Chapter - 1

Introduction

1.1 Intermolecular interactions

The knowledge of intermolecular forces is required for the understanding of chemical complexes such as clathrates, hydrogen-bonded networks, biological molecules such as DNA, RNA etc. Molecular forces are broadly classified into (a) intramolecular forces and (b) intermolecular forces. Intramolecular forces are those that help in the formation of a molecule and are usually a few orders of magnitude larger than intermolecular forces, such as, hydrogen bonds. For example the energy required to break an O-H bond in water is 222kcal/mol as compared to 9kcal/mol of the intermolecular hydrogen bonds. The present work concerns with intermolecular forces and hence no further reference will be made to intra-molecular forces.

Typical intermolecular forces that are encountered are (a) London dispersion forces (b) Debye forces (c) Keesom forces and (d) Hydrogen bonds. The former three are collectively known as van der Waals forces.

London dispersion forces are interactions between instantaneous dipole and induced dipoles. Debye forces are forces of interaction between a permanent dipole and the corresponding induced dipole while Keesom forces arise from interactions between two permanent dipoles. Understanding of van der Waal’s forces is of particular interest in bio-mimicry (Autumn et al., 2002).

Hydrogen bonds are stronger than van der Waals bonds and more directional too. They are the characteristic interactions of biomolecules that are also seen in non-biological systems. A brief introduction to hydrogen bonds is presented below.
1.2 Hydrogen Bonds

Although the term hydrogen bond has been in use for over a century, a clear definition is yet to be made and being discussed (Arunan et al., 2011). Usually, hydrogen bonds are thought of as interactions of the X-Y-H-Z kind with bond energies between 0.5 to 40 kcal/mol (Novakovskaya, 2012). This definition takes into account the basic fact that in hydrogen bonds, one hydrogen atom is bound to two other atoms. This unique property of hydrogen to bridge between two atoms (intramolecular bonds) or two molecules (intermolecular bonds) gives rise to the associative properties of hydrogen bonded liquids. There are a number of definitions of hydrogen bond, but most of them acknowledge the importance of the presence of a highly electronegative Y atom. The presence of a highly electronegative Z gives rise to (usually) a strong hydrogen bond while a weakly electronegative Z atom can lead to a weak hydrogen bond where the boundaries between hydrogen bond and van der Waals bond are blurred.

There have been many other definitions of hydrogen bonds based on (a) the physical forces involved (b) hydrogen donors and acceptors (c) hydrogen bond distances and hence energies etc. The various methods employed to study hydrogen bonds includes measurement of physical properties such as density, boiling point etc., spectroscopic techniques such as NMR, FTIR, neutron diffraction, Raman etc., computational and theoretical methods.

The significance of weak intermolecular interactions such as hydrogen bonds is when intermolecular interactions dominate over other effects in a system. Hydrogen bonds contribute to the anomalous properties of water (Stokely et al., 2010), partially to the stabilization of the secondary, tertiary and quaternary structures of proteins, to the structures of polymers, to control molecular aggregates (Gallant et al., 1991) and to
adhesion (Tareste et al., 2007) among many other phenomena.

Various reviews, monographs and textbooks on hydrogen bonds are available (Forlani, 2007; Hirsch, 2003; Jacobsen and Brasch, 1965; Guardia et al., 2005).

1.3 Hydrogen Bonds in Biological Systems

Depending on the strength of interactions the distance between nearest neighbors in biological systems is between 1.5 to 3.5 Å. Hence hydrogen bonds in these systems fall broadly into this range of bond lengths. The importance of hydrogen bonds in biological systems stems from the fact that bio molecules are either hydrogen bonded or, like in the case of lipids, react biologically through a hydrogen bonding interaction (Sharkhel et al., 2004). Self assembly of bio molecules also depends on hydrogen bonds (Lin and Mao, 2011).

The importance of hydrogen bonds in biological systems arises from the fact that these bonds have relatively small strength. Most biological processes require rapid reactions occurring close to $10^{-9}$s. Hydrogen bonds allow fast association and dissociation so that many combinations of interactions can be checked before the correct association of molecules. It is understood that the specificity of biological systems comes from the trial and error of different sterically complimentary interactions between two molecules, until the best interaction is found.

Some of the early accounts of hydrogen bonds are by Latimer and Rodebush (1920), Bernal and Megaw (1935), Huggins (1936 and 1942) and the book on chemical bonding by Pauling (1939). This was followed by studies indicating the importance of hydrogen bonding in the $\alpha$ and $\beta$ structures of protein (1951) and in base pairing in DNA helix (Watson and Crick, 1953).
Hydrogen bonds are responsible for the structure of proteins and also for the stability of complex biological molecules such as DNA. Proteins have many physiological functions and constitute mainly of C, H, O and N along with other elements. All proteins are made of 20 basic building blocks called amino acids. Proteins are characterized by their primary secondary and tertiary structures. The primary structure of protein is defined by the sequence of amino acids connected by peptide bonds. Two strands of the primary structure are often interlinked through hydrogen bonds to give rise to the secondary structure, which is usually in the form of coils or pleats. The alpha helix and beta strands are different ways of saturation of the hydrogen bond donors and acceptors in the peptide backbone. The three dimensional structure of protein is called the tertiary structure and is usually globular in shape and hydrogen bonds are among the factors that stabilize the tertiary structures of proteins. Quaternary structure of proteins refers to a larger collection of proteins.

When studying the molecular interactions in large molecules such as proteins, it is an accepted convention to study small molecules with similar interactions as prototypes of the larger molecules (Zhang et al., 2008). The hydrogen bonds in proteins and amino acids can be understood by studying the hydrogen bonds in binary mixtures of liquids, which exhibit similar bonds. Hence the study of intermolecular interactions in proteins can be done through the study of proteins, amino acids and binary liquids that exhibit similar hydrogen bonds.
1.4 Bonding and Bio-MEMS

Interaction of biomolecules with substrates is of current interest with far reaching implications. For example, the interaction of proteins with substrates has applications in nanotechnology, biomaterials and biotechnology (Grey, 2004). Studies show that proteins undergo conformal changes when they come into contact with a surface, both at the solid-liquid and the liquid-vapour interfaces. The conformation in retained more on charge neutral hydrophilic surfaces than on hydrophobic surfaces. In similar environment of protein tertiary structure, depending on the influence of amino acids residue in protein (interaction of different amino acid with protein), the twenty amino acids residues have been divided in to nine groups (Saha et al., 2005); [Glycine, Serine, Threonine], [Alanine, Valine], [Proline, Phenylalanine, Tyrosine, Tryptophan], [Histidine], [Cysteine], [Methionine, Leucine, Isoleucine], [Aspartic acid, Glutamic acid], [Asparagine, Glutamine] and [Arginine, Lysine]. Those classifications are useful for residue characterization like size, hydrophobicity, aliphatic or aromatic or conformational flexibility of the side chain etc. Adhesion bonding technology for bio-MEMS has been designed for the bonding of glass/photoresist (SU8) structures to glass cover plates for the fabrication of micro-fluidic devices with integrated 3D-micro-electrode arrays, based on the preparation of ultra-thin adhesive layers between precision machined cylinders and roll-to-surface print transfer onto micro-machined substrates (Kentsch et al., 2006). Bio-MEMS include various forms like lab-on-a-chip, microarray chips, microfluidic chips, or μTAS (micro Total Analysis System), drug delivery components and enable miniaturized instruments on chips. Their sizes vary from a few millimetres on edge to 1mm in thickness. Most of these devices require only a tiny volume a few µl of sample liquid for performing synthetic, analytical and detection
1.5 Present study

The present work consists of both computational and experimental studies. Using Gaussian-03 software and by applying Hartree-Fock method, computational studies were performed to determine the most stable conformer of the molecules. The experimental studies undertaken are contact angle measurements, FTIR spectroscopy and dielectric studies using RLC meter and Abbe refractometer. A brief introduction to each of the methods is presented in chapter 2. Substrates relevant to uses in bio-MEMS devices like glass, stabilize mica, nylocast, silicon, epoxy FR4, acrylic, fiber reinforce plastic (FRP), polypropylene, aluminium oxide (Al₂O₃), polyurethene, teflon, indium tin oxide (ITO) and hylam are chosen for the present study. The choice of liquids taken for study is based on the type of interaction seen in biomolecules. Liquids chosen are Alcohols (methanol, ethanol, isopropanol, butanol, hexanol and octanol), aniline and acetone. Binary mixture of aniline-alcohols or acetone-alcohol form the prototype of hydrogen bonds of the N-H-O and C=O--H kinds formation in biomolecules and a parallel studied on amino acid namely aspartic acid, cystein, glutamic acid, phenylalanine, histidine, tyrosine, methionine, arginine, proline, valine, threonine, lysine, leucine, isoleucine, tryptophan, alanine, serine and glycine and protein (ubiquitin and bovine serum albumin) has been taken up. Since amino acids and proteins are well soluble in water, their saturated solutions in water are made to contact with different substrates. By using a modified spin coater technique as discussed in chapter 2, biomolecules are coated on the glass substrate. The literature survey corresponding to the various systems are presented at the corresponding places in the next few chapters.
1.6 Arrangement of thesis

Organization of thesis is based on the systematic studies of hydrogen bond of the type \( \text{N-H-O} \) and \( \text{C=O-H} \) from the organic binary liquid to biomolecules. The second chapter of the thesis consists of details of computational and experimental techniques used for the present study and their application to study of intermolecular interactions is discussed in detail. The third chapter consists of results and discussion on small organic molecules like alcohols, aniline and acetone, their binary mixture and how these systems interact with different solid substrates. The interactions occurring in small molecules are prototypes for those in larger bio molecules. The fourth chapter details the contact angle studies of various bio-molecules such as amino acids and proteins. The interactions between amino acids and protein, amino acid with substrates, amino acids with test liquid (static/dynamic) and amino acids coated layer by layer on glass substrates are presented in the chapter. The fifth and final chapter consists of the overall summary and discussion of the entire work with a few possible areas of further research being identified.