Chapter 1

Introduction
1.1. INTRODUCTION

Solutions are by far much more common than pure substances. It is no wonder that since ancient times humans have been interested in the properties of solutions and how they are modified from those of the pure constituents. The scientific study of these matters dates from the early part of the 19th century and first period of discovery may be said to have culminated in the 1880s with the discovery of Raoult’s law.

The first quantitative law governing the properties of solutions was published by William Henry [1]. Henry was studying the solubility of gases in liquids and found that this solubility was proportional to the gas partial pressure. He didn’t express his results in an equation but published tables of data from which the proportionality could be extracted. An interested review of the current status of Henry’s law has been given by Rosenberg and Peticolas [2].

The next major step was the enunciation of Raoult’s law [3]. In 1887, Francois Raoult published his investigations on the vapour pressure of the solvent in dilute solutions. He studied five solutes in water and fourteen solutes in each of eleven organic solvents and found that the diminuation of vapour pressure of the solvent upon addition of a given (small) amount of solute was proportionally the same for all the cases. The proportionality factor was the mole fraction of the solute. Raoult had previously discovered the laws of freezing point depression and the boiling point elevation, three of so-called colligative properties of dilute solutions.

Another property of solutions, the osmotic pressure was studied by Jacobs Van’t Hoff in 1887 [4]. Here, van’t Hoff was trying to understand the properties of liquids in terms of those of gases, which was considered fairly well understood. He seized on the phenomenon of osmotic pressure as the analog of the pressure of a gas, and derived the equation, \( \Pi = C_2 RT \). van’t Hoff also showed thermodynamically, the relations between the colligative properties found by Raoult and the osmotic pressure, namely, that they were all the alternative ways of counting molecules.

Not long after these developments, the subject of statistical mechanics had began to be developed. Statistical mechanics had brilliant success in the calculation of the properties of gases, especially after the advent of Quantum theory permitted and
the proper description of the internal states of the molecules, but its applications to the condensed phases was less successful. The theory at that time was based on the cell model of liquids which overestimates the correlation between the molecular positions [5]. Later the emphasis of many theories began to fall on the properties and usefulness of molecular distribution functions, in particular the pair correlation functions. This was due, in part, to the thesis of Jan de Boer. De Boer’s work was for pure fluids, not solutions and other authors, in particular, John G. Kirkwood [6] also developed the correlation function method. This work on correlation functions, when generalized to mixtures, led to two equivalent although superficially different, formally exact theories of solutions, due to Mayer and McMillan [7] and Kirkwood and Buff [8].

The Kirkwood-Buff (KB) theory was considered the most important theory of solutions. It was published in 1951. In the original paper, Kirkwood and Buff derived some new relationships between thermodynamic quantities and molecular distribution functions for multicomponent systems. As such, the KB theory is the most general and most powerful theory of solutions. In essence, it provides a direct relationship between thermodynamic properties such as compressibility, partial molar volumes and derivatives of the chemical potentials, in terms of so-called KB integrals. Thus, the theory may be used to compute the thermodynamic quantities based on the knowledge of the pair correlation function. Unfortunately, almost nothing was known at that time on the pair correlation functions in any mixture. Therefore, the KB theory, though general and potentially powerful, remained practically dormant for many years. For almost 20 years, there were only a few studies on KB theory. The first turning point occurred in the beginning of 1972 when the Kirkwood-Buff theory was found useful in interpreting some properties of water and aqueous-solutions. The main idea was to apply the KB theory of solutions, to pure one-component systems viewed as a mixture of various quasi-component systems. A more dramatic turning point for the Kirkwood-Buff theory occurred in 1978 after the publication of the inversion of the Kirkwood-Buff theory.

In the most general sense thermodynamics is the study of energy, its transformations and its relationship to the properties of matter. The thermodynamic properties of solutions have two major objectives. One of these is to describe the properties of component molecules when it exists in what is called an equilibrium
state, a condition in which its properties shows no tendency to change. The other objective is to describe processes in which the properties of matter undergo changes and to relate these changes to the energy transfers in the form of interactions, which accompany them [9].

Equilibrium chemical thermodynamics aims to establish exact relationships linking theoretically interesting properties to experimentally determinable quantities. In the last quarter of the 20th century, significant advances in instrumentation, particularly availability of fast computers and computer attached instruments, led to commercially available apparatus for accurate determination of thermodynamic properties. Modern techniques provide the means for probing fine details of structural interactions in chemically interesting systems [10].

The calculation of the physicochemical properties of liquids was found to depend on the interactions between the molecules involved in the solutions. These interactions influence the bulk properties of the solutions [11]. A deeper knowledge of the solvent at a molecular level is essential for the understanding of many chemical and biological processes in solution. The investigation on physicochemical properties of aqueous and non-aqueous solutions have been found to provide useful information about the physical nature and strength of intermolecular interactions in these solutions [12-14].

The thermophysical characterization of liquids and liquid solutions, i.e., the knowledge of a complete set of properties over wide temperature and pressure ranges is significant for many purposes. The mixing or separation of substances is very common in chemical production and scientific research [15,16]. The volume change on addition of solute to solvent, at constant pressure is one of the most interesting, yet certainly still one of the least understood. It was Hildebrand and Scott [17] who have emphasized its importance in 1962. This renewed interest is closely related to the advances in the theories of solutions which were made in 1960’s. The subject will continue to be of interest in future simply because the experiments are relatively easy to perform with great precision and because the volume change is a sensitive indicator to the accuracy of theories of solution [18].
There are interesting challenges for chemists, physicists, engineers, biotechnologists and many other researchers working in different fields. Our studies are directed to investigate aqueous solution systems by thermodynamical properties, which provide useful, indications about specific intermolecular interactions. An investigation in the liquid state poses a fascinating and challenging problem to both theoreticians and experimentalists. The molecules in the liquid cannot be studied either as free molecules in gases or as the structurally fixed crystalline arrangement of solids. Our knowledge of liquids is largely empirical, since liquid has solid like behaviour in certain aspects while it shows gaseous property in some other aspects. Liquid is said to have a short-range order and long-range disorder. A certain range of disorder gives liquid the characteristic property of fluidity [19]. The concept of cell theory of the liquid state [20,21] implies some amount of organisation of structure in it. Because of these, level interpretation based on experimentally observed properties instead of theoretical models. Many experimental techniques like Differential Scanning Calorimeter (D.S.C.), Neutron Magnetic Resonance (N.M.R.), Raman Spectroscopy, Infra-Red Spectroscopy, Ultra-Violet (U.V.) are used to get detailed information at molecular level in liquids. Measurements of some of the thermodynamic properties like density, viscosity, ultrasonic speed and refractive index of liquids provides an insight into investigating the intermolecular arrangement in liquids and help to understand the thermodynamic and acoustic properties of solutions. The ultrasonic speed and thermodynamic data derived from it have been widely used for this purpose. Measurement of sound velocity and derived parameters from it offer a convenient method for determining certain thermodynamic properties of liquids not easily obtained by other means. Thermodynamic methods are important because changes in properties caused by variations of temperature, composition and pressure can be studied without any reference to assumption models or hypothesis. The problem becomes more interesting when the interactions between like and unlike molecules in solutions are probed by these techniques. From these thermodynamic studies, the assessment of the molecular interaction between the components has been obtained [20,21]. Most of the physical thermodynamic and acoustic properties of mixtures have been theoretically derived by many workers [22-27] from those of the components by assuming the dissimilar molecules are non-interacting. This case
exists in mixtures where both the components are non-polar. In the binary mixture where one component is polar and the other is non-polar, interaction has been observed. Even if the interaction between polar and non-polar molecules is weak, there will be considerable change in the molecular environment. In such a case the physical and thermodynamic properties are likely to be affected by the intermolecular hydrogen-bonding, dipole-dipole, dipole-induce dipole and charge transfer interaction between the unlike molecules due to the variation in the relative concentration of the components [28,29].

This is a universal fact that proteins are essential constituents of all living organisms. Most tasks performed by living cells require proteins. The variety of functions that they perform is astonishing. They take part in various biological reactions. In animals, for example, proteins are the primary structural components of muscle, connective tissue, feathers, nails, and hair. In addition to serving as structural materials in all living organisms, proteins are involved in such diverse functions as metabolic regulation, transport, defense, and catalysis. The functional diversity exhibited by this class of biomolecules is directly related to the combinatorial possibilities of the monomeric units, the 20 amino acids. There are 20 natural amino acids which are the constituents of all proteins. Amino acids are the subunits of proteins. Each protein is formed by a chain of amino acids linked together through peptide bonds. The chain of amino acids takes on different shapes to form different proteins. The various shapes allow proteins to take on different characteristics in cells. Each amino acid is composed of a constant (always remain the same) group and a variable amine group as shown below (Fig. 1.1):

![Figure 1.1: General Structure of α-Amino Acids.](image)

Amino acids are amphoteric molecules; that is, they can act as either an acid or a base (Fig. 1.2).
This is due to an internal transfer of a hydrogen ion from the –COOH group to the –NH₂ group to leave an ion with both a negative charge and a positive charge. This is called a zwitter ion (Fig. 1.3).

Within a protein, multiple amino acids are linked together by peptide bonds (Fig. 1.4), which are formed by a biochemical reaction that extracts a water molecule as it joins the amino group of one amino acid to the carboxyl group of a neighboring amino acid.

There are 20 common amino acids that are responsible for forming proteins. They can be classified into 4 main families based on their chemical characteristics: acidic, basic, uncharged polar and non-polar.

Below is the list of 20 amino acids found in Proteins:
**Table 1.1:** The 20 Proteinogenic amino acids.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Structure</th>
<th>3-letter code</th>
<th>1-letter code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td><img src="image1" alt="Alanine structure" /></td>
<td>Ala</td>
<td>A</td>
</tr>
<tr>
<td>Arginine</td>
<td><img src="image2" alt="Arginine structure" /></td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
<td>Asparagine</td>
<td><img src="image3" alt="Asparagine structure" /></td>
<td>Asn</td>
<td>N</td>
</tr>
<tr>
<td>Aspartic acid (aspartate)</td>
<td><img src="image4" alt="Aspartate structure" /></td>
<td>Asp</td>
<td>D</td>
</tr>
<tr>
<td>Cysteine</td>
<td><img src="image5" alt="Cysteine structure" /></td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Glutamine</td>
<td><img src="image6" alt="Glutamine structure" /></td>
<td>Gln</td>
<td>Q</td>
</tr>
<tr>
<td>Glutamic acid (glutamate)</td>
<td><img src="image7" alt="Glutamate structure" /></td>
<td>Glu</td>
<td>E</td>
</tr>
<tr>
<td>Glycine</td>
<td><img src="image8" alt="Glycine structure" /></td>
<td>Gly</td>
<td>G</td>
</tr>
<tr>
<td>Histidine</td>
<td><img src="image9" alt="Histidine structure" /></td>
<td>His</td>
<td>H</td>
</tr>
<tr>
<td>Isoleucine</td>
<td><img src="image10" alt="Isoleucine structure" /></td>
<td>Ile</td>
<td>I</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Structure</td>
<td>3-letter code</td>
<td>1-letter code</td>
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</tr>
<tr>
<td>Leucine</td>
<td><img src="image" alt="Leucine structure" /></td>
<td>Leu</td>
<td>L</td>
</tr>
<tr>
<td>Lysine</td>
<td><img src="image" alt="Lysine structure" /></td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Methionine</td>
<td><img src="image" alt="Methionine structure" /></td>
<td>Met</td>
<td>M</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td><img src="image" alt="Phenylalanine structure" /></td>
<td>Phe</td>
<td>F</td>
</tr>
<tr>
<td>Proline</td>
<td><img src="image" alt="Proline structure" /></td>
<td>Pro</td>
<td>P</td>
</tr>
<tr>
<td>Serine</td>
<td><img src="image" alt="Serine structure" /></td>
<td>Ser</td>
<td>S</td>
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<tr>
<td>Threonine</td>
<td><img src="image" alt="Threonine structure" /></td>
<td>Thr</td>
<td>T</td>
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<tr>
<td>Tryptophan</td>
<td><img src="image" alt="Tryptophan structure" /></td>
<td>Trp</td>
<td>W</td>
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<tr>
<td>Tyrosine</td>
<td><img src="image" alt="Tyrosine structure" /></td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Valine</td>
<td><img src="image" alt="Valine structure" /></td>
<td>Val</td>
<td>V</td>
</tr>
</tbody>
</table>
During synthesis each polypeptide molecule bends in three-dimensional space as its amino acid components (called amino acid residues) interact with each other, largely through non-covalent interactions. Proteins and carbohydrates constitute an important class of biological molecules. The tendencies of proteins to decompose denature, and aggregate are critical problems in the biotechnology, food, cosmetics, and pharmaceutical industries. For a protein to be biologically active, it must have the correct structure at all levels.

Proteins consist of strands of amino acids folded into a specific shape. The different protein structures can be classified by four levels of folding, each successive one being constructed from the preceding one.

**Primary Structure** - The very basic strand of amino acids is the called the primary structure (Fig. 1.5).

![Figure 1.5: Primary Structure of proteins.](image)

**Secondary Structure** - The hydrogen-bond interaction among strands of amino acids gives rise to the first level of folding, alpha-helices and beta-sheets (Fig. 1.6).

![Figure 1.6: Secondary Structure of proteins.](image)

**Tertiary Structure** - Interaction between alpha-helices and beta-sheets comprise the second level of folding, protein domains. These protein domains are then strung together through third level folding to form small globular proteins. The combination of second and third level folding yields tertiary structure (Fig. 1.7).
**Quaternary Structure** - In order to achieve enhanced function, small globular proteins often come together to form protein aggregates. This fourth level of protein structure is called the quaternary structure (Fig. 1.8). A famous example of quaternary structure is hemoglobin.

The sequence of amino acids in the primary structure must be right. The secondary and tertiary structures are very important. Proteins act only when they retain their natural conformations, e.g., in enzymes the active site must have the right conformation. In primary structure, there are covalent bonds. But all other structures are maintained by weak solvation, non-covalent interactions or hydrogen bonding. Protein molecules may undergo disruption of their overall structure when subjected to heat or small changes in the environment. The chemical (oxidation, deamidation, and hydrolysis) and physical (unfolding and aggregation) changes of proteins during the formulation process, and storage not only reduce biological activity, but they can also cause adverse reactions [30-36]. In order to protect proteins against denaturation and aggregation, there have been efforts at various levels, which include chemical modification of proteins and controlling the protein environment using co-solvents. To perform their function all of these substances, generally known as co-solvents or osmolytes like sugars, amino acids, salts, polyols, and nucleic acids, must be used at relatively high concentrations.

The ability of carbohydrates to stabilize proteins has been attributed to the preferential hydration of protein that occurs when it is dissolved in a sugar solution.
This phenomenon is due to the fact that carbohydrates, in general, do not interact directly with the protein molecules, and are therefore called non-perturbing osmolytes. Among several osmolytes, sugars have been known to stabilize the protein conformation against chemical denaturation or reaction, thermal denaturation, and loss of their biological activity, which can be caused by an increase in temperature, a change in pH value and the addition of various chemicals [41]. Sugars belong to a class of osmolytes that in nature are synthesized to protect organisms against the stresses of high osmotic pressure and freezing, and sugar synthesis is a good example of a defensive reaction of many organisms. Sugars are commonly employed in freeze-drying formulations of therapeutic proteins to preserve their activity [42].

The properties and mechanism of the sugar bio-protective phenomenon are still under discussion. Several hypotheses have been proposed, but none of them can be considered as fully accepted. They mainly relate to the following properties: (i) sugar are able to replace more water molecules, stabilizing the 3D protein structure during dehydration, and create sugar-protein hydrogen bonds replacing the water protein hydrogen bonds [43], (ii) sugars destructure the hydrogen bond network of water molecules and prevent the formation of ice because sugars bind to a number of water molecules and therefore have a destructuring effect [44], (iii) sugars are co-solvents thus inducing greater thermodynamic stabilization (perhaps protect protein from conformational disorders) [45], and (iv) sugars possess a higher glass transition temperature, which results in a stronger decrease of the protein dynamical fluctuations [46]. Numerous experimental works and molecular dynamics simulations have been performed on the structure and dynamics of ternary protein, sugar and water solutions [47] and more generally the stability of lysozyme [48].

In order to better understand the physico-chemical properties of sugar, in the framework of the bio-protective solvent stability and to understand how globular proteins in their flexible native state at room temperature are influenced by solutes, we performed the study of effects of carbohydrates on proteins.

The other important class of osmolytes which are known to stabilise the native structure of proteins are drugs. The action of drugs on the human body is called pharmacodynamics, and what the body does with the drug is called pharmacokinetics. The drugs that enter the human tend to stimulate certain receptors, ion channels, act
on enzymes or transporter proteins. As a result, they cause the human body to react in a specific way. Once the receptors are activated, they either trigger a particular response directly on the body, or they trigger the release of hormones and/or other endogenous drugs in the body to stimulate a particular response. Drugs interact with receptors by bonding at specific binding sites. Most receptors are made up of proteins, and the drugs can therefore interact with the amino acids to change the conformation of the receptor proteins. These interactions are very basic, just like that of other chemical bondings:

Ionic bonds - Mainly occur through attractions between opposite charges; for example, between protonated amino (on salbutamol) or quaternary ammonium (e.g. acetylcholine), and the dissociated carboxylic acid group. Similarly, the dissociated carboxylic acid group on the drug can bind with amino groups on the receptor. This type of bond is very strong, and varies with the inverse of the distance between the atoms so that it can act over large distances. Cation-π interactions can also be classified as ionic bonding. This type of interaction occurs when a cation, e.g. acetylcholine, interacts with the negative π bonds on an aromatic group of the receptor. Ion-dipole and dipole-dipole bonds have similar interactions, but are more complicated and are weaker than ionic bonds.

Hydrogen bonds - There is a small but significant attraction between hydrogen atoms and polar functional groups (e.g. the hydroxyl [-OH] group). These so-called hydrogen bonds only act over short distances, and are dependent on the correct alignment between functional groups.

Receptors are located on all cells in the body. The same receptor can be located on different organs, and even on different types of tissues. There are also different subtypes of receptor which elicit different effects in response to the same agonist. For example, there are two types of histamine receptor: H1 and H2. Activation of the H1 subtype receptor causes contraction of smooth muscle, whereas activation of the H2 receptor stimulates gastric secretion. It is this phenomenon that gives rise to drug specificity. Of course, drugs do not only act on receptors: they also act on ion channels, enzymes, and cell transporter proteins.

In an attempt to understand the hydration of protein groups, the volumetric properties of short peptides, amino acids, alcohols, sugars, amino alkanes, carboxylic
Acids and other small molecules containing atomic groups identical to those in proteins have been under intensive scrutiny during the past several decades. The volumetric properties of solutions of amino acids, peptides and proteins have been investigated over sixty years. The standard partial molar volume equals the partial molar volume of a solute at infinite dilution; therefore, it is also called the limiting partial molar volume. At infinite dilution, the solute-solute interaction is negligible; therefore, the standard partial molar volume and its temperature-dependence provide valuable information of the solute-solvent interactions.

The stabilization of native conformations of biological macromolecules is commonly related to several non-covalent interactions including hydrogen bonding, electrostatic and hydrophobic interactions. These interactions are affected by the surrounding solutes and solvent of macromolecules, for this reason, the physico-chemical behaviours of proteins are strongly influenced by the presence of solutes. Because of direct solute-solvent interactions and/or alteration of the water structure, these solutes can change many properties of globular proteins such as their hydration, solubility and the activity of enzymes. However, due to complex conformational and configurational three-dimensional structures of proteins, direct investigations of the solute/solvent effect on these biological macromolecules are very challenging. On the other hand, the interpretation of behaviours of model compounds such as amino acids and peptides are quite helpful in understanding the water-protein interactions in solutions. Especially, viscometric and volumetric properties as well as changes in enthalpy and free energy in water and solutions of organic solvents or salts can provide valuable clues for comprehending the protein unfolding and the hydrophobic interactions of non-polar side chains. The study of carbohydrate-protein interactions and drug-protein interactions is very important for immunology, pharmacology and medicine. Due to the complex molecular structure of proteins direct study is quite difficult. So the amino acids, which are the building blocks of proteins are studied. Although a lot of attention has been given to the behaviour of amino acids in different salt-water mixed solvents, very few studies have been carried out on amino acids in carbohydrate + water and drug + water mixtures.

In physiological media such as blood, membranes, cellular fluids etc, water happens to be involved in an important manner. Water is chosen as a mixed solvent because its presence gives rise to hydrophobic forces [49] which are of prime
importance in stabilizing the native globular structure of proteins [50]. In aqueous solutions, amino acids exist as zwitterions (FIG. 1.3) [51-53]. Electrically charged amino acids, mostly found on protein surfaces, promote appropriate folding by interacting with the water solvent. Polar water molecules form shells around charged surface residue side chains, helping to stabilize and solubilize the protein. The study of zwitterions in aqueous solutions, therefore, offers an opportunity for investigating the molecular phenomenon occurring in physiological media.

1.2. INTERMOLECULAR INTERACTIONS OF PROTEINS

Protein structures are stabilized by different types of forces namely hydrogen, ionic, covalent and hydrophobic bonding (Fig. 1.9). These various non-covalent interactions are very important in biological systems. To understand fully the structure and function of the interactions responsible for stabilizing the native state of protein, it is essential to have knowledge of the interactions responsible for stabilising the native state of proteins. Many biological processes such as aggregation, protein hydration, denaturation etc. are not yet completely understood and remain a subject of extensive investigations [54-57].

Hydrogen bondings are weak forces which arise between a partially positive hydrogen and a partially negative oxygen on the same or different molecule. Ionic bonding can take place between an ionic and cationic side chains resulting in side chain cross linking. The most common form of inter-chain covalent bonding is the disulphide bond formed between the sulphur atoms of two cysteine residues.

![Figure 1.9: Interactions in proteins.](image-url)
Many amino acid residues have hydrophobic (water-hating) side chains. Proteins in aqueous solutions are folded so that most of the hydrophobic chains become clustered inside the folds. The polar side chains which are hydrophilic (water-loving) lie on the outside or the surface of the protein. This is called hydrophobic effect. It is considered to be the major driving force for the folding of globular proteins.

The physicochemical and thermodynamic properties of amino acids and their derivatives are of considerable interest as these biomolecules are the building blocks of all living organisms. These biomolecules incorporate some of the structural features found in the larger and more complex biomacromolecules (proteins). These biomolecules (amino acids, peptides and their derivatives), better known as model compounds, provide valuable information that leads to a better understanding of biological macromolecules or proteins [58-64]. It has been generally recognized that in the absence of experimental and thermodynamic data for these macromolecules (proteins etc.), amino acids and peptides can serve as useful models in estimating their properties [65,66]. Even in situations where experimental data are available, the properties of these smaller units are still found applicable in exploring various aspects of structural organization in the larger biomolecules [67].

Therefore, the study of physico-chemical and thermodynamic properties of the aqueous solutions of amino acid + carbohydrate/drug provides an insight into the structure of proteins and their conformational changes. These properties include volumetric, ultrasonic and viscometric properties. Hence, the study of these properties of aqueous solutions of amino acids is of great importance. In view of the importance, as described above, studies on various physico-chemical and thermodynamic properties of selected liquid solutions (amino acid + carbohydrate/drug + water) have been carried out by the measurements of density, viscosity and ultrasonic speed.

1.3. VOLUMETRIC PROPERTIES

Volumetric properties are regarded as a sensitive tool for understanding the interactions in solutions. From the volumetric data, apparent molar volumes, limiting apparent molar volumes, transfer volumes at infinite dilution of amino acids from aqueous solutions to aqueous mixed solvent solutions at various temperatures have been calculated.
Densities of amino acids in aqueous solutions and in aqueous mixed solvents were measured by using single capillary pycnometer. Using the density data, the apparent molar volumes, \( V_\phi \), of amino acids in aqueous solvent systems can be calculated from solution densities, by using the following relation:

\[
V_\phi = \frac{1000(\rho_o - \rho)}{m \rho \rho_o} + \frac{M}{\rho} 
\]

(1.1)

where, \( m \) is the molal concentration of the solute (amino acid), \( \rho \) and \( \rho_o \) are the densities of the solution and the solvent (aqueous-carbohydrate/drug), respectively; and \( M \) is the molar mass of the solute (amino acid). At infinite dilution, the apparent molar volumes, and partial (limiting) molar volumes, are identical. The values of limiting apparent molar volume, \( V^\circ_\phi \), have been obtained using method of linear regression of \( V_\phi \) vs. \( m \) of amino acid in carbohydrate/drug + water solvents from the following relation [68]:

\[
V^\circ_\phi = V^\circ_\phi + S_v m 
\]

(1.2)

where, the intercept, \( V^\circ_\phi \), is free from solute-solute interactions and therefore provides a measure of solute-solvent interactions, whereas the experimental slope, \( S_v \), provides information regarding solute-solute interaction.

Limiting apparent molar properties of transfer provide qualitative as well as quantitative information regarding solute-solvent interactions without taking into account the effects of solute-solute interactions [69,70].

The transfer volumes, \( V^\circ_{\phi, tr} \), of amino acid from water to aqueous-carbohydrate/drug solutions were calculated by using the following relation

\[
V^\circ_{\phi, tr} = V^\circ_{\phi, aq-carbohydrate/drug} - V^\circ_{\phi, water} 
\]

(1.3)

The temperature dependence of partial molar volumes, \( V^\circ_\phi \), may be expressed in terms of the absolute temperature (\( T \)) by the following relation

\[
V^\circ_\phi = a + b T + c T^2 
\]

(1.4)

where \( a, b, c \) are the coefficients and \( T \) is the temperature. The partial molar expansibilities, \( E^\circ_\phi \) [71] are obtained by the following equation
Another useful parameter, proposed by Hepler [72] provides information about the structure-making or structure-breaking ability of a solute in aqueous solution. This is called Hepler’s constant and is calculated by the following relation

\[
\left( \frac{\partial^2 E_\phi}{\partial T^2} \right)_p = \left( \frac{\partial^2 \dot{V}_\phi}{\partial T^2} \right)_p = 2C
\]

If the sign of second derivatives of the limiting apparent molar volume with respect to the temperature is negative, the solute is a structure maker; otherwise it is a structure breaker.

The partial molar volume of the amino acids can be studied by a simple model given by following equation

\[
\dot{V}_\phi^\circ (\text{amino acid}) = \dot{V}_\phi^\circ (\text{int}) + \dot{V}_\phi^\circ (\text{elect})
\]

where \( \dot{V}_\phi^\circ (\text{elect}) \) is the electrostriction partial molar volume due to the hydration of amino acid and can be calculated from experimentally measured values of \( \dot{V}_\phi^\circ (\text{amino acid}) \), and \( \dot{V}_\phi^\circ (\text{int}) \) is the intrinsic partial molar volume of the amino acid and has been calculated from equation

\[
\dot{V}_\phi^\circ (\text{int}) = (0.7 / 0.634) \dot{V}_\phi^\circ (\text{cryst})
\]

where \( \dot{V}_\phi^\circ (\text{cryst}) = \text{mol.wt.} / d_{\text{cryst}} \) is the crystal molar volume, 0.7 is the packing density for molecules in organic crystals and 0.634 is the packing density for random packing spheres. The value of \( \dot{V}_\phi^\circ (\text{int}) \) for the amino acid can be estimated from above equation (1.8) using \( d_{\text{cryst}} \) values for the amino acids taken from the work of Berlin and Pallansch [73].

\( \dot{V}_\phi^\circ (\text{elect}) \) can be calculated from experimentally measured values of \( \dot{V}_\phi^\circ (\text{amino acid}) \),

\[
\dot{V}_\phi^\circ (\text{elect}) = \dot{V}_\phi^\circ - \dot{V}_\phi^\circ (\text{int})
\]
The change in volume due to electrostriction can be related to the number of water molecules, \( n_{H1} \), hydrated to the amino acid by following equation

\[
n_{H1} = V^\circ_{\phi}(\text{elect.})/(V^\circ_{\phi,e} - V^\circ_{\phi,b})
\]

(1.10)

where, \( V^\circ_{\phi,e} \) is the molar volume of electrostricted water and \( V^\circ_{\phi,b} \) is the molar volume of bulk water. The reported value of \((V^\circ_{\phi,e} - V^\circ_{\phi,b})\) is \(-3.3 \times 10^{-6}\) m\(^3\) mol\(^{-1}\) at 298.15 K.

A linear regression analysis of values \( V^\circ_{\phi} \) vs \( n_c \) (number of carbon atoms in amino acid) in water and in aqueous mixed solutions was carried out using the following relation

\[
V^\circ_{\phi} = V^\circ_{\phi}(NH_3^+, COO^-) - n_cV^\circ_{\phi}(CH_2)
\]

(1.11)

where, \( V^\circ_{\phi}(NH_3^+, COO^-) \) and \( V^\circ_{\phi}(CH_2) \) represent the zwitterionic end group and the methylenic group contributions, respectively, to \( V^\circ_{\phi} \) value. It may be noted that the values of evaluated here represent the mean contribution of CH and CH\(_3\) groups to \( V^\circ_{\phi} \) values of amino acids

\[
V^\circ_{\phi}(CH_3) = 1.5V^\circ_{\phi}(CH_2)
\]

(1.12)

\[
V^\circ_{\phi}(CH) = 0.5V^\circ_{\phi}(CH_2)
\]

(1.13)

**1.4. ULTRASONIC PROPERTIES**

Ultrasonic study on the amino acids with aqueous solution of carbohydrates and drugs provides useful information in understanding the behaviour of intra-molecular and intermolecular associations, complex formation and related structural changes in liquid systems. 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 [74,75].

The apparent molar compressibility, \( K_{s,\phi} \) of these solutions were calculated by using the relations
Chapter 1

\[ K_{s,\phi} = \frac{1000(\kappa_s \rho_o - \kappa_s^o \rho)}{m \rho \rho_o} + \frac{\kappa_s M}{\rho} \]  \hspace{1cm} (1.14)

where \( m \) is the molal concentration of the solute (amino acid), \( \rho \) and \( \rho_o \) are the densities of the solution and the solvent (aqueous-carbohydrate/drug), respectively; and \( M \) is the molar mass of the solute (amino acid), \( \kappa_s \) and \( \kappa_s^o \) are the isentropic compressibilities of the solution and the solvent (aqueous-carbohydrate/drug), respectively, calculated using the relation

\[ \kappa_s = 1/u^2 \rho \]  \hspace{1cm} (1.15)

The limiting apparent molar compressibility, \( K_{s,\phi}^o \) and the slope, \( S_h \) have been obtained using method of linear regression of \( V_{\phi}^o \) and \( K_{s,\phi} \) vs. \( m \) of amino acid in carbohydrate/drug + water solvents from the following relations

\[ K_{s,\phi} = K_{s,\phi}^o + S_h m \]  \hspace{1cm} (1.16)

where the intercept, \( K_{s,\phi}^o \), is free from solute-solute interactions and therefore provides a measure of solute-solvent interactions, whereas the experimental slope, \( S_h \) provides information regarding solute-solute interaction.

The transfer compressibility, \( K_{s,\phi,\text{tr}}^o \) of amino acid from water to aqueous-carbohydrate/drug solutions were calculated by using the relation

\[ K_{s,\phi,\text{tr}}^o = K_{s,\phi,aq-carbohydrate/drug}^o - K_{s,\phi,\text{water}}^o \]  \hspace{1cm} (1.17)

where \( K_{s,\phi,\text{water}}^o \) is the limiting apparent molar compressibility of amino acid in water.

Further, the number of water molecules hydrated to the amino acids were calculated by using the method given by Millero et.al. [76]

\[ n_H = -K_{\phi}^o (\text{elect.})/(V_{\phi,b}^o - K_{s,\phi,b}^o) \]  \hspace{1cm} (1.18)

where, \( K_{s,\phi,b}^o \) is the isothermal compressibility of bulk water. Value of \( V_{\phi,b}^o \). \( K_{s,\phi,b}^o \) is 0.81 \( \times 10^{-5} \) m\(^3\) mol\(^{-1}\) GPa\(^{-1}\). The electrostriction partial molar compressibility \( K_{s,\phi}^o \)
(elect) can be calculated from the experimentally measured values of $K_{s,\phi}^o$ (amino acid) from the following equation

$$K_{s,\phi}^e (\text{elect}) = K_{s,\phi}^o (\text{amino acid}) - K_{s,\phi}^o (\text{int})$$  \hspace{1cm} (1.19)

where $K_{s,\phi}^o (\text{int})$ is $K_{s,\phi}^o$ (isomer) for L-phenylalanine ($3 \times 10^{-6}$ m$^3$ mol$^{-1}$ GPa$^{-1}$). Since $K_{s,\phi}^o (\text{int})$ is expected to be small and it is less than $5 \times 10^{-6}$ m$^3$ mol$^{-1}$ GPa$^{-1}$ for ionic crystals and many organic solutes in water. So, we can assume $K_{s,\phi}^o (\text{int}) = 0$.

Therefore, for $K_{s,\phi}^o (\text{int}) = 0$, equation (1.19) becomes

$$K_{s,\phi}^e (\text{elect}) = K_{s,\phi}^o (\text{amino acid})$$  \hspace{1cm} (1.20)

A linear regression analysis of values $K_{s,\phi}^o$ vs $n_c$ (number of carbon atoms in amino acid) in water and in aqueous mixed solutions was carried out using the following relation

$$K_{\phi}^o = K_{\phi}^o (NH_3^+, COO^-) - n_c K_{\phi}^o (CH_2)$$  \hspace{1cm} (1.21)

where, $K_{\phi}^o (NH_3^+, COO^-)$ and $K_{\phi}^o (CH_2)$ represent the zwitterionic end group and the methylenic group contributions, respectively, to $K_{s,\phi}^o$ value. It may be noted that the values of evaluated here represent the mean contribution of CH and CH$_3$ groups to $K_{s,\phi}^o$ values of amino acids

$$K_{\phi}^o (CH_3) = 1.5 K_{\phi}^o (CH_2)$$  \hspace{1cm} (1.22)

$$K_{\phi}^o (CH) = 0.5 K_{\phi}^o (CH_2)$$  \hspace{1cm} (1.23)

1.5. VISCOMETRIC PROPERTIES

The study of viscous behaviour of macromolecules in solutions is important in understanding the mechanism of transport processes. Viscosity and its derived parameters provide valuable information regarding the shape and size of the molecules [77]. The viscosity data have been utilized to calculate the values of
viscosity B-coefficients, free energy of activation and related thermodynamic parameters. The viscosity data were analyzed by using Jones-Dole [78] equation of the form

\[ \eta_r = \frac{\eta}{\eta_o} = 1 + Am^{1/2} + Bm \]  \hspace{1cm} (1.24)

where \( \eta_r \) is the relative viscosity of the solution, \( \eta \) and \( \eta_o \) are the viscosities of solution and the solvent (carbohydrate/drug + water), respectively, \( m \) is molality of amino acid in carbohydrate/drug + water solvent, \( A \) and \( B \) are the Falkenhagen [78-80] and Jones-Dole [78] coefficients, respectively. Coefficient \( A \) accounts for the solute-solute interactions and \( B \) is a measure of structural modifications induced by the solute-solvent interactions [81-83]. The values of \( A \) and \( B \) have been obtained as the intercept and slope from linear regression of \( [(\eta_r - 1)/m^{1/2}] \) vs. \( m^{1/2} \) curves, which were found almost linear for these systems.

A linear regression analysis of values \( B \) vs \( n_c \) (number of carbon atoms in amino acid) in water and in aqueous mixed solutions was carried out using the following relation

\[ B = B(NH_3^+,COO^-) - n_c B(CH_2) \]  \hspace{1cm} (1.25)

where, \( B(NH_3^+,COO^-) \) and \( B(CH_2) \) represent the zwitterionic end group and the methylenic group contributions, respectively, to \( B \) value. It may be noted that the values of evaluated here represent the mean contribution of CH and CH\(_3\) groups to \( B \) values of amino acids

\[ B(CH_3) = 1.5B(CH_2) \]  \hspace{1cm} (1.26)

\[ B(CH) = 0.5B(CH_2) \]  \hspace{1cm} (1.27)

The viscosity data have also been used for calculating free energies of activation per mole of solute and free energies of activation per mole of solvent according to transition state theory of the relative viscosity proposed by Feakins et al. [84]. According to this theory, the \( B \)-coefficient is given by the relation

\[ B = \frac{[(\bar{V}_1 - \bar{V}_2) + \bar{V}_1(\Delta \mu_2^{o^*} - \Delta \mu_1^{o^*})/RT]}{1000} \]  \hspace{1cm} (1.28)
where $\overline{V}_1^o$ is the apparent (partial) molar volume of the solvent (aqueous- carboxyhydrate/drug) and $\overline{V}_2^o (= V_\phi^o)$ is the limiting apparent (partial) molar volume of the solute, respectively. The free energy of activation per mole of solvent ($\Delta \mu_1^{\#}$) has been calculated by using the Eyring viscosity [86] relation

$$\Delta \mu_1^{\#} = RT \ln(\eta_o \overline{V}_1^o / hN) \quad (1.29)$$

where $h$ and $N$ are Planck’s constant and Avogadro number, respectively. Equation (1.28) rearranges to give free energy of activation per mole of the solute, $\Delta \mu_2^{\#}$

$$\Delta \mu_2^{\#} = \Delta \mu_1^{\#} + \left(\frac{RT}{\overline{V}_1^o}\right) \left[1000B - (\overline{V}_1^o - \overline{V}_2^o)\right] \quad (1.30)$$

The entropy of activation, $\Delta S^*$ [86] has been calculated using the following relation

$$\Delta S^* = -d(\Delta \mu_2^{\#})/dT \quad (1.31)$$

$\Delta S$ is obtained from the negative slope of the plots of $\Delta \mu_2^{\#}$ against $T$ by using a least-square treatment.

The activation enthalpy, $\Delta H^*$ [86] has been calculated by using the following relation

$$\Delta H^* = \Delta \mu_2^{\#} + T \Delta S^* \quad (1.32)$$

1.6. REVIEW OF THE LITERATURE

Various biological processes involve volume changes and the hydration of molecules. Their complete understanding needs a proper idea for the state and behaviour of the molecules in the medium [87]. Because of the structural complexities of proteins and the non-feasibility of their direct thermo dynamic studies, amino acids are often used as model compounds because they are building blocks of proteins.

Amino acids as structural components of proteins participate in all the physiological processes of a living cell. Therefore, many physical and chemical
studies have been done to elucidate interaction mechanisms between amino acids and organic compounds in cell fluids or compounds with the same functional groups as those existing in biomolecules of living organisms [88-91].

In the past 30 years, there have been extensive thermodynamic property studies on amino acid-water simple salt systems [92-101] but few studies on the thermodynamic and transport properties of amino acids in protein–aqueous media [102-107].

In previous studies the enthalpies of solution of the simple amino acid such as glycine in aqueous solution of amides [108], urea [109], alcohols [110] and electrolytes [111] were examined. The results obtained were discussed from the point of view of molecular interaction on the basis of the McMillan-Mayer model [112] as modified by Franks [113] and Desnoyers [114]. It is well known that McMillan-Mayer enthalpic interaction coefficient can be considered as measures of intermolecular interaction in solution [115]. The enthalpic pair interaction coefficient being the measure of interaction between the molecules/ions under investigation and solvent water. All the processes that stabilized hydrogen bonds of water (e.g., hydrophobic hydration) weaken the direct interaction between the polar groups of interacting solute molecule/ions [115]. The study of model compounds in aqueous solutions are of fundamental importance in understanding intermolecular interaction in liquid [116,117] and the effect of these compounds on water structure [118]. These studies have been carried out by a number of researchers [119-126,76,92].

Work on the volume properties of proteins and some amino acids have shown that they undergo a decrease in volume upon dissolving in water [127-135,73]. This decrease in volume upon dissolution is similar to what occurs for electrolytes [136] and can be attributed to electrostriction due to water-protein, and water-amino acid interactions. The number of water molecules is determined from the electrostriction of protein [137] and amino acids [138,139] (using methods that have been applied to electrolytes) [140]. Results indicated that proteins behave as electrolytes. The effect of pressure on the specific volumes [133] indicates that the so-called bound water behaves more like water solvated to soluble organic solutes [137].

The study of some of the thermodynamic properties of proteins can be facilitated by investigating the behaviour of their constituents, viz., amino acids and
peptides. Some of these properties are found to be implicated in several biochemical processes such as protein hydration [141,133], aggregation [142,143], denaturation [144,145], antigen–antibody reactions [146], etc. The property of partial specific volume of protein is believed to be implicated in the phenomenon of anaesthesia. According to the concept of critical volume [147,148], the reversal of anaesthesia under applied pressure has been viewed as important evidence in favour of this concept [149].

Several important works on volumetric investigations of amino acids and peptides have been reported in the past at normal laboratory temperature (298.15 K) [143-156,76]. These studies have contributed significantly toward an understanding of water-dipolar ion interactions. Volume contributions of the glycyl residue and of various side groups are now known with greater degree of accuracy and have been used in additivity schemes for computing improved volumetric data for proteins [157]. The expansibility data for biological macromolecules are believed to be beneficial for the study of living systems under different physiological conditions such as fever or hypothermia [158].

In living organisms interactions of carbohydrates with proteins play a key role in a wide range of biochemical processes. The influence of the solute and media on the ability of carbohydrate to specifically interact with proteins on the basis of model system has been explained by many workers.

Parfaryak, Davydover and Lebedeva [159] have measured the stability constants and thermodynamic parameters of the interactions of \( d \)-maltose and sucrose with some amino acids (glycine, \( dl \)-alanine, \( dl \)-leucine and \( l \)-serine) at 298.15 K by calorimetric titration. The apparent molal volumes of the disaccharides in dilute aqueous solutions of the amino acids have been determined from density measurement at 298.15 K. These results were interpreted in terms of the influence of the nature of the solutes, their specific conformations, hydration and on the ability of the disaccharides to form associated complexes with the amino acids. In contrast to \( d \)-maltose, sucrose was found to be associated with the amino acids and these associated species are preferentially entropy stabilized.

Liu et al. [160] have measured the enthalpies of mixing of three kinds of aqueous amino acid solutions (glycine, \( l \)-alanine and \( l \)-serine) with aqueous sorbose
solution and aqueous fructose solution and their respective enthalpies of dilution have been determined at 298.15 K. The results were interpreted from the point of view of solute-solute interactions. They also calculated the enthalpies of mixing of aqueous glucose solutions and of aqueous amino acid solutions (glycine, \textit{l}-alanine, \textit{l}-serine, \textit{l}-valine, \textit{l}-proline and \textit{l}-threonine) and their respective enthalpies of dilution at 298.15 K [161]. The experimental data were analyzed in terms of the McMillan–Mayer model to obtain interaction coefficients. The results were interpreted from the point of view of solute-solute interactions. Their results showed that the discrepancies of heterotactic enthalpic interactions coefficients mainly depend on the differences in the structures of the amino acids studied. Glycine is the simplest amino acid in nature. \textit{l}-alanine has one H-atom of the \textalpha-carbon replaced by a methyl group compared to glycine, which increases the hydrophobic properties of \textit{l}-alanine. Thus, the enthalpic interaction coefficients between \textit{l}-alanine and the saccharides are larger than those of glycine. The side chain of \textit{l}-serine has polar group –\text{CH}_2\text{OH}, which makes the enthalpies of mixing more complex. Thus, it is possible for \textit{l}-serine molecule to interact with saccharide molecules side by side. This means the zwitterionic groups and –\text{OH} group of \textit{l}-serine molecule can interact with different –\text{OH} group of the same saccharide molecule. In this situation, the non-polar group of \textit{l}-serine and saccharide becomes closer and the hydrophobic interactions are enhanced. So, the enthalpic pairwise interaction coefficient of \textit{l}-serine interacting with saccharide is less negative than that of glycine interacting with saccharide, but the introduction of –\text{OH} leads to the more negative values than those of \textit{l}-alanine.

Tarlok et al. [162] have reported partial molal volumes at infinite dilution of glycine, \textit{dl}-alanine, \textbeta-alanine, \textit{dl}-\textalpha-amino-n-butyric acid, \textit{l}-valine, \textit{l}-leucine, \textit{l}-isoleucine, \textit{l}-phenylalanine, \textit{l}-serine, \textit{l}-threonine, \textit{l}-proline and \textit{l}-glutamic acid by density measurements using pycnometer at different temperatures. These data in conjunction with the reported values at other temperatures have been utilized to estimate the precise partial molal expansibilities at infinite dilution. These parameters were used to predict the structural hydration effect, i.e., the structure-making and structure-breaking effects of solutes in solutions. The precise values of partial molal expansibilities have been used to convert the partial molal adiabatic compressibilities of the solutes to partial molal isothermal compressibilities at infinite dilution which is
an ideal parameter for interpreting the results. They concluded that all the amino acids were acting as structure-breakers in these solutions.

Mixed aqueous solvents are used extensively in chemistry and other fields to control factors like stability, reactivity and solubility of systems [163,164]. Singh and Banipal [164] studied partial molar adiabatic compressibilities and viscosities of glycine, \( \textit{dl}-\alpha\)-alanine, \( \textit{l}\)-valine, \( \textit{l}\)-leucine and \( \textit{l}\)-phenylalanine in water and in mixed aqueous solutions of glycerol at 298.15 K. The data were used to calculate partial molar adiabatic compressibilities at infinite dilution, and viscosity \( B \)-coefficients and their corresponding transfer functions. The activation energy for viscous flow in aqueous and mixed aqueous glycerol solutions had been calculated from \( B \)-coefficients and partial molar volume data. The hydration numbers, interaction coefficients and side chain contributions had also been calculated. The results were interpreted in terms of solute–co-solute interactions. An attempt had also been made to correlate these parameters with the stability of protein in aqueous glycerol solutions. The partial molar adiabatic compressibilities of transfer values at infinite dilution were found to be positive for the studied amino acids and their magnitude increased with increase in concentration of glycerol. viscosity \( B \)-coefficient of transfer values were also found to be positive for these amino acids, which after passing through maxima \( \approx 3.5 \, m_B \) decrease at higher concentration of glycerol.

Iqbal and Ahmed [165] calculated the partial molar volumes of ten amino acids, \( \textit{dl}\)-alanine, \( \textit{l}\)-arginine, glycine, \( \textit{l}\)-histidine, \( \textit{l}\)-isoleucine, \( \textit{l}\)-lysine \( \text{HCl} \), \( \textit{l}\)-methionine, \( \textit{l}\)-proline, \( \textit{l}\)-serine and \( \textit{dl}\)-threonine in water using density measurement at 308.15 K. Amino acid–water interactions are interpreted from partial molar volume data with particular reference to structural features of solute molecules, such as hydrogen bonding, side group interactions, etc. Hydration volumes were estimated using different values of intrinsic volumes. Isobaric expansibilities were also calculated which indicate correlation between isobaric expansibilities and size and between isobaric expansibilities and hydrophobicity of solute molecules. The values of partial molar volume of \( \textit{dl}\)-alanine and \( \textit{l}\)-serine were found to be about the same while their molar masses differ by 16 mass units. The side group in \( \textit{l}\)-alanine is \(-\text{CH}_3\) and in \( \textit{l}\)-serine it is \(-\text{CH}_2\text{OH}\). Thus, a lower value of partial molar volume of \( \textit{l}\)-alanine was expected. On the contrary, nearly the same values for both of these represented the shrinkage due to hydrogen bonding between the \(-\text{OH}\) of serine and surrounding
solvent molecules, an interaction missing in the case of $l$-alanine. Similarly, partial molar volume of $l$-methionine was much smaller than $l$-isoleucine expected on the basis of molar mass correlation due to hydrogen bonding between –S– of methionine and the surrounding solvent molecules. The hydrophobic portion of these molecules did not seem to be very dissimilar from each other and hence volume change due to these may not be too dissimilar as well. Data concerning $l$-lysine:$l$-arginine pair and $l$-threonine:$l$-proline pair also tend to highlight similar specific interactions by the side groups. The hydration numbers were found to be positive.

Sinha et al. [166] studied the apparent molar volumes and viscosity $B$-coefficients of $l$-alanine in water and silver sulphate-water mixtures from measurements of density and viscosity at different temperatures. The limiting values were calculated by a linear extrapolation using the least-squares method. These data were used to discuss the structure-making or structure-breaking ability of amino acids. The activation parameters of viscous flow for the ternary solutions were also derived and explained in terms of transition state theory. The limiting value of apparent molar volume and viscosity $B$-coefficient values indicated the presence of strong solute-solvent interactions, which further strengthen at higher molarities of silver sulphate in the ternary solutions, but decrease at higher temperatures. Also, the trend in Hepler’s constant and $\Delta \mu _2 ^\#$ values in the different aqueous silver sulphate solutions suggested $l$-alanine to be net structure promoter in aqueous silver sulphate solutions.

Pal et al. [167] studied the volumetric and ultrasonic properties of glycine in aqueous solutions of mannose, maltose and raffinose at different temperatures. Partial molar volumes and partial molar adiabatic compressibilities of glycine at infinite dilution have been calculated. These values were used to calculate the number of water molecules hydrated to glycine molecule. Transfer volumes and transfer adiabatic compressibilities at infinite dilution have also been calculated. These parameters have been interpreted in terms of solute-cosolute interactions on the basis of cosphere-overlap model. Pair and triplet coefficients have also been calculated from transfer parameters. Glycine has positive Partial molar volumes and negative partial molar adiabatic compressibilities at infinite dilution in aqueous saccharide solutions at different temperatures showing the presence of strong solute-solvent interactions. The values of transfer volumes were found to be positive in the case of mannose and maltose at higher concentrations of saccharides indicated the overlap of
the hydration co-sphere of the ion \((NH_3^+, COO^-)/(--NH_2)\) of the glycine and –OH group of the saccharides. This suggested that the glycine is a structure breaker in higher mass % of mannose and maltose. The negative values of transfer volumes in case of raffinose indicated the weak structure-breaking effect of glycine in aqueous raffinose solutions. Hydration number values decrease with increase in the concentration of mannose or raffinose except with maltose, which indicated the increase in solute-cosolute interaction. Similar observations had been obtained by them in case of \(l\)-alanine [168].

Viscosities and densities of aqueous solutions of glycine, \(dl\)-alanine, \(dl\)-\(\alpha\)-amino-n-butyric acid, \(dl\)-valine, \(dl\)-leucine have been measured as a function of amino acid concentration at different temperatures by Yan et al. [169]. The calculated standard partial molar volumes \(V^\phi\) and \(B\)-values were split into the contributions from \((NH_3^+COO^-)\) and \(CH_2\) groups by linear correlations. Free energies of activation, \(\Delta\mu_2^\#\) of aqueous amino acids were obtained by application of the transition-state theory to the \(B\)-coefficient data, and the corresponding activation enthalpy, \(\Delta H^\#\) and entropy, \(\Delta S^\#\) were also given. The results showed that the values of partial molar volume were constant over the studied temperature range within the experimental uncertainty. \(B\)-coefficients values increased in the order: Gly < Ala < Abu < Val < Lue. This showed that the \(B\)-values increase with increasing alkyl chain length of the \(\alpha\)-amino acids. The positive \(dB/dT\) values confirmed that these amino acids act as structure-breakers in these solutions.

Cibulka et al. [170] studied the partial molar volumes of glycine and \(l\)-alanine in water at temperatures (298 to 443) K and at pressures upto 30 MPa. (at pressures close to the saturation line of water, at pressures in the range from (15 to 17) MPa, and at 30 MPa. Values of an analogue of isothermal compressibility, \(k_{T,2}^\phi = -(1/V^\phi_{m,2})(\partial V^\phi_{m,2}/\partial P)_T\) were evaluated. Maxima on the curves \(\partial V^\phi_{m,2}(T)\) and \(k_{T,2}^\phi(T)\) were also observed and discussed. The new data along with literature values of standard molar volumes and heat capacities were used for generating the recommended parameterization of an equation of state for standard molar thermodynamic properties of the aqueous-amino acids. The values of \((\partial V^\phi_{m,2}/\partial P)_T\)
and \( \frac{\partial V_{m,2}^o}{\partial T} \) were found to be positive at low pressures. Maxima appear on the \( \frac{\partial V_{m,2}^o}{\partial T} \) curves: for the low pressure set the maximum is located at \( T_{max} = 355 \text{ K} \) for glycine (aq) and at slightly higher temperature \( T_{max} = 375 \text{ K} \) for \( l \)-alanine (aq), in accordance with lower hydrophilic/hydrophobic ratio in \( l \)-alanine molecule compared to glycine. Temperature \( T_{max} \) for glycine (aq) is independent on pressure, that for \( l \)-alanine (aq) seems to increase with increasing pressure (\( T_{max} = 382 \text{ K} \) for the middle pressure set, no maximum is observed on the isobar 30 MPa upto 408 K).

Pal and Chauhan [171] studied the volumetric behaviour of amino acids (glycine, \( l \)-alanine, \( l \)-valine and \( l \)-leucine) and their group contributions in aqueous lactose solutions at different temperatures. The density was used to compute apparent molar volume, \( V_\phi \), partial molar volume at infinite dilution, \( V_\phi^o \) and experimental slope, \( S_\nu \) were obtained and interpreted in terms of solute-solvent and solute-solute interactions. These data were used to calculate the \( \frac{\partial V_\phi^o}{\partial T} \) values. The partial molar volume of transfer, \( V_{\phi,tr}^o \) from water to aqueous lactose solutions at infinite dilution had also been calculated. In addition to this, the linear correlation of \( V_\phi^o \) with number of carbon atoms in the alkyl chain of amino acids was utilized to determine the respective contributions of (\( \text{NH}_3^+ \),\( \text{COO}^- \)), and \( \text{CH}_2 \) groups to \( V_\phi^o \). The values of \( V_\phi^o \) were found to be large positive suggesting the presence of strong solute-solvent interactions in the concerned medium. The order of increase was: \( l \)-leucine > \( l \)-valine > \( l \)-alanine > glycine. The \( V_{\phi,tr}^o \) values were positively and increased positively with increase in mass % of saccharide. The \( E_\phi^o \) values were positive at different concentration of lactose and at all temperatures in case of all the amino acids favouring the solute-solute interactions. Amino acids with larger alkyl groups (\( l \)-alanine, \( l \)-valine, \( l \)-leucine) were under hydrophobic hydration in the presence of lactose as compared to glycine. From \( V_{\phi,tr}^o \) and group contribution data, authors concluded that the interactions of the –OH group of lactose with the zwitterionic groups of the amino acids dominated compared to those of the hydrophobic group of lactose interactions.
Pal [172] studied the partial molar adiabatic compressibilities, $K_{s,\phi}$, of glycine, l-alanine, l-valine and l-leucine in aqueous and mixed aqueous solutions of lactose at different temperatures. From these data, partial molar adiabatic compressibilities at infinite dilution ($K_{s,\phi}^\circ$) were evaluated to calculate corresponding transfer function. Also, the contributions of NH$_3^+$, COO$^-$ and CH$_2$ groups have been calculated by the linear correlation of $K_{s,\phi}^\circ$ with number of carbon atoms in the alkyl chain of amino acids. The transfer partial molar adiabatic compressibilities at infinite dilution, $K_{s,\phi, tr}$, were found to be positive. The results indicated the existence of strong solute-solvent interactions, i.e., hydrophilic-ionic and hydrophilic-hydrophilic interactions. These solute-solvent interactions increased with the increase in the concentration of lactose. The decrease in the magnitude of transfer partial molar adiabatic compressibilities from glycine to l-leucine indicated the dominance of hydrophobic-hydrophobic interactions between the increasing side chains of amino acids. It was observed that the hydrophilic-hydrophobic and hydrophobic-hydrophobic interactions increased as the side chain length of the amino acids increased.

Pitkänen et al. [173] measured the densities, partial molar volumes and viscosities of aqueous solutions of glycyl betaine at different temperatures over the concentration range 0.05 to 5.0 mol·L$^{-1}$. The partial molar volumes showed that betaine existed partly as a monohydrate and partly in its anhydrous form. The proportion of the anhydrous form increased with increasing temperature. Also, an associated form of betaine appeared in concentrated betaine solutions, possibly with water as a bridging group. The significance of the viscosity $B$-coefficient was discussed. The signs of $B_{st}$, the increment of the viscosity $B$-coefficients arising from structural changes of water, were negative and the signs of $dB/dT$, the temperature derivative of $B$, were positive. These authors concluded that betaine is a water structure breaker especially at lower temperatures, and this effect decreased to insignificance at higher temperatures.

Jha and Kishore [174] studied the thermodynamic interactions of homologous series of amino acids namely glycine, alanine, valine and leucine with sorbitol. They calculated apparent molar volume, $V_\phi$, apparent molar isentropic compressibilities, $K_{s,\phi}$ and enthalpies of dilution of the four amino acids in aqueous-sorbitol solutions.
These data have been used to calculate limiting apparent molar volumes, $V_\phi^\circ$, limiting apparent molar compressibilities, $K_{s,\phi}^\circ$, and infinite dilution enthalpies of dilution. From these values, the standard partial molar transfer properties were also calculated. The linear correlation of for homologous series of amino acids has been utilised to calculate the contribution of the charged end groups ($NH_3^+, COO^-$), the ($CH_2$) group and the alkyl chain of the amino acids. The results for the standard partial molar volumes of transfer, compressibilities and enthalpies of dilution from water to aqueous-sorbitol solutions have been correlated and interpreted in terms of ion-polar, ion-hydrophobic and hydrophobic-hydrophobic group interactions. Results suggested that an enhancement of the hydrophilic/polar group interactions operating in ternary systems of amino acid, sorbitol and water.

Kumar et al. [175] studied the densities and viscosities of glycine and $l$-valine at 308.15 K and 318.15 K in aqueous tripotassium citrate solutions at various concentrations of tripotassium citrate. The viscosity data was analyzed by using Jones-Dole equation. The activation parameters of viscous flow were obtained to throw light on the mechanism of viscous flow. The values of apparent molar volume, partial molar volume at infinite dilution and relative viscosities of each amino acid in various aqueous tripotassium citrate solutions were evaluated from the density and viscosity data. The partial molar volumes of transfer from water to aqueous tripotassium citrate solution at infinite dilution were also calculated. These data were used to calculate the pair and triplet interactions. The results were discussed in terms of solute-solute and solute-solvent interactions and the structural changes of the solutes in solutions. The $V_\phi^\circ$ values of glycine and $l$-valine in aqueous-tripotassium citrate solutions were found to be positive and increase with increase in the salt concentration and temperature, thereby showing the presence of strong solute-solvent interactions. The positive but smaller values of $S_v$ as compared to $V_\phi^\circ$ suggest that solute-solute interactions are weaker than the solute-solvent interactions. The pair interaction coefficients, $V_{xy}$ and quartet interaction coefficient, $V_{xyyy}$ are positive for both amino acids, whereas triplet interaction coefficient parameters, $V_{xxy}$ are both negative at both temperatures. The $V_{xyy}$ has lower negative values for glycine whereas larger negative values in case of $l$-valine. Large and positive $B$-values as compared to
A-values indicated that the ion-solvent interactions are strong. The values of $\Delta \mu_2^\circ \#$ were positive and larger than $\Delta \mu_1^\circ \#$ for both the amino acids except for l-valine in 0.6 mol kg$^{-1}$ tripotassium citrate.

Siddique and Naqvi [176] obtained the viscosities values of l-histidine and l-arginine in 1 m aqueous solutions of sodium acetate, potassium acetate and calcium acetate at different temperatures. The Falkenhagen coefficient, $A$ and Jones-Dole coefficient, $B$, relative viscosity, and specific viscosity of the solutions were also determined using the measured viscosities. The results were interpreted in terms of solute-solute and solute-solvent interactions occurring in the system under investigation and also discussed in terms of the structure-making /breaking ability of the solute in these salt solutions. The structure-making/breaking abilities of the solutes in these systems were strongly influenced by temperature. It was concluded from the values of the $B$-coefficients that the l-lysine monohydrochloride shows stronger ion-solvent interactions than the other two studied amino acids, and the higher value of the $B$-coefficient in 1 m CA solutions showed that the dehydration effect of Ca$^{2+}$ is more than that of Na$^+$ and K$^+$ on these amino acids.

Riyazudddeen et al. [177] studied the interactions of l-threonine and l-alanine in aqueous glucose and sucrose solutions at different temperatures. They studied densities and speeds of sound of l-theronine and l-alanine in aqueous solutions of glucose and sucrose. The partial molar volumes, $V_\phi^\circ$, transfer partial molar volumes, $V_{\phi,tr}^\circ$, partial molar isentropic copressibilities, $K_{s,\phi}^\circ$ and transfer partial molar isentropic compressibilities, $K_{s,\phi,tr}^\circ$, were measured using the experimentally measured values of density and speed of sound. The trends of experimental and computed parameters were discussed in terms of ionic-hydrophilic, hydrophilic-hydrophilic and hydrophilic-hydrophobic interactions operative in these systems. The higher $V_\phi^\circ$ and $V_{\phi,tr}^\circ$ values of l-alanine and l-threonine in aqueous sucrose solution that those in aqueous-glucose solution was due to the stronger ionic-hydrophilic and hydrophilic-hydrophilic interactions between zwitterions and OH groups of sucrose than those with OH groups of glucose molecules. The decrease in $V_\phi^\circ$ and $V_{\phi,tr}^\circ$ values of l-alanine/l-threonine in aqueous glucose/sucrose solution with increase in
temperature, indicated the corresponding decrease in number of electrostricted water molecules in the solutions. The positive $K_{s,\phi, tr}^\circ$ values suggest that the ionic-hydrophilic groups and hydrophilic-hydrophilic group interactions dominated in the studied systems. The decrease in $K_{s,\phi, tr}^\circ$ values with an increase in temperature indicated that the water molecules are released from the secondary salvation layer of $l$-alanine and $l$-threonine zwitterions into the bulk water as the temperature increases.

Hakin et al. [178] measured the densities and heat capacities for aqueous solutions of glycine, $l$-alanine, $l$-serine and $l$-threonine at different temperatures. Apparent molar volumes and heat capacities and the associated standard state partial molar properties were also calculated. Constant pressure variations of revised Helgeson, Kirkham, and Flowers equations had been fitted to calculate standard state volumes and heat capacities over the temperature range. These equations had been used to estimate standard state volumes and heat capacities, and hence equilibrium constants, for aqueous amino acids systems at higher temperatures.

Kulikova and Parfenyuk [179] studied the influence of side chain $l$-$\alpha$-amino acids ($l$-alanine, $l$-phenylalanine, $l$-leucine and $l$-serine) on their interaction with $d$-glucose in dilute aqueous solutions by titration calorimetric and UV absorption spectroscopy measurements. The obtained results showed that the ability of $l$-$\alpha$-amino acids to form intermolecular complexes with $d$-glucose in aqueous solution is affected by the size and nature of their side chains. It has been found that $d$-glucose is able to form thermodynamically stable 1:1 complexes with some $l$-$\alpha$-amino acids. Complex formation is accompanied by small exothermic effects. The stability constant values indicate that the studied amino acids are able to form 1:1 molecular complexes with $d$-glucose, except for $l$-leucine. The data presented in this work showed that the entropic contribution is the major factor governing the complex formation between the studied amino acids and the monosaccharide. This may be connected with extensive hydration of the solutes and a significant structural reorganization of the solvent during complex formation. Negative binding enthalpies may be caused by specific intermolecular and van der Waals interactions.

Ali et al. [180] studied the volumetric, viscometric and refractive index behaviour of glycine, $l$-alanine, $l$-valine and $l$-leucine at different concentrations of
aqueous-glycerol solution at different temperatures. The experimental data were used to calculate the apparent molar volumes, $V_\phi$, the infinite dilution apparent molar volumes, $V_\phi^*$, the partial molar volumes of transfer, $V_{\phi,fr}$, of amino acids from aqueous to aqueous glycerol solutions as well as the viscosity $A$ and $B$ coefficients of the Jones-Dole equation of the amino acids. The free energies of activation of viscous flow, $\Delta \mu_1^0$, and $\Delta \mu_2^0$, per mole of solvent and solute, respectively, were obtained by application of the transition state theory of $B$-coefficient data and the corresponding activation enthalpy, $\Delta H^*$ and entropy, $\Delta S^*$ were also determined. The $V_\phi^*$, $B$-coefficient, and $\Delta \mu_2^0$ were found to vary linearly with increasing number of carbon atoms in the alkyl chain of the amino acids and they were split into contributions from the zwitterionic end groups, $\text{NH}_3^+\text{COO}^-$ and methylene groups of amino acids. The results were interpreted in the light of the solute-solute and solute-solvent interactions in the mixed solvents. The apparent molar volumes were found to decrease with increasing concentration of amino acid in aqueous glycerol and increase with increasing temperature for all the amino acids under study. The $B$-coefficients are larger than the $A$-coefficients except for glycine at 308.15 and 313.15 K, suggesting stronger solute-solvent interactions as compared to solute-solute interactions. $\Delta \mu_2^0$ values are positive and much larger than the $\Delta \mu_1^0$ values. The $\Delta H^*$ values of these mixtures are positive and increase regularly with increasing concentration of amino acid, thereby, suggesting that the formation of activated species for viscous flow becomes difficult as the amount of amino acid in the mixture increases. The small values of $\Delta S^*$, which increased with increasing concentration of amino acids, for all the studied mixtures, suggest that the net order of the system decreases as the concentration of amino acid in the mixture increases. The values of refractive index increased with increasing concentration of the amino acids in the mixture which reflects that the refractive index is directly related to the interactions in the mixture.

Banipal et al. [181] studied apparent molar volumes, and viscosities, of glycine, $dl$-$\alpha$-alanine, $dl$-$\alpha$-amino butyric acid, $l$-leucine and $l$-phenylalanine in water and in different concentration range of aqueous sodium acetate solutions at 298.15 K. The standard partial molar volumes, $V_\phi^*$, obtained from $V_\phi$, were used to calculate the
corresponding volume of transfer at infinite dilution, \( V_{\phi,ir}^{\circ} \), from water to aqueous sodium acetate solutions. \( B \)-coefficients were calculated using the Jones-Dole equation. The side-chain contributions to \( V_{\phi}^{\circ} \) and \( B \)-coefficients had also been calculated. The \( V_{\phi,ir}^{\circ} \) values were positive for the studied amino acids and increased almost linearly in the lower concentration range up to \( 1.0 \text{ m} \). This suggested that the ion-ion interactions dominated in these systems. The decreasing magnitude of transfer volumes from glycine to \( l \)-leucine indicated the building up of the ion-hydrophobic interactions that contributed negatively to the transfer values. The \( B \)-coefficient values for amino acids in water and in aqueous sodium acetate solutions followed the order: glycine < \( dl - \alpha \)-alanine < \( dl - \alpha \)-amino butyric acid < \( l \)-leucine < \( l \)-phenylalanine. The authors concluded that various thermodynamic parameters for the studied amino acids in aqueous-sodium acetate solutions suggested that ion-ion interactions are much stronger than ion-hydrophobic group interactions over the entire concentration range of sodium acetate. The ion-nonpolar (hydrophobic) group interactions increased from glycine to \( l \)-leucine with the increasing size of the alkyl side chain. A comparison of \( V_{\phi,ir}^{\circ} \) values with the literature showed that the effect of the acetate ion followed the same order as in the Hofmeister series.

Riyazudddeen et al. [182] studied the ultrasonic velocities and densities of \( l \)-phenylalanine, \( l \)-leucine in aqueous NaCl and aqueous NaNO\(_3\) solutions from (298.15 to 328.15) K. The ultrasonic velocity values increase with an increase in the molality of the amino acid as well as temperature. The density values increase with an increase in the molality of the amino acid and decrease with an increase in temperature for the systems under investigation. The isentropic compressibilities decrease with an increase in the molal concentration of the amino acid as well as temperature. They also studied the viscosities [183] of \( l \)-phenylalanine, \( l \)-leucine in aqueous NaCl and aqueous NaNO\(_3\) solutions from (298.15 to 328.15) K. They found that viscosity coefficient values of these systems increased with increase in molal concentration of amino acids in solutions. These coefficients had been found to be positive for all the systems.

Ali et al. [184] determined the densities, viscosities and refractive indices of aqueous-dimethylsulfoxide (DMSO) solutions and of solutions of amino acids namely
dl-valine, l-isoleucine and l-proline (0.01-0.05 M) in aqueous-DMSO at different temperatures. The density data have been used to calculate apparent molar volume, limiting apparent molar volume and the slope. The viscosity data were analysed by means of Jones-Dole equation. The free energies of activation per mole of solvent and per mole of solute were obtained by using transition state theory. The refractive index data was used to calculate molar refractive for all the three amino acids in aqueous DMSO solvent mixtures. All these parameters were used to study solute-solvent and solute-solute interactions in these mixtures.

Drug-macromolecular interaction is an important phenomenon in physiological media [185]. Thermodynamic properties like partial molar volumes and viscosity B-coefficient are convenient parameters in interpreting molecular interactions in solution phase [186]. It is noted that drug action can be achieved with a small amount of drug as high concentration are rarely achieved in vivo, therefore, ion-solvent interactions are the controlling forces in dilute solution.

Matubayasi and Ueda [187] proposed that the volume of anaesthetic molecules in the membrane is of prime importance for the mechanism of anaesthesia, and it occurs when the volume of anaesthetic molecules reaches a critical value in the cell membrane.

Lande et al. [188] reported the findings on three drugs (i) psuedoephedrine hydrochloride (PHE) which is stereoisomer of ephedrine and has similar sympathomimatic action, (ii) phenylepherine hydrochloride (PSE) which is a sympathomimatic agent, which has predominately alpha–adrenergic activity and is without stimulating effects on central nervous system and (iii) salbutamol sulphate (SBS), which is a direct acting sympathomimatic agent with predominately beta–adrenergic activity. Evaluation of partial molar expansibility \( \left( \frac{\partial V^o_{\phi}}{\partial T} \right) \), needs \( V^o_{\phi} \) values at various temperatures [189]. The sign of second derivatives of partial molar expansibility gives information regarding structure-making or breaking tendency. The observed \( V^o_{\phi} \) and B-coefficient values in these drugs are greater than the corresponding values for inorganic ions. These values are in line with similar structured drug molecules. The \( V^o_{\phi} \) follows the order, PHE < PSE < SBS. First two drugs were taken as their chlorides and the third was taken as its sulfate. They would,
therefore, behave as electrolyte in water. PSE and PHE are present as univalent and SBS as a divalent cation. The size and shape factors are more favorable for SBS, which showed large values of $V^\circ$. The apparent molar expansibility was found to increase with temperature, suggesting caging effect [190]. Positive sign [29] of \( \left( \frac{\partial^2 V^\circ}{\partial T^2} \right)_p \) suggests the structure-maker and negative sign suggests the structure-breaker solute. All the above three drugs showed positive values, therefore, they all were the structure-makers.

Banipal et al. [191] measured the partial molar volumes, $V^\circ$ and viscosity $B$-coefficients from density and flow time measurements for some sulpha drugs namely sulphanilamide, sulphanilic acid and sulphosalicylic acid dihydrate in water and in aqueous solutions of sodium chloride at temperatures from (288.15 to 318.15) K by the use of a vibrating tube digital densimeter and micro-ubbelohde type capillary viscometer. The transfer volume at infinite dilution calculated from partial molar volumes had both positive and negative values. The overall positive values at higher concentrations of sodium chloride were in the following order: sulphosalicylic acid dihydrate > sulphanilic acid > sulphanilamide, which is also the order of hydrophilicity of these drugs. The interaction coefficients, partial molar expansibilities, $E^\circ$ and second order derivative, \( \left( \frac{\partial^2 V^\circ}{\partial T^2} \right)_p \) were also calculated. The transfer $B$-coefficient values were calculated from viscosity $B$-coefficient data. Transition state theory was used to calculate the activation free energy for the viscous flow, $\Delta \mu^\circ_{2\#}$ of solutions. The related activation parameters like $\Delta H^\circ$ and $\Delta S^\circ$ were also calculated. The negative $S_v$ values in the case of sulphanilamide and sulphanilic acid in water as well as in aqueous sodium chloride solutions were attributed to the self association of the molecules studied. The \( \left( \frac{\partial^2 V^\circ}{\partial T^2} \right)_p \) values suggested that sulphanilamide and sulphosalicylic acid dihydrate were structure-breakers in both water and aqueous sodium chloride solutions, but sulphanilic acid was a structure maker in water and a structure-breaker in sodium chloride solutions. Negative values of excess molecular volume also justified the self-association of sulphanilamide and sulphanilic acid. The results of activation free energy for the viscous flow of solutions suggested that the formation of transition state was less favoured in the presence of studied sulpha drugs.
Perlovich et al. [192] measured the densities of some drug and pro-drug substances in 1-octanol at 298.15 K. They measured the densities of phenol, acetanilide, benzamide, benzoic acid, phenacitin, \( i \)-(acetylamino)-benzoic acid, \( i \)-hydroxy-benzamide and \( i \)-acetaminophen (where \( i = 1,2,3 \)) in 1-octanol. They calculated apparent molar volumes, \( V_{\phi} \) and partial molar volumes at infinite dilution, \( V_{\phi}^o \). The correlated equations obtained revealed a connection of \( V_{\phi}^o \) with vander waals’ molecular volumes, \( V_{2}^{vdw} \) and \( V_{\phi}^o \) with molecular volumes in the crystal lattices, \( V_{2}^{mol} \). These equations also showed that \( V_{\phi}^o \) values were two-times more sensitive to variations of van der Waals’ molecular volumes and one and a half times more sensitive to changing \( V_{2}^{mol} \). The resulting correlation equations helped to estimate \( V_{\phi}^o \) values on the basis of only \( V_{2}^{vdw} \) or \( V_{2}^{mol} \) (X-ray data) whose values were known, without carrying out any densimetric experiments.

Their work also included the analysis of the influence of the nature and location of substitutes on the free volume per molecule both in the octanolic solutions, \( V_{2}^{free} \), and in the crystals, \( V_{2}^{free} \) (cr); the determination and analysis of the increments of \( V_{\phi}^o \) and \( V_{2}^{free} \) between the unsubstituted molecules and their different isomers; the introduction and comparison of the parameters characterizing molecular packing density in the solutions and the crystals.

Perlovich et al. [193] studied naproxen by classical thermo analytical methods namely sublimation calorimetry, solution calorimetry and the solubility method. Temperature dependence of a saturated vapor pressure was obtained and the sublimation enthalpy, \( \Delta H_{sub}^o \) and entropy, \( \Delta S_{sub}^o \) and their relative fraction of the total process were calculated. These parameters for naproxen were compared to the respective data of other naphthalene derivative.

The crystal lattice energy of naproxen was calculated by two force fields and compared to the experimental data and contributions of different motifs of the naproxen molecules to the total packing energy were analyzed. The Gibbs energy of solvation as well as enthalpic and entropic terms in aliphatic alcohols were studied for naproxen, and compared to the model substances and other non-steroid anti-inflammatory drugs (benzoic acid, diflunisal and flurbiprofen). The major driving
force of the solvation process is the enthalpy. The respective contributions of the specific and the non-specific solvation interactions in terms of absolute and relative values were investigated.

Iqbal and Chaudhry [194] studied the densities and viscosities of salicyl amide, salicylic acid and acetyl salicylic acid (non-steroidal anti-inflammatory drugs) in two aprotic solvents namely dimethyl sulfoxide and acetonitrile at different temperatures in various molality range. The data were used to calculate apparent molar volumes, $V_\phi$ and viscosity $B$-coefficients. The partial molar volumes and hydration numbers were also calculated. The effect of temperature on the thermodynamic properties was studied by determining partial molar expansibilities, $E_\phi^\circ$ and Hepler’s constant, $(\partial^2V_\phi^\circ / \partial T^2)$. Free energies of activation for viscous flow of the solution, $\Delta\mu_2^\circ$ were obtained by the application of transition state theory of solutions to the $B$-coefficient data and the corresponding activation enthalpies, $\Delta H^\circ$ were also reported. Their study revealed a strong drug-solvent interaction because their partial molar volumes were positive. The positive Hepler’s constant, $(\partial^2V_\phi^\circ / \partial T^2)$ suggested that all the three drugs were structure-makers in the aprotic solvents used in these systems. The values of $B$-coefficients were also positive for these compounds. It was indicative of the structure-promoting affinity of the three drugs studied. The Gibb’s free energy values of solute molecules, $\Delta\mu_2^\circ$ were positive for all the three drugs indicating the strong interactions between the drug and solvent molecules in the ground state than in the transition state.

Dhondge et al. [195] reported the density and viscosity values for aqueous solutions of the drugs like Metformin Hydrochloride (MH), Ranitidine Hydrochloride (RH) and Tramadol Hydrochloride (TH) at different temperatures within the concentration range (0 to 0.15) mol.kg$^{-1}$. The density and viscosity data were used to obtain apparent molar volume of solute, $V_\phi$ and relative viscosity, $\eta_r$, of aqueous solutions at different temperatures. The limiting apparent molar volume of solute, $V_\phi^\circ$, limiting apparent molar expansivity, $E_\phi^\circ$, thermal expansion coefficient, $\alpha$, hydration number, $n_{H}$, viscosity $A$- and $B$-coefficients, experimental slope, $S_v$ at different temperatures and temperature coefficient of $B$, i.e. $(dB/dT)$ at 298.15 K were also calculated. The $V_\phi^\circ$ values increase with increase in temperature for all the studied
aqueous solutions indicating the presence of strong ion-solvent interactions. The values of $E^\phi_\psi$ are all positive for all the systems and increase with increase in temperature. All the solutions show the positive values of Hepler’s constant suggesting that all the solutes act as structure-makers when dissolved in water. The increasing order is: $TH < MH < RH$. The largest values of $\alpha$ for MH suggests that water around it is loosely bound, which gives rise to the higher value of expansivity. The viscosity $A$-coefficients for all the drugs studied in this work are small, positive and temperature insensitive. The values of $B$-coefficients increase with increase in temperature for the $(MH + H_2O)$ system. For the $(RH + H_2O)$ system, it increases marginally with increase in temperature initially but decreases marginally with further rise in temperature. In the case of $(TH + H_2O)$ system, it decreases initially with rise in temperature and with further rise in temperature, it further decreases marginally. The values of $(dB/dT)$ at $T = 298.15$ K is small and positive for $(MH + H_2O)$. For $(RH + H_2O)$ and $(TH + H_2O)$ systems, the values of $(dB/dT)$ at $T = 298.15$ are found to be small and negative. Thus, it can be concluded that MH acts as weak structure breaker and RH and TH act as a weak structure makers. The values of $n_3$ decreases with increase in temperature for the $(MH + H_2O)$ and $(RH + H_2O)$ systems. This suggests that the extent of the co-sphere water around the ions decreases if the temperature is increased. On the other hand in case of $(TH + H_2O)$ system, hydration number increases with rise in temperature.

Pal and Chauhan [196] studied the volumetric interactions of amino acids and peptides with the drug pentoxyfylline in aqueous solution at various temperatures. Pentoxyfylline is used to improve blood flows through peripheral blood vessels. The density of three amino acids and two peptides (glycine, $l$-alanine, $l$-valine, glycylglycine and glycylglycylglycine) were measured using DSA 5000 instrument at different temperatures in aqueous solution of this compound. The apparent molar volume, $V_\phi^0$, partial apparent molar volume, $V_\phi^0$, transfer partial molar volume, $V_{\phi,T}^0$, partial molar expansibility, $E^\phi_\psi$, thermal expansion coefficient, $\alpha$ and Hepler’s constant, $(\partial^2 V_\phi^0/\partial T^2)$ were calculated from the density data. These parameters were used to interpret the solute-solute and solute-solvent interactions of amino acids/peptides in aqueous pentoxyfylline solution. The dependence of these parameters upon concentration and temperature clearly suggested the role of amino acids/peptides and pentoxyfylline in solute-solvent interactions.
Therefore, in living organisms, proteins (in other words their constituents amino acids) interact with carbohydrates and this interaction has been recognized to play a key role of biochemical processes [160]. Carbohydrates play a vital role in the biological and food industries. It is widely recognized that carbohydrates as co-solutes help in stabilizing biological macromolecules. Experimental findings indicate that this action is performed either as a result of direct interactions between them and/or through alteration of the water structure [197,198]. Carbohydrates located at cell surfaces act as receptors with regard to the bioactive structures of hormones, enzymes, viruses, antibodies, etc. [199]. Also, the studies of protein-carbohydrate interactions are very important for immunology, biosynthesis, pharmacology and medicine.

Also, drug-biomolecular interactions are important in physiological media such as blood, membranes, cellular fluids, etc. The processes of drug transport, protein binding, anesthesia, etc. are a few examples where drugs and bio-macromolecules appear to interact in an important and significant manner [200,201]. Most of the compounds of medicinal interest undergo a number of complicated interactions of varied nature [201,202]. Drug action has been widely recognized to be the ultimate consequence of physicochemical interactions [202,203], between the drug and functionally important biomolecules in the living organisms. Despite years of investigations many important drug actions and their mechanisms are not fully understood. Because of the complexities associated with macromolecules, it is generally difficult to carry out direct studies on physiological media with a view of drug action mechanism. Hence, the studies of amino acids in aqueous-drug media would be very useful in understanding the drug-biomolecular interactions in physiological media.

A complete understanding of the mechanism of stabilization of proteins by these additives (carbohydrates/drugs) still lacks in the literature. Therefore, a systematic study of amino acids-carbohydrate/drug interactions in aqueous solutions can provide valuable information about their behavior in solution and hence, the stability and interactions of larger biomolecules in solutions. Also, the knowledge of thermodynamic properties of amino acid-carbohydrate/drug interactions in aqueous medium will be helpful for the development and design of new separation and purification processes of biomolecules in biotechnology and other related fields.
1.7. OBJECTIVES OF THE RESEARCH WORK

- To provide an understanding of the interactions of amino acids with carbohydrate/drugs in aqueous media.
- To provide physicochemical and thermochemical data on amino acids/peptides in these mixed aqueous media, which would be helpful in estimating physicochemical and thermodynamic properties of macromolecules (proteins) in these media.
- To be helpful in ascertaining the mechanism of drug action in physiological media.
- To provide better understanding of several important biochemical and physiological processes.
- To establish useful correlation between physicochemical/thermodynamic properties of amino acids/peptides and many biological/physiological phenomena involving biopolymers (proteins).
- To be useful for the development and design of new separation and purification processes of biomolecules in biotechnology and other related fields.
REFERENCES


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