INTRODUCTION

1.1 General Introduction

Saccharides and their derivatives are essential components of living systems. These are important due to their hydroxyl (–OH) rich periphery, coordinating ability, homochirality and stereospecificity. Saccharides interact with the aqueous environment through numerous hydroxyl groups and build hydrogen bonds\(^1\)\(^-\)\(^4\). Depending upon their molecular weight, saccharides can be divided into three subgroups, such as mono-, oligo- and high molecular weight poly-(cellulose, chitin, chitosan, starch, amylose, amylopectin, agarose, and xylan) saccharides. As integral parts of some coenzymes, nucleic acids (DNA and RNA), glycoproteins and glycolipids, they perform multiple roles from the storage and transport of energy to participation in the immune system, fertilization, development, pathogenesis, signaling, cell-cell recognition, and molecular and cellular communication. Saccharides located at cell surface are receptors with regard to the bioactive structures of hormones, enzymes, viruses, antibodies, etc\(^5\)\(^-\)\(^12\).

To get a better understanding of biological phenomena, low molecular weight model compounds such as alcohols, saccharides, peptides, nucleic acid bases, nucleosides and nucleotides have been studied because of the complexities of biomolecules\(^13\). The hydration characteristics of saccharides in aqueous solutions are of direct relevance for understanding the role of glycoproteins and glycolipids in molecular recognition. Therefore, an increasing interest in biophysical and biochemical research is presently being directed toward the novel subdiscipline “glycobiology”\(^14\)\(^-\)\(^17\).

Saccharides and polyhydroxy compounds are well known protein / enzyme’s stabilizing agents. In an aqueous environment, co-solutes such as disaccharides cause an increase in the thermal denaturation temperature of proteins\(^18\). Lee & Timasheff\(^19\) studied the thermal transitions of \(\alpha\)-chymotrypsin, chymotrypinogen and ribonuclease in sucrose and argued that the sucrose stabilize proteins against thermal damage. However, the mechanism by which stabilization is imparted still belongs to a realm of speculation\(^20\)\(^-\)\(^23\).

There has been a growing interest for disaccharides especially concerning their hydration, glass transition and biopreservation properties and much less work was devoted to their crystallization in aqueous medium\(^24\)\(^-\)\(^26\). Trehalose is well-known for its ability to
preserve life in cells, organisms, and biomolecules under conditions of extreme drought or low temperatures. There are evidences that high concentrations of trehalose in the tissues of certain insects, yeasts, lactobacillus, mushrooms, and desert plants allow them to survive for decades or centuries, in a state of suspended animation under conditions of water deficiency, resuming rapidly their active metabolism when rehydrated. Disaccharides such as maltose and sucrose have shown similar properties but trehalose is found to be more effective. Several mechanisms for the antidesiccant properties of saccharides have been proposed, but their protective mechanisms are not well understood.

Saccharides are often used in pharmaceutical, food, and biomedical applications to prepare a glassy matrix for long term storage of biological materials. Rheological properties of saccharide solutions are applied for the control of gelling processes and osmoregulation of tissues and organs in cryoprotection. Maltose consisting of two glucose units linked by a glycosidic bond is widely used in the food industry. The micro- and macro-scopic studies of sweetner-H₂O interactions are useful to understand sweet taste chemoreception. Mechanism of taste chemoreception is thought to depend upon the role of water, molar volume and stereochemistry of the molecule, which affect their fit within the water structure. Various solution properties (apparent specific volume and apparent molar isentropic compressibility) of different sapid substances have been studied, but the mechanism of taste chemoreception is still not fully understood.

The hydration characteristics of saccharides and their interactions with electrolytes and non-electrolytes in aqueous media are of significant thermodynamic and biochemical importance. Most biological systems contain ionic solutes (Na⁺, K⁺, Ca²⁺) which play a vital role in various metabolic activities. It has been reported that synthesis, metabolism, decomposition and trans-membrane transport of saccharides is related to the concentration of Na⁺, H⁺ and other metal ions in body fluid. The Mg-sugar complex has a stabilizing effect on the DNA double-helix, sugar-metal complexes participate in the ATP cell energy cycle. Due to the fact that saccharides readily undergo isomerization and conformational changes, their complexation with metal ions result in the formation of a mixture of products, although interactions involved are weak, but selective.

The presence of salts modifies important properties of aqueous saccharide solutions related to their protective roles such as viscosity, water sorption, crystallization rate and glass
transition temperature, $T_g$. The addition of disodium tetraborate (borax) to aqueous solutions of saccharides and other polyols results in various complexes between the borate ion and the saccharides\textsuperscript{45-47}. Aqueous solutions of sodium acetate are widely used in molecular biology applications, purification and precipitation of nucleic acids, protein crystallization, staining of gels in protein gel electrophoresis, and HPLC\textsuperscript{48-49}. Sodium gluconate occurs naturally in plants and foodstuffs, is non-toxic, the gluconic acids salts are utilized in mineral supplementation where the acid acts as a carrier of metal ions of therapeutic importance\textsuperscript{50}.

Physicochemical studies of saccharides in mixed aqueous solutions are of great importance to understand the solvation behaviour of saccharides as a function of concentration and temperature and how the solute-solvent interactions get modified in the presence of co-solutes (additives)\textsuperscript{33,40}. Therefore, thermodynamic and transport properties of solutions characterizing the solvation behavior of saccharides are needed which will be useful for better understanding the various phenomena like protein stabilities, taste chemoreception, antidesiccation properties, etc.

Saccharides have a very high affinity for water and their hydrogen bonding interactions with aqueous solvents occurs through their hydroxyl groups. The hydrogen bonding between saccharide and water is more extensive than that between water molecules themselves and these interactions (hydrogen bonding) are stereo specific in nature. Saccharides influence the surrounding water molecules and that in return, affects the structure of the dissolved saccharide molecules\textsuperscript{51-52}. The nature of these interactions is responsible for most biological functions of saccharides such as energy transport and storage, sweet taste chemoreception, signal transmission, biomolecular recognition, protein stabilization, gel phase formation, etc\textsuperscript{20,34-35,53}. So, for understating the role of water in characterizing the hydration behaviour of saccharide in mixed aqueous solutions, a brief description about the structure of water is needed, which is described in the next section.

1.2 Structure of Water

Water, due to its ubiquitous presence on the Earth and in living organisms, is the key compound for our existence on this planet and it is involved in nearly all chemical, biological and geological processes\textsuperscript{54-55}. In most cases, water contains dissolved salts and it is in such aqueous solutions that the chemistry of life takes place. Despite the apparent simplicity of the
water molecule, the condensed phases of water, such as liquid water and ice, are the most mysterious substances in our world\textsuperscript{56-58}. Being the solvent of life, water has attracted the most scientific attention among liquids, in the past several decades. In addition, water also plays an important role as a mediator in interactions in proteins, which stabilize their structure\textsuperscript{59-60}. The most important property of liquid water is its unique ability to form a network of self-associated molecules through hydrogen bonding and that causes the anomalousness in physical properties of liquid water\textsuperscript{61-62}. In the past years, a large number of studies have been carried out in order to understand the structure of water and dynamics of the hydrogen bonding network in water that give rise to different unique properties\textsuperscript{63-65}.

Bernal and Fowler\textsuperscript{66} and later Morgan and Warren\textsuperscript{67}, carried out the X-ray diffraction measurements on liquid water and revealed that the water is tetrahedrally coordinated through hydrogen bonds, similar to that in ice I (Fig. 1.1). Tammann\textsuperscript{68} suggested that there should be as many different 'kinds' of water as there are different phases of ice. The complexity of liquid water has given a great variety of proposed water models which are classified as; (a). Continuum model, (b). Mixture models.

\textbf{1.2.1 Continuum Model}

It assumes almost completely hydrogen bonded water molecules in a continuous network, where the distortion of hydrogen bonds results in a continuous distribution of hydrogen bond distances, energies and angles. First structural theory work was done by Bernal and Fowler\textsuperscript{66}, and later Pople\textsuperscript{69} established the modern continuum theory of water and this theory accounts for the heat capacity and thermal energy of water. The thermodynamic properties of liquid water will, of course, depend on the energetics of the bending and stretching of the hydrogen bonds; it will also depend on the intra- and inter-molecular vibration frequencies, which will be considerably altered by the deformations of the hydrogen bonds.

Recently, Random Network Model (RNM), which is a highly successful continuum model, has been proposed by Rice and Sceats\textsuperscript{70} and further developed by Henn and Kauzmann\textsuperscript{71}. It does not involve changes in the number of hydrogen bonds. RNM model is a good model for determining the heat capacity contribution due to water-water interactions and also this model gives a good insight into the reorganization of water around the non-polar and polar solutes. This model also provides a simple mechanism for energy absorption, variations of dielectric properties and X-ray radial distribution function with temperature as a
variable. However, in view of the restrictions placed on molecular motions, this model fails on logic to explain the trend in entropy data and the hydration properties of non-polar molecules.

Fig. 1.1 Schematic representation of crystal structure of ice I at low pressure

1.2.2 Mixture Models

These models visualize liquid water as a collection of different hydrogen bonded species in which each water molecule can fluctuate through states where one or both of its H atoms are engaged in hydrogen bonding. Depending on the type of species involved, the mixture models have been classified as; (i) Interstitial Model proposed by Samoilov and Forslind (ii) Water Hydrate Model proposed by Pauling (iii) Flickering Cluster Model proposed by Frank and Wen, and Frank. The most popular and commonly used model, especially in the field of solution chemistry is the ‘Flickering Cluster’ Model.

Flickering Cluster Model

According to Frank and Wen, and Frank water is a mixture of clusters of hydrogen bonded water molecules with voids therein (bulk water) and free unbonded monomers (dense water) in equilibrium with each other (Fig. 1.2). The formation of hydrogen bonds and the disruption of hydrogen bonds in such a polymeric complex is considered as cooperative phenomenon. The physical basis of this cooperativity is that hydrogen bond formation involving one bonding site of a water molecule contributes delocalization energy to the molecule, which favours additional bonding at other potential hydrogen bonding sites. According to this model, the presence of flickering clusters of water molecules (Fig. 1.2) of short half-life time \(10^{-11} \text{ to } 10^{-10} \text{ s}\) reflects local energy fluctuations. The occurrence of some type of dynamic monomer-polymer equilibrium in water allows an acceptable explanation of the free energy of activation of transport processes in water, whether
calculated from self-diffusion, viscous flow, dielectric relaxation, or ultrasonic absorption data.

Fig. 1.2 Frank-Wen Flickering Cluster Model of Liquid Water

Nemethy and Scheraga\textsuperscript{73} used a statistical thermodynamic treatment to calculate the Helmholtz free energy, internal energy and entropy as a function of temperature of liquid water, assuming that each of the hydrogen bonded molecules could be associated to a discrete energy level in the flickering clusters. Hagler \textit{et al.}\textsuperscript{83} and Lentz \textit{et al.}\textsuperscript{84} have modified this model to remove some major inconsistencies prevalent in it as pointed out by Nemethy\textsuperscript{85}. Walrafen\textsuperscript{74} and Hartman\textsuperscript{86} provide the strongest support for the mixture model from the Raman Spectroscopic studies of HOD. These studies reveal a pronounced shoulder in the high frequency side along with the intense low frequency band. Further these studies show that as the water is heated, the intensity of the high frequency component increases compared to that of low frequency component.

The high frequency component was associated to the monomeric species and the main band of the low frequency to the hydrogen bonded species. It was further argued that the increasing intensity of the high frequency component with increase in temperature reflects the conversion of hydrogen-bonded molecules to the non-hydrogen bonded molecules (Fig. 1.3). The mixture model thus appears to be consistent with the vibrational spectroscopic data.

Recently Robinson and Cho\textsuperscript{87} have also reported that on average, water is composed of dynamically transforming microdomains of two very different bonding types i.e. regular tetrahedral water-water bonding similar to that of ordinary ice i.e. Ih and other is a more dense non-regular tetrahedral bonding similar to that in ice II. Although many water models are
successful in predicting selected liquid water and ice polymorph properties, there is still a need to construct a model of general applicability\textsuperscript{88-89}.

Fig. 1.3 (a) Argon-Ion-Laser-Raman spectrum from a 6.2 M solution of D\textsubscript{2}O in H\textsubscript{2}O at 25\textdegree C. (b) Quantitative mercury-excited Raman spectra from a 6.2 M solution of D\textsubscript{2}O in H\textsubscript{2}O at a series of temperature.

1.2.3 Computer Simulations

Different experimental techniques such as neutron scattering, X-ray scattering, infrared absorption, and NMR have been used to probe the microscopic structure of water\textsuperscript{90-94}. The information gathered from these experiments and from theoretical calculations\textsuperscript{95-96} has led to the development of around twenty "models" that attempt to explain the structure and behavior of water. With the advent of the supercomputers, computer simulation methods based on statistical thermodynamic and molecular dynamic theories of mixtures, to understand the structure of liquid water have become accessible\textsuperscript{97-98}.

Most commonly used computer simulation methods are: (1) Monte Carlo (MC)\textsuperscript{99}, (2) Stochastic Dynamics\textsuperscript{100} (3) Molecular Dynamics (MD)\textsuperscript{101} (4) Normal mode analysis\textsuperscript{102} (5) Energy minimization\textsuperscript{103}. Among these, Monte Carlo and Molecular Dynamics provide the most promising approaches for structural characterization of liquid water\textsuperscript{101}. The former
method offers the possibility of generating canonical ensembles of given temperature, but it is entirely restricted to a study of static structural properties. MD, which is nominally microcanonical, can probe both static and kinetic behavior, so in this method, the temporal evolutions of the translational and rotational trajectories of the individual molecules are followed under the joint influence of the forces and torques exerted on it by all other molecules. These trajectories, then yield the time autocorrelation function and hence various kinetic properties of the liquid water\(^9\).

Results of both MC and MD methods indicate that there exists a marked tendency of liquid water toward hydrogen bond order, but with much less predictable geometry. However, the study of ice by crystallography alone would have suggested this. The potential used in the MC or MD calculations are all pair wise additives i.e., the energy of a given assembly is expressed as a sum of the interaction energies between all pairs of molecules in the assembly. It has been stressed that none of these pair potentials can account for both gas and condensed phase properties of water\(^10\). It is evident from quantum mechanical calculations on water trimers, tetraters and pentamers that hydrogen bonding in these clusters is stronger than in dimer, which suggests the cooperative nature of hydrogen bonding interactions\(^10^4\).

Alder and Wainwright\(^10^5\) were the first researchers to perform the MD simulation, followed by Barker and Watts\(^10^6\) and later by Rahman and Stillincer\(^10^7\) and they published the first computer simulations of liquid water. Rahman and Stillincer\(^10^7\) using MD technique examined both static structural properties and the kinetic behavior at nominal temperature, 34.3 °C. They concluded that the model employed in the research work can be adapted to simulation of a simple solute.

Sokol et al.\(^5^7\) carried out the MD simulation of water in solid, liquid and gaseous state. They performed simulations for two density cases, normal ($\rho = 1\text{g cm}^{-3}$) and lower density ($\rho = 0.251\text{ g cm}^{-3}$), for four different temperatures. The calculated structural and dynamical properties were also compared to earlier experimental and simulated results.

Krishnan et al.\(^10^8\) carried out the computer simulation based on sophisticated and empirical model of interaction developed by Rick et al.\(^10^9\). Krishnan and coworkers\(^10^8\) have obtained the minimum energy structures of water clusters up to a size of ten and reported that the cluster structures agree well with experimental data. They were able to identify the hydrogens that form hydrogen bonds based on their charges alone, a feature that was not
possible in simulations using fixed charge models. They also used fluctuating charge model in order to study the structure of liquid water at ambient conditions.

Beveridge *et al.* \(^{110}\) used metropolis MC method to study the hydrophobic interaction of alkyl and phenyl groups in water and solvent effects on the conformational stability of the alanine dipeptide and the dimethyl phosphate anion in water.

Jorgensen\(^{95}\) has developed transferable intermolecular potential functions (TIPS) suitable for use in liquid simulations for water. This potential has been used by Jorgensen and Madura\(^{111}\) in MC simulation on liquid water to study the effect of temperature on vaporization, hydrogen bonding, density, isothermal compressibility and radial distribution functions. Recently, Mahoney and Jorgensen\(^ {112}\) by applying classical MC statistical mechanical simulations optimized a five-site, fixed charge model of liquid water with emphasis on improving the computed density as a function of temperature and the position of the temperature of maximum density. The obtained results further support a two state mixture models for the liquids.

Wang *et al.*\(^{113}\) have performed DFT (density functional theory)-based AIMD (ab initio molecular dynamics) simulations of liquid water with different GGA (generalized gradient approximation) and vdW (van der Waals density) functionals and different densities, comparing the structural and diffusive properties. It was concluded that a better treatment of the exchange interaction together with vdW correlations might provide a better agreement with experimental results in terms of structure, density, and diffusivity of liquid water.

Recently, Kalinichev and Bass\(^ {114}\) performed MC computer simulations under thermodynamic conditions corresponding to available X-ray and neutron diffraction measurements of the supercritical water structure and found a good agreement between their results with other recent experimental and simulated data under similar thermodynamic conditions.

From theoretical and simulation methods\(^ {115-116}\), various other workers also have reported that linear molar volume of relevant inherent structures contributing to the liquid phase increases with the decrease in temperature. Owicki and Scheraga\(^ {117}\) observed a broad, smooth distribution of hydrogen-bond energy for liquid water, rather than relatively discrete sets of the bonded and unbonded interaction energies.

Grunwald\(^ {118}\) developed a model for the structure of the liquid water network using the concept of the two state models. The model is consistent with free energy and its temperature
derivatives, the dielectric constant, the probable number of hydrogen bonded nearest neighbors, intermolecular vibration frequencies and dielectric relaxation dynamics.

Dore have emphasized that the simulation methods will need to be used in conjunction with experimental data for more understanding of water and its anomalies. Inspite of enormous experimental and theoretical studies to explore water structure, a compact and useful description is yet to be achieved.

1.3 Structure of Aqueous Solutions

According to ‘Flickering Cluster’ model, water molecules can be considered to be in dynamic equilibrium between the bulky tetrahedrally hydrogen bonded clusters and monomer molecules, as represented by: \((H_2O)_n \Leftrightarrow n(H_2O)\), where \((H_2O)_n\) and \(n(H_2O)\) represent 'bulky' and 'dense' states, respectively. The statistical degree of ice-likeness (or whatever its structure in water) is considered to be proportional to the half-life \((10^{-11}\text{s})\) of the clusters, which may shift the equilibrium in either direction. The solute which causes a shift so as to increase the average half-life of the cluster is termed as a ‘structure maker’ and a solute, which has an effect in the opposite direction, is called a ‘structure breaker’.

The concept of ‘structure-making’ and ‘structure-breaking’ by solutes has been proved to be useful in understanding the effects of solutes on the water structure. These effects can be reflected experimentally by observing the changes brought about by the solutes in the kinetic properties of water such as fluidity, reorientation time, viscosity, conductance, etc. For instance, structure makers are shown to decrease the fluidity of water by causing an increase in reorientation time and increase in viscosity and result in positive excess partial molar heat capacities in water. The reverse is true for structure breakers.

Hydration of different kinds of solutes, depending upon their nature is classified as: (i) Ionic and polar group hydration; (ii) Hydrophobic hydration; (iii) Hydrophobic interactions.

1.3.1 Ionic and Polar Group Hydration

 Ionic hydration is one of the fundamental physicochemical processes related with many research fields in chemistry including stability of biomolecules and chemical reactions. For example, the crystal lattice of salts like NaCl is held together by very strong electrostatic attractions between ions. However, water readily dissolves crystalline NaCl because of very strong electrostatic attractions between \(Na^+/or Cl^-\) ions and water dipoles. This leads to very stable hydrated \(Na^+\) and \(Cl^-\) ions, ion-solvent interactions greatly exceed the tendency of \(Na^+\) and \(Cl^-\) ions to attract each other. When an ion is introduced into water, it interferes with the
local ordering of the water molecules in its neighbourhood and tends to impose a new order on them. Ions of simple electrolytes such as LiF and MgCl$_2$ having high charge density act as net structure makers and the ions of electrolytes like CsI and KBr with low charge density behave as net structure breakers. In order to explain these phenomena, Frank and Wen$^{72}$ visualized a picture (Fig. 1.4) in which an ion is surrounded by three concentric regions of water molecules as described below:

(i) An innermost structure-formed region 'A' consisting of polarized, immobilized and electrostricted water molecules.

(ii) An intermediate structure-broken region 'B' in which water is less ice-like i.e. more randomly ordered than the normal water;

(iii) An outer region 'C' in which water molecules have the normal liquid structure, which is polarized in the usual way as the ionic field at this range will be relatively weak.

![Fig. 1.4 Frank-Wen multizone model of ion-hydration in aqueous solutions](image)

In the electrostrictive region, ionic charge on the ion completely orients the surrounding water molecules, which become immobilized and firmly packed around the ion in comparison to bulk water and this phenomenon is called as positive hydration. The water in the second and third solvation layers is less immobilized and orientation of water molecules is diminished but it is enough to interfere with the formation of normal bulk water. The decreased structure in region 'B' is presumably due to approximate balance of the two competing forces, namely, the normal structure-orienting influence of the neighbouring water molecules and the radially-orienting influence of the electric field of the ion which acts simultaneously on any water molecule in this region. The latter ionic influence predominates
in region ‘A’ and the former in region ‘C’ and between ‘A’ and ‘C’, according to Frank and Wen\textsuperscript{72}, there is a region of finite width in which more orientational disorder should exist than in either ‘A’ or ‘C’. Thus, water in this region has less structure (or there are fewer hydrogen bonds) and ion can be classified as structure-breaking or negative hydration ion.

The relative size of these three regions depends on the charge, size and composition of the ion. In general, the ions with low charge density have relatively weak electrostatic fields which makes the region ‘A’ very small thereby causing the net decrease in the structure. On the other hand, ions of high charge density increase the region ‘A’ and might induce additional structure (entropy loss) of some sort beyond the first water layer, thereby causing a net structure-making effect.

Friedman and Krishnan\textsuperscript{121} have proposed a model for aqueous ionic solutions primarily based on the ideas of Frank and Evans\textsuperscript{122}. Gurney\textsuperscript{123} and Samoilov\textsuperscript{79} with the assumption that around each solute particle $x^z$ ($z =$ charge on the ion) there is a region termed as cosphere, having the thickness of one solvent molecule in which solvent properties are affected by the presence of the solute. These effects are characterized by the thermodynamics of the process: $n$ [Solvent(pure bulk liquid)] $\rightarrow$ $n$[Solvent (in cosphere state next to $x^z$)] where, $n$ is the number of solvent molecules in the cospheres. Friedman and Krishnan\textsuperscript{121} model emphasizes on the contribution of the various cosphere states to the thermodynamic properties of solutions, while Frank and Wen model\textsuperscript{72}, and more recently Stillinc
er and Ben-Naim model\textsuperscript{124} of ionic solutions being rather qualitative, are supported by the experimental studies\textsuperscript{125} of kinetic properties, X-ray and neutron scattering. Friedman and Krishnan\textsuperscript{121} have compiled the various thermodynamic properties and have given reasonable explanation of these properties based on their cosphere model.

Polyfunctional solutes e.g. saccharides exhibit very complex behaviour in aqueous solutions which is difficult\textsuperscript{3,126-127} to rationalize as both hydrophilic and hydrophobic types of hydration are contributing.

### 1.3.2 Hydrophobic Hydration

Non polar solutes (hydrocarbons and ions) having non polar groups e.g. substituted hydrocarbons and alkyl-ammonium salts, behave quite differently in water as compared to the ionic solutes, as these are readily soluble in most non polar solvents, but only sparingly soluble in water\textsuperscript{128-129}. In thermodynamic terms, the low solubility of non polar solutes means that the transfer of the solute molecules from the pure liquid alkane to dilute aqueous
solution. This process is accompanied by large decrease in entropy which makes the free energy of their solution positive in spite of their exothermic dissolution in water. It seems natural to regard the insolubility as being ‘entropy controlled’. This behaviour of non polar solutes is apparently uniquely characteristic of aqueous solutions. The negative entropy and enthalpy change and positive change in heat capacity upon the addition of non polar solute to water indicates that the packing of water molecules around the non polar solutes is somewhat tighter than in pure water called a ‘structure increase’ or enhancement of structure of water (Fig. 1.5). This region is also known as iceberg formation or hydration of second kind or hydrophobic hydration although the structure is not necessarily identical with that of ice. These models assume the formation of short-lived aggregates of water molecules around the solutes, having more or less well defined structure, called as ‘icebergs’, ‘flickering clusters’, ‘clathration shells’, ‘polyhedral cages’ similar to those found in the crystalline hydrates formed by some small non polar molecules. These models are consistent with the view that considers, inert non polar solutes as ‘structure makers’. The formation of polymeric aggregates involves strengthened hydrogen bonding, which would give negative contribution to $\Delta H^\circ$. The solutes imposed structure melts away on heating the solutions which involves breaking of some of the excess hydrogen bonds resulting in an increase in the heat capacity.

Ben-Naim also discussed the stabilization of water structure caused by the addition of non electrolytes that arise from the difference in the change of the chemical potentials of the clusters and of the monomeric water molecules. He also pointed out that the penetration of solute molecules into the cavities of the relatively open structure of the clusters enhances the stabilization effect, although filling up of the cavities is not an essential requirement for the existence of a stabilizing effect. A number of hydrates of the noble gas atoms, hydrocarbons, halogens and of many other simple molecules are known to exist. Frank has pointed out that the induced ice-likeness, cannot consist of clathrate formation in the liquid phase, although ice-like structure could be transitory fragments of clathrate-like aggregates.

According to Privalov and Gill, there are two temperatures $T_H$ and $T_S$ of universal nature that describe the thermodynamic properties for the dissolution of liquid hydrocarbon into water. $T_H$ is the temperature at which heat of solution is zero, which is around 20 °C for a number of liquids, $T_S$ is the other universal temperature around 140 °C where entropy of transfer of non polar compounds into water is zero, and the process of transfer is most
unfavourable. Further at any temperature other than $T_S$, the net effect of hydration of non polar solutes favours the transfer of non polar molecules from the compact state into water. Thus, according to their views, the hydration effect of non polar solutes stabilizes the dissolved state and this itself cannot be regarded as the cause of their hydrophobicity.

Fig. 1.5  Diagrammatic representation of
(a) Hydrophobic hydration and (b)-(e) hydrophobic interactions;
(b) Kauzmann-Nemethy-Scheraga contact interaction;
(c) Globular protein folding;
(d) Proposed solvent-separated interaction; and
(e) Possible stabilization of helix by interaction as in (d)

Muller$^{128}$ has given another view that at and above $T_S$ water retains though to a lesser extent, the same capacity for structural reorganization which makes it a unique solvent at 25 °C. This modified ‘hydration shell hydrogen bond model’ (HSHB) assumes that the progressive breaking of hydrogen bonds on heating accounts for about half of the observed heat capacity of bulk water, and the bond breaking process is perturbed by the introduction of a solute that give rise to $\Delta C_{\text{p,2}}$. 

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According to Lumry et al.\textsuperscript{77,134} and Ben-Naim\textsuperscript{132} there is an exact compensation for the entropy and enthalpy changes that arises from change in the state of water in the presence of non polar solutes i.e. ordering of water, and thus this effect does not contribute to the hydration Gibbs energy. However, Privalov and Gill\textsuperscript{131} and Murphy et al.\textsuperscript{135} have pointed out that large heat capacity change plays the key role in regulating the hydration free energy change and the principal feature of hydration process.

Borisover et al.\textsuperscript{136} suggested a new method of estimating the hydrophobic effect contribution to the thermodynamic functions of hydration of non electrolytes. Their results indicate that enthalpies of the hydrophobic effect for alkanes are negative which are similar to that reported by Belousov and Panov\textsuperscript{137} but contrary to Abraham’s opinion\textsuperscript{138} according to which enthalpy is positive. Borisover et al.\textsuperscript{136} from these studies also indicated that the rare gases exhibit hydrophobic hydration.

Costas et al.\textsuperscript{139} have considered that the water molecules around solute undergo a relaxation process, which lowers the Gibbs energy, enthalpy and entropy of the system and is responsible for large $\Delta C_{P,2}$. The origin of hydrophobicity lies in high cohesive energy of water being counteracted by relaxation of water molecules in the neighborhood of the hydrophobic solute.

Madan and Sharp\textsuperscript{129} have reported that non polar solutes have a large positive heat capacity of hydration, $\Delta C_p$, while polar groups have a small negative $\Delta C_p$ of hydration. The increase in heat capacity of non polar solutes can be as large as 12 J K\textsuperscript{-1} mol\textsuperscript{-1} per first shell water at 25 °C, a surprisingly large change when compared to the total heat capacity of water, 75 J K\textsuperscript{-1} mol\textsuperscript{-1}. As a consequence of the large heat capacity increase, the low solubility of apolar groups in water at higher temperatures (>90 °C) is caused by unfavourable enthalpic interactions and not because of unfavourable entropy changes. The hydration of polar groups is accompanied by a small but significant decrease in $\Delta C_P$. Thus the solubility of polar groups in water is driven by favourable enthalpic interactions at both higher and lower temperatures. Talukdar and Kundu\textsuperscript{140} have studied the salt effect on the hydrophobic hydration and concluded that hydrophobic cations induce more hydrophobic hydration in aqueous NaNO$_3$ solution than in pure water.

1.3.3 Hydrophobic Interactions

The tendency of non polar groups to aggregate in aqueous solutions in order to minimize energetically unfavourable interactions with water is termed as the ‘hydrophobic
bonding or ‘hydrophobic interaction’. The entities can either be parts of independent molecules or ions, e.g., glycerides, phospholipids, surfactants, and dyestuffs, or side chains, covalently bonded to a common backbone, e.g., polypeptides or proteins. They play fundamental role in the stabilization of micelles, biomembranes, and native globular protein structures. These interactions are generally involved in many molecular recognition processes such as the specific binding of substrates in the active site of enzymes, quaternary protein structure formation, protein-DNA interactions and host-guest binding. Hydrophobic interaction is also one of the important factor in hydrophobic chromatography, a novel methodology for the purification of a variety of biological and industrial materials. These subjects are however problematic even on a semantic level.

Ide et al. have reported that non polar substitutents bring about structuring of water surrounding these groups. NMR studies carried out by Bagno et al. also showed that water-water bonding in the surrounding of hydrophobic side substituents are stronger than these within bulk water. Hechte et al. has also reported the increase in water clusters around alkyl side chain with increase in alkyl side chain length. A distinction is made between bulk and pairwise hydrophobic interactions in water. Bulk hydrophobic interactions, which lead to surfactant aggregates, such as micelles and lipid bilayers, are the resultant of the fact that insufficient water is available for the formation of independent hydration shells, so that association cannot be prevented. These aggregates are formed with a high cooperativity and London dispersion energy contributes significantly to their stabilization. Pairwise hydrophobic interactions on the other hand do take place via destructive overlap of independent hydration spheres. London dispersion interactions play a much less important role, since for these direct interactions, the hydration of solute is required. Ludemann et al. have reported very recently that till date there is no evidence for a qualitative difference between pair and bulk hydrophobic interactions.

The association of non polar moieties is assumed to be partial reversal of the solution process. The thermodynamic parameters for hydrophobic interactions, i.e. \( \Delta H^\circ > 0 \), \( \Delta S^\circ > 0 \), \( \Delta C_p^\circ < 0 \), \( \Delta G^\circ < 0 \) have opposite signs as compared to those of hydrophobic hydration. The hydrophobic interaction is accompanied by disordering of water structure, i.e. partial melting of the so called ‘iceberg’ or clathrate like structure that are produced when the separate non polar species are first introduced into water. The hydrophobic interactions are found to be unusual as compared to normal processes because these are temperature
dependent. At 25 °C, the transfer of non polar solutes to water is not principally opposed by the enthalpy, rather it is due to excess entropy\textsuperscript{131,133,144}. It arises from the water of solvation around the solute rather than from solute-solute interactions. Water surrounding the non polar solute prefers to hydrogen bond with other water molecules rather than to ‘waste’ hydrogen bonds by pointing them towards the non polar species\textsuperscript{151-152}. However, at higher temperatures the aversion of non polar solutes for water becomes ordinary and less entropy driven. The heat capacity change for transfer of non polar solutes from the pure liquid to water is large and positive\textsuperscript{56,131,133}, which implies that the enthalpic and entropic contributions are highly temperature dependent. The free energy is a nonlinear function of temperature, increasing at low temperature and decreasing at higher temperature. Hence there will be a temperature at which solubility of non polar species in water is a minimum. The free energy of transfer of non polar solute into water is extrapolated to be most positive in the temperature range 130-160 °C\textsuperscript{133-135,153}. Hydrophobic interactions are found to be at their maximum value at 140 °C and are determined entirely by enthalpic contributions. Hence water ordering is not the principal feature of the aversion of non polar residues of water.

1.4 Specific Molecular Hydration

The hydration effects in aqueous solutions of non polar solutes have been shown to be largely non-specific and are characterized by microheterogeneities (reflected in marked concentration dependence of physical properties of these solutes). Solvent molecules adjacent to non polar solutes interact with their external atomic groups thereby becoming structurally, dynamically and thermodynamically distinct from bulk water. The opposite is the case for highly polar solutes where specific interactions between proton donor or acceptor sites (e.g. −OH, −N−, −O, >C=O) on neutral molecules and water molecules occur. However, interactions which predominate in aqueous solutions of polyfunctional solutes are of rather different nature from those occurring in both hydrophobic and polar solutes.

The hydration characteristics of a saccharide molecule depend on its stereochemistry. It has been proposed that the anomic effect and the ratio of axial versus equatorial hydroxyl groups of a saccharide govern its hydration\textsuperscript{154}. “Specific hydration model” emphasized that in addition to the number of potential hydration sites, the relative position of the −OH groups at C-2 and C-4 appears to be a key factor. This is fully consistent with quantitative studies of kinetic medium effects\textsuperscript{3,16} and relevant thermodynamic measurements\textsuperscript{155}. Since the hydration interaction competes with the intermolecular hydrogen bonding in water, solute molecules
which are able to interact with the solvent without major perturbations in the solvent structure are likely to interact preferentially. Galema et al.\textsuperscript{156} have reported that molecular dynamics (MD) studies in aqueous solutions provide important insights into the hydration characteristics of saccharides.

Warner\textsuperscript{157} has pointed out that in many biological molecules, the spacing of ether, ester, hydroxyl and carbonyl oxygen atoms is about 4.8 Å, which is very close to the next-nearest oxygen spacing (4.75 Å) in a hypothetical ice I lattice at 298 K. This suggests that, provided there exists in liquid water some resemblance to an ice-like structure, molecules like biotin, 1,4-quinone, β-D-glucose and triglycerides could be cooperatively hydrogen bonded into water structure without undue modification of the existing hydrogen bonded system (Fig. 1.6).

**Fig. 1.6** Correlation between oxygen spacings in organic molecules and in the ice lattice. Numbers in the formulae indicate interaction points with the correspondingly numbered oxygens in the ice lattice.

Figs. 1.1 & 1.2 - Ref. 73, Fig. 1.3 – Ref. 74, Fig. 1.4 - 72, Fig. 1.5 - Ref. 56, Fig. 1.6 - Ref. 74.
References


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