alpha\), are highest in case of L-serine as compared to that of L-valine which suggest that water around L-serine is loosely bound which is responsible for the higher value of expansivity.
From compression data, the plots of apparent molar isentropic compression, $K_{\phi,s}$, versus molality, $m_A$, for L-serine/L-valine in water and in different concentrations of thiamine-HCl at different temperatures indicates that the values of $K_{\phi,s}$ are negative at all temperatures and concentrations of thiamine-HCl. Furthermore, the magnitude of negative values of $K_{\phi,s}^0$ for both these amino acids become less negative with increasing concentration of thiamine-HCl. On addition of thiamine-HCl, the electrostriction interactions between these amino acids and water molecules are suppressed due to increasing interactions between them. As a result, the electrostricted water is released from the solvation sphere. This results in higher $K_{\phi,s}^0$ values for both these amino acids in aqueous solution of thiamine-HCl compared to their values in water. The negative values of $S_k$ suggest the solvation of ions due to weak ion-ion interactions, which is also evident from our $S_v$ data. The $\Delta n K_{\phi,s}^0$ values are positive for both the L-serine/L-valine with different concentrations of thiamine-HCl. The values of $n_H$ calculated from both volumetric and compression methods indicates that the hydration number of the studied amino acids in the presence of thiamine-HCl is less than those in water. This establishes the fact that thiamine-HCl has a dehydration effect on both L-serine and L-valine, i.e., water molecules is replaced by thiamine-HCl molecules with increasing concentration of thiamine-HCl in solution. From volumetric and compression pair interaction coefficients, $V_{AB}$ and $K_{AB}$, are positive for both L-serine and L-valine in thiamine-HCl solutions. This signifies that interactions between amino acids and thiamine-HCl are mainly pairwise. An overall analysis of pair and triplet interaction coefficients in the present study reveals that pairwise interactions are dominating in (L-serine/L-valine + thiamine-HCl + water) solution.

From viscometric data, a representative 3D-plot of viscosity with molality as a function of temperature for L-serine in 0.10 mol kg$^{-1}$ aqueous thiamine-HCl solutions indicates that the viscosity increases with increase in molality of solute but decrease with increase in temperature. Similar trend has been found for L-valine. As the viscosity is largely associated with its molar mass, therefore, the solute having a larger molar mass will tend to move more slowly, thereby, leading to a greater value of viscosity (L-valine > L-serine).

The variation of relative viscosity, $\eta_r$, with molarity, $C$, for both these amino acids in aqueous thiamine-HCl solutions shows that the $\eta_r$ increase with increase in molality of
amino acids but decrease with increase in temperature. Also, the viscosity $B$-coefficients are found to be positive for these amino acids and increase with increase in concentration of solvent, indicates that L-serine/L-valine-thiamine-HCl-water interactions are dominating over L-serine-L-serine/L-valine-L-valine interactions and also the structure making tendency of the these amino acids with thiamine-HCl. Furthermore, according to Feakin’s model, the greater the value of $\Delta \mu_2^0$, the greater is the structure-promoting tendency of a solute and positive $\Delta \mu_2^0$ values for both the L-serine and L-valine in the studied thiamine-HCl solutions suggest it to be a net structure promoter/maker. This is also supported by the conclusion drawn from volumetric studies discussed above as well as shows an agreement with the results of the $dB/dT$ trend.

**Chapter VI**

**Volumetric, acoustic and viscometric properties of L-aspartic acid in water and aqueous solution of 1,2-propanediol at different temperatures**

In this chapter, experimental values of density, speed of sound and viscosity of L-aspartic acid in water and in (5%, 10%, 15% and 20%) aqueous solution of 1,2-propanediol (PD) at $T = (298.15, 303.15, and 308.15)$ K have been measured. These experimental values have been used for calculation of different volumetric, acoustic and viscometric parameters. From volumetric data, the apparent molar volume has been calculated and the variation of apparent molar volume with molality of L-aspartic acid for all composition of PD shows that the apparent molar volume increase with increase in PD concentration as well as temperature. The $V^0_\phi$ increase with increase in temperature which may be attributed to the release of water molecules from the secondary solvation layers of L-aspartic acid into the bulk of the solvent, resulting into the expansion of solution, as inferred from larger values of $V^0_\phi$ at higher temperatures. The values of $\Delta n V^0_\phi$, are positive which indicates that ion-hydrophilic interactions between zwitterionic groups ($\text{NH}_3^+$, $\text{COO}^-$) of L-aspartic acid and -OH groups of PD, and hydrophilic-hydrophilic interactions between -CH$_2$COOH group of L-aspartic acid and -OH groups of PD, dominate over hydrophilic-hydrophobic, ion-hydrophobic and hydrophobic-hydrophobic interactions. The positive values of $E^0_\phi$ reveals that the hydration shell of the amino acid increases in volume with a change of the solvent (PD + water) due to increase in the ion-hydrophilic and hydrophilic-
hydrophilic interactions between L-aspartic acid and PD molecules. The values of $(\partial E^0/\partial T)_p$ are found to be negative for lower concentrations of PD and positive for higher concentration of PD which indicates that the structure making behavior of L-aspartic acid increase with increasing concentrations of PD.

From speed of sound measurement, the variation of $K_{\phi,s}$ with molality of L-aspartic acid are linear for all concentrations of PD (0.00 to 20% PD) which are found to be negative at all temperatures and concentrations of PD, the magnitude of which decreases with increase in concentration of PD and temperature. The dependence of $K^0_{\phi,s}$ on mass % of PD shows that on the addition of PD, the electrostriction interactions between L-aspartic acid and water molecules are suppressed due to increasing interactions between L-aspartic acid and PD. The values of $\Delta_\mu K^0_{\phi,s}$ are positive and increase with increase in concentration of PD. This phenomenon can be explained by the co-sphere overlap model developed by Friedman. The values of $n_H$ calculated from both volumetric and compression methods for L-aspartic acid shows that the values for L-aspartic acid are less in presence of PD as compared to their values in water and their magnitude is lowest in higher concentration region of PD. This indicates the increase in solute-co-solute interactions. The values of volumetric and compression pair interaction coefficients, $V_{AB}$ and $K_{AB}$, are positive for L-aspartic acid in presence of PD which shows that interactions between L-aspartic acid and PD are mainly pairwise.

Further, the 3D-plot of viscosity with molality as a function of temperature for L-aspartic acid in 10% aqueous solution of PD indicates that the viscosity increases with increase in molality of solute but decrease with increase in temperature. The plots of viscosity $B$-coefficients with mass % PD as well as temperatures indicates that the viscosity $B$-coefficients are positive for L-aspartic acid and increase with increase in concentration of PD, suggesting the presence of strong solute-solvent interactions in presence of PD. The values of $dB/dT$ are negative which indicate that this amino acid, i.e., L-aspartic acid acts as structure-maker in aqueous PD solvent. The viscosity $B$-coefficients of transfer, $\Delta_\mu B$, increase with increase in concentration of PD. The $B/V^0_\phi$ ratio lying in between (0 and 2.5) depicts unsolvated solutes, while a higher value indicates solvated solutes with primary solvation sphere. In present case, the value in between 3.21 to 5.13 suggests that L-aspartic acid or its constituent ions remain solvated with primary solvation spheres in solution.