CHAPTER II

GENERAL INTRODUCTION (REVIEW OF THE EARLIER WORKS)

II.1. HOST –GUEST INCLUSION COMPLEX:

In the field of supramolecular chemistry, host–guest chemistry deals with the complexes that consist of molecules or ions that are present together in the complexes by forces that differ from full covalent bonds. Host–guest chemistry covers the area of molecular recognition and interactions by forming noncovalent bonding.[1] Noncovalent bonding is very weak to build up 3D structure of large biomolecules, as for example proteins. But it takes part in many biological processes in human body and living organisms in which large molecules bind together specifically but not strongly to one another. These non covalent interactions can be specified into four classes: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions.[2]

The formation of inclusion complexes by the interaction between host and guest molecules is thermodynamically favorable because in the formation of the complex the overall Gibbs free energy of the system is lowered. Now it is the interest of modern chemists to determine the thermodynamic properties and energy of these types of complexes which have vital importance in supramolecular chemistry and nanoscience. Many host molecules used for this purpose are pillarene, cucurbitril, cyclodextrin, crown ethers etc.

II.2. Earlier works with Cyclodextrin:

Cyclodextrin becomes the interest of many chemists as host in the field of supramolecular chemistry due to its unique truncated cone structure and having the ability to form inclusion complexes with a number of guest molecules.

- K. Dinar and his co-workers show that N-sulfamoyloxazolidinones forms IC with with β-cyclodextrin and this fact was proved by molecular

modeling, using PM3, PM6, ONIOM/2 methods, and NBO analysis.[3] N-Sulfamoyloxazolidinones are very interesting compounds which combine an oxazolidinone pharmacophore and a sulfamoyl moiety. It is used as an antibiotic and used as a precursor of 2-chloroethylnitrososulfamides .NBO analysis provide informations that the hydrogen bonds interactions are of type C-H-O with stabilization energies smaller than 2 kcal/mol indicating that the host–guest interactions are weak.

- Plants give us a enormous type of molecules that are used to recover healing: these are mainly fibre, vitamins, phytosterols, some sulphur-containing compounds, carotenoids, organic acid anions and polyphenolics. This vital molecules need to be protected from environmental hazards to avoid losing their structural morfology and bioactivity. E. Pinho and his co-workers investigated that cyclodextrin improve the biological, chemical and physical properties of bioactive molecules.[4]

- A water-soluble inclusion complex of hypericin with β-cyclodextrin polymer was prepared by hydrophobic interactions between them by W. Zhang etal.[5] Hypericin (HY) is basically a natural polycyclic quinone from Hypericum perforatum, usually termed as St John's wort. This natural product is an useful antidepressant and anxiolytic. It is also used to prevent activity of virus and as a photosensitizer. It also helps in photodynamic therapy of cancer. HY is highly lipophilic and insoluble in water, which creates intravenous injection problematic and hold backs its medical applications. β-cyclodextrin polymer is employd as a solubilising agent for hypercin in this article. The inclusion complex (HY-CDP) was identified by ^{1}H NMR, FTIR, and UV–Vis spectroscopies.

- The mechanism of preparation of inclusion complex f between 2-hydroxy-1-naphthoic acid and β-cyclodextrin in liquid, solid and virtual states was

studied by K. Sivakumar and his co-workers.[6] 2-Hydroxy-1-naphthoic acid (2H1NA) is an aromatic compound having bicyclic structure which comprises of two benzene rings fused together. This has various applications in different fields like agriculture, construction, pharmaceutical industries, photographic, rubber and textile chemicals. The binding constant of the inclusion complex in aqueous solution is calculated by cyclic voltammetry analysis at pH 2.75. A sharp increase in the anodic peak current and peak potential with gradual increase in β-CD concentration is characteristic to the inclusion complex formation between them.

- Sulfanilamide forms inclusion complex with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin and it was investigated by A. Tacic et al.[7] Sulfanilamide is a vital drug which has an efficiency to prevent bacteriostatic effect on different pathogenic microorganisms. This activity is related with spirited antagonism with p-aminobenzoic acid, this is also essential part of folic acid. The application of this drug sulfanilamide is inadequate on account of its poor solubility in the aqueous solution. Sufanilamide forms inclusion complex with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin by the process co-precipitation. The insertion of this drug sulfanilamide was established with the help of FTIR, ^{1}H-NMR, XRD and DSC experiments. Phase-solubility techniques were also employed to confirm the configuration of the inclusion complex between sulfanilamide and cyclodextrins. The stability of this drug and its inclusion complexes was investigated by UVB irradiation using a photochemical reactor by application of the UV–Vis method.

- Y. Gao et al. Prepared inclusion complexes of three ionic liquids 1-dodecyl-3-methylimidazolium hexafluorophosphate, 1-tetradecyl-3-methylimidazolium hexafluorophosphate and 1-hexadecyl-3-methylimidazolium hexafluorophosphate with β-cyclodextrin.[8] The

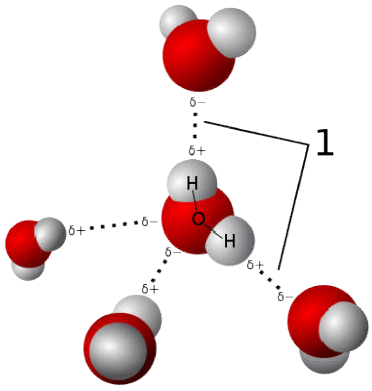
surface tension study proved that there were two types of inclusion formations, 1:1 and 1:2. These inclusion complexes were further identified by XRD, ^{13}C CP/MAS NMR, ^{1}H NMR, rotating frame Nuclear Overhauser Effect Spectroscopy (ROESY), and thermogravimetry (TGA). The obtained inclusion complexes are fine crystalline powder. The hydrophobicity has a vital role in supporting the formation of ICs. The decomposition temperature of these ICs was lower than those of their precursors.

- M.D.Cagno and his co-workers investigated the complexation of ibuprofen as model drug with various β-cyclodextrins.[9] The solutions of cyclodextrins were arranged in phosphate buffer. The pH value was maintained at the range of 7.4-7.6 and the solutions were isotonic with NaCl. A thermal activity monitor was used for isothermal titration calorimetry (ITC). ^{1}H NMR analysis was performed to explore the structures of the complexes. ITC analysis proved that each type of-cyclodextrin had its definite values of enthalpy and mass equilibrium constant for the inclusion complex formation processes with the drug molecules. ^{1}H NMR spectroscopy of the complexes revealed noteworthy differences in chemical shifts that interaction between the cyclodextrins and ibuprofen molecules differ to some extent may be due to different three-dimensional arrangements of ibuprofen in the cyclodextrin cavity, initiated by the different substituent bonded to the glucose rings. These differences were in agreement with the thermodynamic parameters of the complexes.

II.3. Various non covalent interactions between the host and guest molecules

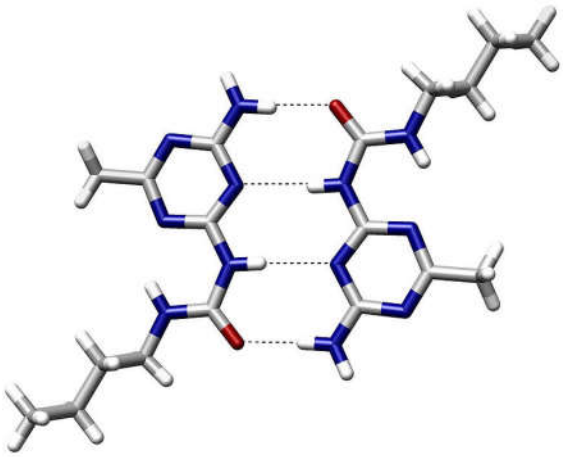
- **Hydrogen Bond:** The hydrogen bond may be described as the electrostatic force of attraction between two polar atoms of a molecule which generally forms when a hydrogen (H) atom is covalently bonded to

a highly electronegative atom i.e. nitrogen (N), oxygen (O), and fluorine (F).[10] Hydrogen bonding can be classified into two types- intermolecular (forms between different molecules) and intramolecular(forms within the same molecule). The energy of hydrogen bond ranges from 1 to40 kcal/mol and it depends upon the nature of the electronegative atoms that is involved in bonding with hydrogen.[11] Thus the energy of the hydrogen bond is in between Vander Waal Bond and Covalent bond. Hydrogen bond is present in in inorganic molecules such as water, ammonia, hydrogen fluoride and also in organic molecules such as DNA and proteins. Intermolecular hydrogen bonding results many characteristic properties of molecules as for example high boiling point of water (100 °C) in comparison to the other members of group 16 hydrides in which hydrogen bonding is absent.[12] Intramolecular hydrogen bonding builds up the secondary and tertiary structures of proteins, enzymes, hormones nucleic acids. It is also present in the structure of many polymers, both synthetic and natural. The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation.



Model of hydrogen bonds between molecules of water

The hydrogen bond can also be defined as an electrostatic dipole-dipole interaction between molecules. Hydrogen bonding also has some common property with covalent bonding such as it is directional in nature and strong than London forces. The interatomic distances in case of it is shorter than the sum of the van der Waals radii, and a minimum number of interacting molecules are responsible for the formation of such bonding. The covalent character of the bond increases with the increase of the electronegativity of the group involved in bonding. The length of hydrogen bonds is a factor of the following properties namely bond strength, temperature, and pressure. The bond strength also depends upon temperature, pressure, bond angle, and environment. The usual length of a hydrogen bond in water is approximately 197 pm.[13] The bond angle generates in this type of bonding mainly depends upon the electronegative group attached with hydrogen.

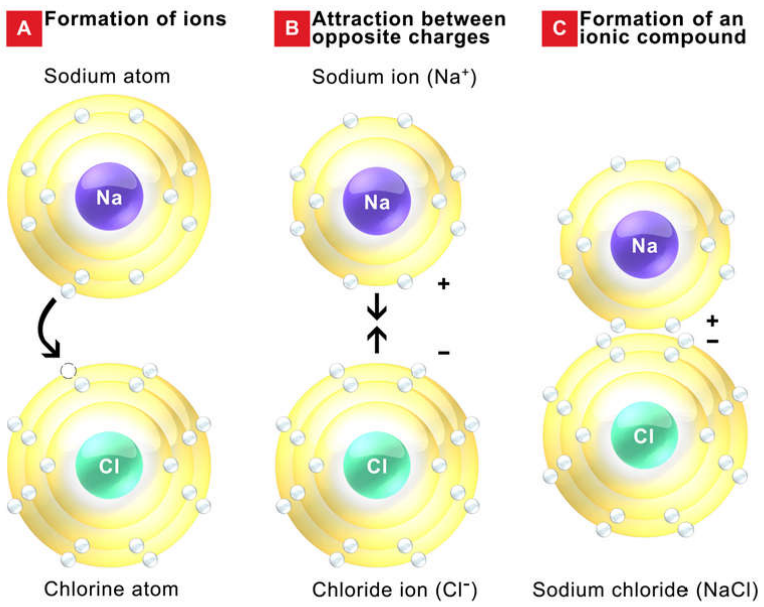


An example of intermolecular hydrogen bonding in present in a self-assembled dimer complex

- **Ionic Bonding-** It can be described as a type of chemical bond that includes the electrostatic attraction between two oppositely charged ions; it is also the primary interaction that exists in ionic compounds. The ions can be defined as atoms that have either accepted one or more electrons (these are called as anions and they are negatively charged) or atoms that

have donated one or more electrons (these are called as cataions and they are positively charged). This type of transitions of electrons are termed as electrovalence.[14] Generally, metal atom converts into the cataion and the non metal atom plays the role of anaion, but ions with more complex nature can also formed. Thus, an ionic bond formation involves the transfer of electrons from a metal to a non-metal with a target to fulfill the valence shell for both atoms. But all the ionic compounds have partial covalent nature, or electron sharing. The bonds are usually termed as "ionic bonding" when the ionic character of the bond is higher than the covalent character. In case of ionic bonding great electronegativity between the two atoms exsist. Polar covalent bonds are those bonds which have both ionic and covalent character.

These compounds can conduct electricity both in molten state and in solution. Ionic compounds usually posses a high melting point and boiling point. High charge and small size of the cataion and large size of the anaion exerts strong cohesive forces and it reflects in higher melting point and boiling point. These compounds are also soluble in water.



Formation of an Ionic Bond

Redox reaction also can result ionic bonding with those atoms of the elements with low ionization energy with a tendency to achieve stable electronic configuration like inert gases. Cations are formed in this way. The atoms of nonmetallic elements having positive electron affinity accepts these electrons and form anions. The attraction which is electrostatic in nature between the anions and cations forms solid compounds having crystallographic lattices with the ions stacked one by one in an alternating way. In this type of lattice, it is impossible to differentiate separate molecular units and these compounds are not molecular compounds. But these ions can aggregate together to form other complex ions like the acetate anion or the ammonium cation. Ionic compounds usually comprises of lattice structures.[15] The charge and size of the ions determine the arrangement in lattices and also the structure. Some structures are repeated in a number of compounds; such as the structure of the rock salt sodium chloride are similar with many alkali halides, and binary oxides like MgO.

The quantity of energy released in forming a solid crystalline ionic compound from gaseous ions is known as the lattice energy. The the lattice energy value can be calculated from the Born-Haber cycle. It can also be determined from the Born-Landé equation as the sum of the electrostatic potential energy, determining interactions between cations and anions, and a short-range repulsive potential energy term. The Madelung constant gives the idea of the geometry of the crystal.

- **Van der Waals Forces-** This type of forces may be illustrated as the interactions that depends upon the distance and generally occurs between atoms or molecules. This type of forces are completely different from ionic or covalent bonds, these attractions are also not similar with chemical bonds. The van der Waals forces generally weaken at longer distances between interacting molecules.

There is vital importance of Van der Waals forces in the field of supramolecular chemistry, structure related to biology, polymeric materials, nanoscience, surface chemistry, and condensed matter physics. It can also explain various charecteristics of organic compounds and molecular solids, also it suggests about the solubility in polar and non-polar solvents. In absence of other forces, the Van der Waals force becomes repulsive rather than attractive when two atoms adjacent to each other are termed as the van der Waals contact distance.

Vander waals force is the weakest force, having a strength ranges from 0.4 to 4kJ/mol. this force arises from a transient shift in electron density. In an atom the electrons rotate in different orbits and protrons and neutrans are present in the nucleus. The density of electrons sometimes shifts in an atom. As a result a transient charge develops in the atom. Which in turn attracts or repels other electrons of a nearby atom. If the interatomic distance between two nearby atoms is more than 0.6 nm the force is weak and generally not observed. But if the the interatomic distance is less than 0.4 nm the force is found to be repulsive.

The van der Waals forces described above are usually anisotropic in nature. But it is not found in case of two noble gas atoms. Anisotropic suggests that they are a function of relative orientation of the molecules. In the case of induction and dispersion interactions they are attractive in nature, whatever is the orientation, but the magnitude of the electrostatic interaction changes with rotation of the molecules. Thus the nature of electrostatic force either can be attractive or repulsive, and it depends upon the mutual orientation of the molecules. If we consider the molecules in the liquid and gaseous phase they exhibit random motion and the electrostatic force is diminished as they have thermal rotation. It can be concluded that the thermal averaging effect is much less articulated in case of the attractive induction and dispersion forces.

The chief distinctiveness of van der Waals forces is:

a) They are feebleer than standard covalent and ionic bonds.

b) Van der Waals forces are preservative and cannot be inundated.

c) These forces are not directional in nature.

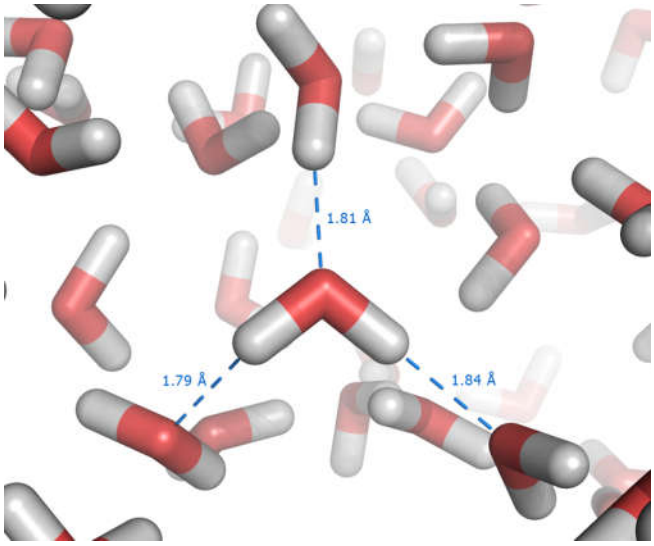
d) The range of van der walls force is short and the interactions between the nearby particles require to be judged (all other particles can be ignored). Van der Waals force is higher when the molecules are close.

e) Van der Waals forces do not depend upon temperature apart from dipole – dipole interactions.

- **Hydrophobic Effect-** The hydrophobic effect may be defined as the tendency of nonpolar substances to form aggregation in aqueous solution. The exact word meaning of the term hydrophobic is "water-fearing. The hydrophobic effect is liable for the division of a mixture of oil and water into its two components. This effect controls the shape and structure of biology, such as cell membranes and vesicles formation, primary and secondary structure of protein, incorporation of membrane proteins into the nonpolar lipid environment .Thus this effect is very important in biology. The compounds which exhibit this effect are termed as hydrophobes. The hydrophilic groups avert phase separation of the molecules by placing the hydrophobic groups in water and they form strong hydrogen bonds with water molecules. Hydrophobic effect is responsible for the self assemble of different molecules.

  The source of the hydrophobic effect is not clearly known. In some theory this hydrophobic interaction is often mentioned as an entropic effect which arises due to the disturbance of highly vibrant hydrogen bonds between molecules of liquid water by the action of nonpolar solute. Generally most organic compounds having a long hydrocarbon chain or non polar part is unable to form hydrogen bonds with water. Insertion of

such non polar parts into water results disruption of the hydrogen bonding chain that forms between water molecules. The arrangement of hydrogen bonds are changed accordingly in order to minimize interruption of the hydrogen bonded three dimensional network of water molecules, and as a result there forms water "cage" about the nonpolar surface. The water molecules which are involved in the formation of the "cage" have limited mobility. The velocity of molecules forming such salvation cell or cage is restricted to almost 10%. At normal temperature dissolved xenon shows a mobility restriction of 30%. If the size of the nonpolar molecules is large, then the orientation of the motion of the water molecules in the cage like structure may be constrained by a value of two to four times. Usually, restriction in the mobility significantly affects the translational and rotational entropy of water molecules; consequently the whole process becomes unfavorable in terms of the free energy of the system. The aggregation of nonpolar molecules decreases the surface area depicted to water and diminish their troublesome effect.



Dynamic hydrogen bonds between molecules of liquid water

The measurement of the hydrophobic effect can be done by measurement of the partition coefficients of the non-polar molecules between water and hydrophobic solvents. The partition coefficients is usually converted to free energy of transfer including enthalpy and entropy measurements, $\Delta G = \Delta H - T\Delta S$. These quantities are experimentally establishd with the help of calorimetry. The hydrophobic effect is generally controlled by entropy at room temperature as the mobility of water molecules in cage like shell decreases in the environment of the non-polar solute; but the enthalpic component of transfer energy was positive, which means the water-water hydrogen bonds become strong in the solvation cage owing to the decreased mobility of water molecules. With the increase of temperature, the water molecules have higher mobility; this increase of energy is accompanied by the quantity entropy.

The hydrophobic effect is very vital as it controls the secondary structure of proteins. The water-soluble proteins contain a hydrophobic core with side chains are covered from water, and this leads to stabilization of the folded structure. Side chains of organic compounds having polar groups are-depicted to the surface and thereby interacting with the water molecules present in surface. The protein show folded secondary structure and the reason behind this is the minimization of of the hydrophobic portion and besides this development of hydrogen bonds into the protein help in stabilizing the protein structure. The DNA tertiary structure can also be explained by the hydrophobic effect.

II.4. Theory of Different Investigations

II.4.1. SURFACE TENSION

Surface tension is the most significant of the characteristic properties of liquids. The surface of a liquid is in a state of tension and any attempt to make a penetration along any line in the surface will require an application of force to hold the separate portions of the surface together. The force is called the surface

tension, denoted usually by the symbol γ. It is expressed as the force in dynes per unit length acting at right angles to the line along the surface of the liquid.

The Surface tension is numerically the same as the surface energy per unit area of the liquid. It is a function of temperature. The surface tension of a liquid generally decreases with rise in temperature. Eotvos gave a quantitative relation between these two.

$$(MV)^{2/3}\gamma=K(T_C-T) \qquad (II.1)$$

Where M is the molecular weight and V is the specific volume of the liquid, γ is the surface tension, K is a constant. T_C and T represent the critical temperature and temperature of the liquid respectively.

Surface tension (γ) measurement can be used to obtain valuable information about the formation of inclusion complex. Cyclodextrin is used as the host molecule in all the research works. The Surface tension (γ) of aqueous cyclodextrin doesn't show any remarkable change with increasing concentrations. But after the addition of guest molecules such as amino acid, ionic liquids, drug molecules the γ values show remarkable change. Due to the insertion of guest molecules the γ value changes. The γ value either shows an increase or decrease depending upon the structure of the guest molecule. Each plot also indicates that there is single break point at certain concentrations. Finding of break point in surface tension curve not only indicates formation of IC but also gives information about its stoichiometry, *i.e.*, appearance of single, double and so on break point in the plot indicates 1:1, 1:2 and so on stoichiometry of host:guest ICs. The concentration and corresponding surface tension at which maximum inclusion took place (break point at the curve) have been calculated by solving the equations of two intercepting straight lines.

II.4.2. CONDUCTANCE

The conductance of solutions is generally governed by the formula as in the case of metallic conductor.

$\kappa=1.L/R.a$ (II.2)

Where, κ is the specific conductance, R is the resistance, l and a are length and area of the metallic conductor respectively.

The specific conductance of the solution (L) is the conductance of the solution enclosed between the two electrodes of 1 suare cm. area and 1 cm. apart. The conductance depends upon the number of ions present and hence on the concentration of the solution. To compare the conductance of different solutions, it is necessary to take the concentration of the solutions into consideration. It is done by using equivalent conductance, λ. The equivalent conductance is defined as the conductance of a solution containing 1 gm. Equivalent of the dissolved electrolyte such that the entire solution is placed between two electrodes 1 cm apart. Λ is always evaluated through the measurement of L.

$\Lambda=1000\kappa/C$ (II.3)

Where c is the concentration of the solution.

The studies of conductance measurements were pursued vigorously during the last five decades, both theoretically and experimentally and a number of important theoretical equations have been derived. We shall dwell briefly on some of these aspects in relation to the studies in aqueous, non-aqueous, pure and mixed solvents. The successful application of the Debye-Hückel theory of interionic attraction was made by Onsager to derive the Kohlrausch's equation representing the molar conductance of an electrolyte. For solutions of a single symmetrical electrolyte the equation is given by:

$$\Lambda=\Lambda_o-S\sqrt{c} \qquad \text{(II.4)}$$

Where, $S=\alpha\Lambda_o+\beta$

$$\alpha=\frac{(z^2)k}{3(2+\sqrt{2})\varepsilon_r kT\sqrt{c}}=\frac{82.406\times10^4 z^3}{(\varepsilon_r T)^{\frac{3}{2}}}$$

$$\beta = \frac{z^2 eFk}{3\pi\eta\sqrt{c}} = \frac{82.487 z^3}{\eta\sqrt{\varepsilon_r T}}$$

The equation took no account for the short-range interactions and also of shape or size of the ions in solution. The ions were regarded as rigid charged spheres in an electrostatic and hydrodynamic continuum, i.e., the solvent.

With the help of conductivity (κ) study the inclusion phenomenon can be confirmed. The guest molecules which exist in ionic form in aqueous solution show considerable value of κ. As aqueous cyclodextrin solution was added to the aqueous solution of guest molecules, the κ was observed to show decreasing trend probably because of encapsulation of the guest molecules inside the cavity of cyclodextrin. At certain concentrations of both host and guest single break was found in each of the conductivity curve, which indicates the formation of inclusion complexes. The concentration and corresponding conductivity at which maximum inclusion took place (break point at the curve) have been calculated by solving the equations of two intercepting straight lines.

II.4.3. pH measurement

pH can be defined as the negative logarithm of the molar concentration of H_3O^+ ions in aqueous solution.

$pH=-\log_{10}[H_3O^+]$

With the increase or decrease of H_3O^+ ions in solutions the pH value decreases or increases respectively. The increase or decrease of pH value by one unit results the concentration of H_3O^+ ions in solutions to decrease or increase by 10 times. The more a solution is acidic the less is the pH value of the solution.

The relation of pH and pOH with the ionic product of water(pKw) is as follows:

$pH + pOH = pKw$ (II.5)

For pure water at 25°c , pH +pOH= 14

For pure water at 25°c, pH= pOH=7.

For neutral solution the pH value is always equal to 7.

For acidic solution, the pH value is always <7.

For basic solution, the pH value is always >7.

The range of pH scale is from 0-14. But with the change of temperature, the pKw value of water changes and as a result the range of pH values also changes.

pH measurement is applied in determining the inclusion of the amino acids leucine,isoleucine, asparagines, aspartic acid into the cavity of both the cyclodextrin molecules. The above mentioned four amino acids exsist as zwitterions in the aqueous solution. pH values were measured by Mettler Toledo Seven Multi pH meter having uncertainty ±0.001. The amino acid solutions are prepared by mass fraction. The aqueous solution of both the cyclodextrin molecules are gradually added to the amino acid solutions. The increase of the pH values with increasing concentration of amino acids and host molecules clearly show the variation in their zwitterionic forms, *i.e.*, the amine and carboxylic acid groups exist in ionic forms $-NH_3^+$ and $-COO^-$ respectively.

II.4.4. DENSITY

The physicochemical properties of liquid mixtures have attracted much attention from both theoretical and engineering applications points of view. Many engineering applications require quantitative data on the density of liquid mixtures. They also provide information about the nature and molecular interactions between liquid mixture components.

The density of a compound can be described as mass per unit volume. This physical quantity is denoted by ρ. The mathematical expression of density is

$$\rho = m/V$$

Where ρ is the density, m is the mass, and V is the volume. If the substance is pure, the magnitude of density is equivalent to its mass concentration. The density value differs from material to material. Osmium and iridium are most dense elements at standard temperature and pressure but certain metals may be denser.

In different measuring systems like CGS or SI the value of density is different and it becomes complicated to compare the values. It is more relevant to replace it with another quantity "relative density" or "specific gravity", which has no dimension. The later can be described as the ratio of the density of the material to that of a standard material, normally water. Consequently a relative density less than one means that the substance will float in water.

The density of a element is a function of temperature and pressure. This difference is characteristically small for solids and liquids but higher for gases. If we increase the pressure on an object its volume will decrease thereby increasing its density value. But if we raise the temperature of a compound its density value gradually falls as the volume increases.

The inverse quantity of the density of a compound is often termed as its specific volume; this term is quite used in thermodynamics. Density is mentioned as an intensive property that is it does not depend upon the mass of the substance.

The density in case of all homogeneous objects is equal to its total mass divided by its total volume. The mass of a substance is usually taken by a standard balance; the volume can be determined directly (using the concept of the geometry of the compound) or by some indirect method. The determination of the density of a fluidcan be done using hydrometer, a dasymeter or a Coriolis flow meter respectively. In a similar way, hydrostatic weighing is basically

depending upon the idea of displacement of water in case of a submerged object calculating the density of the compound.

II.4.5. APPARENT AND PARTIAL MOLAR VOLUMES

The molar volume of a pure compound may be evaluated using density data. It is not easy to determine the volume contributed to solvent upon addition of one mole of an ion. The reason behind this is that the ion changes the volume of the solution after addition as they changes the structure of the solvent molecules and also the volume compresses due to the influence of ion's electrostatic field. When electric fields of the order of 10^9-10^{10} V m^{-1} are applied on a solution the compression of ions occur and the molecules present become insignificant. The apparent molar volume (ϕ_V) is the measure of the sum of geometric volume of the central solute molecule and changes in the solvent volume due to the interactions with the solute around the co-sphere. The partial molal volume can be obtained from apparent molar volume. The apparent molar volumes, (ϕ_V), of the solutes can becalculated by the following equation

$$\phi_V = \frac{M}{\rho_0} - \frac{1000(\rho - \rho_0)}{c\rho_0} \qquad \text{(II.6)}$$

In the above relation, M is the molar mass of the solute, c is the molarity of the solution; $\rho_o$and ρ are the densities of the solvent and the solution respectively. The partial molar volumes, ϕ_{2v}can be obtained from the equation

$$\phi_{2V} = \phi_V + \frac{(1000 - c\phi_V)}{2000 + c^{3/2}\left(\partial\phi_V / \partial\sqrt{c}\right)} c^{1/2}\left(\partial\phi_V / \partial\sqrt{c}\right) \qquad \text{(II.7)}$$

The extrapolation of the apparent molar volume of electrolyte to infinite dilution and the relation of the concentration with the apparent molar volume have been expressed by four important equations for many years – the Masson equation ,

the Redlich-Meyer equation , the Owen-Brinkley equation, and the Pitzer equation .

According to Masson [16], the apparent molar volume of electrolyte, ϕ_V , changes as a function of square root of the molar concentration and can be expressed as

$$\phi_V = \phi_V^0 + S_V^* \sqrt{c} \qquad \text{(II.8)}$$

here, ϕ_V^0 is the apparent molar volume (equal to the partial molar volume) at infinite dilution and S_V^* the experimental slope. The series of ϕ_V values in water and rest of other ϕ_V data in non-aqueous solvents have been extrapolated to infinite dilution with the help of equation (II.8).

The temperature dependence of ϕ_V^0 for various electrolytes used in this research work can be expressed by the following equation:

$$\phi_V^0 = a_0 + a_1 T + a_2 T^2 \qquad \text{(II.9)}$$

Where a_0, a_1 and a_2 represent the coefficients of a particular electrolyte and T is the temperature in Kelvin.

The standard deviations (σ) were determined using the following equation:

$$\sigma = \sqrt{[\sum (Y_{exp} - Y_{obs})^2 / (N - 1)]} \qquad \text{(II.10)}$$

where N is the number of data points.

ϕ_v and ϕ_v^o values can be calculated from the density of the solutions at 298.15 K using the provided equation. If the magnitude of ϕ_v is found to be positive for the guest molecules such as amino acids and ionic liquids, it indicates strong

solute–solvent interactions. ϕ_v varies linearly with the square root of molal concentration ($\sqrt{c}$) and is fitted to the masson equation, from where ϕ_v^o has been determined. The increase of ϕ_v^o values with increasing mass fraction of both cyclodextrin molecules suggest the ion-hydrophilic group interactions are stronger than ion-hydrophobic group interactions. The guest molecules and aq cyclodextrin from a ternary system. The interaction of charged groups of guest molecules is localized with -OH groups of cyclodextrins. Due to this interaction the electostriction of water results an increase in volume. The greater the hydrophobic part of the guest, the higher is the ϕ_v^o values.

■Contributions of zwitter ionic group (NH_3^+), (COO); (CH), side group (R) of amino acids leucine, isoleucine to the limiting apparent molar volume (ϕ^0_V) :

The ϕ^0_V value of zwitterionic group, (CH), side group (R) of the amino acids were estimated from the following equations [17]

$$\phi^0_V = \phi^0_V(NH_3^+, COO^-) + \phi^0_V(CH) + \phi^0_V(\text{side grp}) \quad \text{(II.11)}$$

$$\phi^0_V(R) = \phi^0_V - \phi^0_V(NH_3^+, COO^-) - \phi^0_V(CH) \quad \text{(II.12)}$$

For asparagines and aspartic acid The ϕ^0_V value of zwitterionic group, (CH), (CH_2) and end group (R) of the amino acids were estimated from the following equations.

$$\phi^0_V = \phi^0_V(NH_3^+, COO^-) + \phi^0_V(CH) + \phi^0_V(CH_2) + \phi^0_V(\text{end grp}) \quad \text{(II.13)}$$

$$\phi^0_V(R) = \phi^0_V - \phi^0_V(NH_3^+, COO^-) - \phi^0_V(CH) - \phi^0_V(CH_2) \quad \text{(II.14)}$$

II.4.6. Viscosity

The viscosity is a very important property of fluids that is gases and liquids. It can be explained as a measure of the resistance to gradual deformation of relative velocity between different layers of fluids by shear stress or tensile stress. In case of liquids it is quite similar to thickness such as, glycerin has a

higher value of viscosity than water. Viscosity refers to the characteristic of the fluid that opposes the relative velocity between the two layers of the liquid in a fluid that have a different value of velocity. If a fluid is allowed to flow through a tube, the molecules which invent the fluid usually travel more quickly near the tube's axis and slowly around its walls; consequently a force (may be pressure disparity among the two ends of the tube) is required apply to conquer the friction between layers of molecules to keep the movement of fluid. The force that is needed to apply is a function of the fluid's viscosity. A fluid that is completely unable to prevent the stress is known as an ideal or inviscid fluid. The magnitude of viscosity becomes zero at low temperature for superfluids. Except this, all other fluids have positive value of viscosity, and usually termed as viscous or viscid. In general concept, however, a liquid is mentioned more viscous if it has a higher value of viscosity than water. But if the viscosity value is less than that of water it is known as mobile liquids.

The term dynamic viscosity of a fluid can be explained as the confrontation to shearing flows and in this case the contiguous layers move parallel to each other having different speeds. In a ideal situation this type of flow is called as a Couette flow, in which a layer of fluid is fascinated between two parallel plates, one stationary and another moving horizontally at steady speed u.

But if the velocity of the upper plate is very low, then the fluid molecules will shift corresponding to it, and their velocity will change as a linear function from zero value at the underneath to u at the upper. Every film of fluid will go quicker than the layer just under it, whereas the friction between them develops a force preventing their comparative motion. Generally, the fluid gives on the upper plate a force directionally opposite to its velocity, and a force of equal magnitude in opposite direction in the underneath plate. An outer force is therefore necessary to apply for keeping the upper plate shifting at steady speed.

The extent F of this force is established as proportional to the velocity u and the area A of every layer, and inversely proportional to the separating distance y:

$$F = \mu A \frac{u}{y}$$

The ratio u/y is expresses the rate of shear deformation or shear velocity.

The fluids that's obey Newton's law, having a value of viscosity μ which does not depend upon the stress, is called as Newtonian. Newtonian may be gases, liquids and others in general conditions and circumstance. But many of the fluids do not obey this law and exhibit significant deviation in many respects. Few examples may be cited as:

- The liquids which show a gradual increase of viscosity with the increase of shear strain generally known as Shear-thickening liquids.
- The liquids which show a gradual decrease of viscosity with the increase of shear strain generally termed as Shear-thickening liquids.
- Thixotropic liquids, which progressively turns less viscous with time after application of shake, agitation, or stress.
- Rheopectic liquids, which progressively turns moreviscous with time after application of shake, agitation, or stress.
- A Bingham plastic which is a solid at normal temperature and low pressure but turns in a liquid when high stress is applied.

In the case of a Newtonian fluid, the viscosity generally is a function of its constituting particles and temperature. The viscosity of gases and other liquids usually depends on temperature and exhibit variation with pressure with a slow rate.

Viscosity is a physical quantity and in SI system its unit is poiseuille (Pl) and in cgs system, it is poise (P). poiseuille is comparable to the pascal second (Pa·s), or (N·s)/m2, or kg/(m·s). When a fluid flows between two plates having a separation of one meter, and one of the two is shoved in a side with a force of one

pascal, and its velocity is x meters per second, then the value of viscosity becomes 1/x pascal seconds. Inspite of this some other factors are also responsible for the variation of viscosity.

The viscosity of different solutions for this thesis has been measured. The relative viscosity (η_r) may be defined as the viscosity of the solution to the viscosity of the solvent. It has been analyzed using the Jones-Dole equation [18].

$$(\eta/\eta_o - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B\sqrt{m} \qquad \text{(II.15)}$$

where $\eta_r = \eta/\eta_o$, η and η_o are the relative viscosities, the viscosities of the ternary solutions (amino acid + aq. CD) and binary aqueous mixture (aq. CD) and m is the molality of the amino acids in ternary solutions. A and B are empirical constants known as viscosity A and B-coefficients, which are specific to solute-solute and solute-solvent interactions respectively, are estimated by least-square method by plotting $(\eta_r - 1)/\sqrt{m}$ against $\sqrt{m}$ and.

- The B-coefficients of zwitterionic group, (CH), side group (R) of the amino acids have been resolved as follows:

$$B = B\,(NH_3^+, COO^-) + B\,(CH) + B\,(\text{side grp}) \qquad \text{(II.16)}$$

$$B\,(R) = B - B\,(NH_3^+, COO^-) - B\,(CH) \qquad \text{(II.17)}$$

II.4.7. REFRACTIVE INDEX

Refractive index is a very important tool to predict the molecular interaction between the host and the guest molecules in solution systems.

The ratio of the speed of light in a vacuum to the speed of light in another substance is defined as the index of refraction (n_D) for the substance.

$$\text{Refractive Index } (n_D) \text{ of substance} = \frac{\text{Speed of light in vacuum}}{\text{Speed of light in substance}}$$

Whenever light changes its velocity as it crosses a boundary from one medium into another, its direction of travel also changes, i.e., it is refracted. The relationship between light's velocity in the two mediums (V_A and V_B), the angles of incidence ($sin\theta_A$) and refraction ($sin\theta_B$) and the refractive indexes of the two mediums (n_A and n_B) is shown below:

$$\frac{V_A}{V_B} = \frac{sin\,\theta_A}{sin\,\theta_B} = \frac{n_B}{n_A}$$

Thus, it is not required to determine the velocity of light in a sample to evaluate its index of refraction. Rather, by measuring the angle of refraction, and providing the index of refraction of the layer that is in contact with the sample, it is possible to evaluate the refractive index of the sample quite accurately.

The refractive index of aqueous solution mixtures can be correlated by the application of a composition-dependent polynomial equation. Molar refraction, was obtained from the Lorentz- Lorenz relation by using, n_D experimental data according to the following expression [19]

$$R=[(n_D^2-1)/n_D^2+2](M/\rho) \qquad \text{(II.18)}$$

Here M is the mean molecular weight of the mixture and ρ is density of the solution. n_D can be expressed by the following relation:

$$n_D=[(2A+1)/(1-A)]^{0.5} \qquad \text{(II.19)}$$

Where A is given by:

$$A=[\{\frac{(n_1^2-1)}{(n_1^2+2)}(1/\rho_1)\}-\{\frac{(n_1^2-1)}{(n_1^2+2)}(w_2/\rho_1)\}+\{\frac{(n_2^2-1)}{(n_2^2+2)}(w_2/\rho_2)\}\rho]$$

In the above equation, n_1 and n_2 are the pure component refractive indices, w_j the weight fraction, ρ *is* the density of the solution, and ρ_1 and ρ_2 the pure component densities.

The molar refraction deviation can be calculated by the following equation:

$$\Delta R = R - \phi_1 R_1 - \phi_2 R_2 \qquad \text{(II.20)}$$

Where ϕ_1 and ϕ_2 are volume fractions and R, R_1, and R_2 the molar refractivity of the mixture and of the pure components, respectively.

The Limiting molar refraction (R_M^o) have been estimated from the following relation[20]

$$R_M = R_M^o + R_S \sqrt{m} \qquad \text{(II.21)}$$

The higher value of R_M and the limiting molar refraction (R^o_M) indicate that the medium is more compact and dense. if the hydrophobic interaction between the host and guest molecules increases, the molar refraction and limiting molar refraction values also increase.

II.4.8. Hydration Number

Hydration number is the **number** of molecules of water with which an ion can combine in an aqueous solution of given concentration. The number of water molecules (n_H) hydrate the amino acids can be estimated from the value of measured standard partial molar volume. The values of ϕ^0_V of the studied amino acids can be expressed as [21]

$$\phi^0_V \text{ (amino acid)} = \phi^0_V \text{ (int)} + \phi^0_V \text{ (elect)} \qquad \text{(II.22)}$$

Here ϕ^0_V (int) is intrinsic partial molar volumes of amino acids and ϕ^0_V (elect) is electrostriction partial molar volume as a result of hydration of the amino acids. The ϕ^0_V (int) consists of two terms, *e.g.*, van der Waals volume and volume due to packing effects. The values of ϕ^0_V (int) for the amino acids were calculated from their crystal molar volume by using the following relationship [21],

$$\phi^0_V \text{ (int)} = (0.7/0.634)\ \phi^0_V \text{ (cryst)} \qquad \text{(II.23)}$$

Where, 0.7 is the packing density in an organic crystal and 0.634 is the packing density of randomly packed spheres. The molar volume of crystals ϕ^0_V (cryst) was calculated using the crystal densities of the amino acids represented by Berlin and Pallansch [22].The hydration number is estimated using the relation

$$n_H = \phi^0_V \text{ (elect)}/(V_e^o - V_b^o) \qquad \text{(II.24)}$$

where V_e^o is the molar volume of the electrostricted water and V_b^o is the molar volume of bulk water. This model implies that for every water molecules taken from the bulk phase to the surroundings of amino acid, the volume is decreased by $(V_e^o - V_b^o)$. The value of $(V_e^o - V_b^o)$ is calculated to be -3.0 or -3.3 at 298.15K respectively.

II.4.9. UV-Visible Spectra

The UV-Visible spectra can be termed as electronic spectroscopy as it involves the promotion of electrons from the ground state to the higher energy state. It is very useful to measure the number of conjugated double bonds and also aromatic conjugation within the various molecules. It also distinguishes between conjugated and non-conjugated systems. For visible and UV-spectrum, electronic excitations occur in the range 200-800mμ and involves the promotion of electrons to the higher energy molecular orbital.

The Absorption Law

Beer Lambert Law:

The change in intensity of light (*dI*) after passing through a sample proportionates to the following:

(i) Path length (*b*), the iarger the path, more number of photons should be absorbed.

(ii) Concentration (*c*) of sample, more molecules absorbing means more photons Absorbed.

(iii) Intensity of the incident light.

Thus, *dI* is proportional to *bcI* or $dI/I = - kbc$ (where *k* is a proportionality constant, the negative sign indicates that there is a decrease in intensity of the light, this makes *b*, *c* and *I* always positive. Integration of the above equation leads to Beer-Lambert's law :

$$- \ln I/I0 = kbc \quad \text{(II.25)}$$

$$- \log I/I0 = 2.303kbc \quad \text{(II.26)}$$

$$\varepsilon = 2.303k \quad \text{(II.27)}$$

$$A = - \log I/I0 \quad \text{(II.28)}$$

$$A = \varepsilon bc \quad \text{(II.29)}$$

A is reffered as absorbance and it is found to be directly proportional to the path length, *b* and the concentration of the sample, *c*. The extinction coefficient is characteristic of the substance under study and of course is a function of the wavelength.

Selection Rules:

(i) The transitions which involve a change in the spin quantum number of an electron during the transition do not occur. Thus singlet –triplet transitions are forbidden.

(ii) The transitions between orbitals of different symmetry do not occur.

When the molecule absorbs UV or visible light, its electron get promoted from the ground state to the higher energy state. In the ground state, the spins of the electrons are essentially paired. In the higher energy state, if the spins of the electrons are paired, then it is called an excited singlet state. On the other hand, if the spins of the electrons are parallel in the excited state, it is called an excited triplet state. The excited triplet state is always lower in energy than the excited singlet state. Therefore triplet state is more stable than corresponding excited singlet state. In the excited triplet state the electrons are far apart in space and thus the repulsions between them is minimized. An excited singlet state is converted to excited triplet state with the emission of energy as light. The transition from singlet ground state to excited triplet state is symmetry forbidden. The higher energy states are designated as higher energy molecular orbitals and also called anti-bonding orbitals. In most of the cases several transitions occur resulting in the formation of several bands.

Chromophore Concept:

Chromophore is any isolated covalently bonded group that shows a characteristic absorption in the UV-visible region.

There are two types of chromophores:

a) Cromophores in which the group contains π electrons and they undergo π-π* transitions. Such chromophores are ethylene and acetylene etc.

b) Chromophores which contain both π electrons and n electrons. Such chromophores undergo π-π* and n- π* transitions. Examples are carbonyls, nitriles, azo compounds, nitro compounds etc.

Auxochrome:

It may be defined as any group which does not itself act as chromophore but whose presence brings about a shift in the absorption band towards the red end of the spectrum. The combination of a chromophore and auxochrome gives rise to another chromophore. Some common auxochromic groups are –OH, OR, -NHR, $-NH_2$,-SH etc.

Bathochromic Effect:

It is an effect by virtue of which the absorption maximum is shifted towards longer wavelength due to presence of an auxochrome or by the change of solvent. Such an absorption shift towards longer wavelength is called red shift or bathochromic effect.

Hypsochromic Effect:

It is an effect by virtue of which the the absorption maximum is shifted towards shorter wavelength. The absorption shifted towards shorter wavelength is called blue shift or hypsochromic shift. It may be caused by the removal of conjugation and change in polarity of the solvent.

Hyperchromic Effect:

It is an effect by virtue of which the intensity of absorption maximum increases.

Hypochromic Effect:

It is an effect by virtue of which the intensity of absorption maximum decreases.

II.4.10. Mass Spectra Study

Mass spectrometry is the most accurate method for determining the molecular mass of the compound and its elemental position. In this process molecules are bombarded with a beam of energetic electrons. The molecules are ionized and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge i.e., m/e ratio. For most ions, the chage is one and thus m/e ratio is simply the molecular mass of the ion.

A parent ion results when one electron is removed from the parent molecule of the substance

$$M\,(g) + e \qquad M^{+}\,(g) + 2e$$

The m/e value of the parent ion is equal to the molecular mass of the compound. In a few cases, the parent ion peak may be the base peak and can be easily identified. In some cases the parent ion peak is not the base peak and present in very small abundance. Many elements occur in nature as their isotope and among them the lightest form generally predominates.

The mass spectrometer has three basic functions:

(i) To vapourise compounds of varying volatility.

(ii) To produce ions from the neutral compounds in the vapor phase.

(iii) To separate ions according to their mass over charge ratio and to record them. The plot of m/e values taken along the abscissa and their relative intensities along the ordinate is called mass spectrum.

$M^+ (g) \rightarrow m_1^+ + m_2$

or $m_1 + m_2^+$

Neutral particles produced in the process of fragmentation cannot be detected in the mass spectrometer.

The instrument needed to produce the mass spectrum of a compound consists of the following compounds

- **Ion source**

The first step to obtain mass spectrum is to ionize the sample under examination. The minimum energy required to ionize a molecule or an ion is called ionization potential. The ions are generally produced in mass spectrometer by bombardment of electrons. The energetic electrons are produced from an electrically heated tungsten filament. Few mg of the substance is produced as the vapour in the source. The vapour is allowed to pass through a slit in the chamber. It is bombarded by a stream of electrons. Due to bombardment the molecules generally loss one electron to form a parent ion radical.

- **Mass analyser**

The positively charged ions produced in the ion chamber are accelerated by application of an potential. These ions enter the mass analyser. The fragment ions are differentiated according to their m/e ratio. The positive ions are

directed through the slit. The ions travel through the whole analyser with high velocity and are separated according to their m/e ratio.

The positive ions travel in a circular path through 180° under a magnetic field H. let an ion having a charge e is accelerated through a voltage V. the kinetic energy of the ions are expressed as:

$\frac{1}{2}mv^2 = eV$

V=potential applied

v= velocity of the ions after acceleration.

In a magnetic field H, any ion will experience force Hev. It produces an acceleration of v^2/r in a circular path of radious r.

Hence, from Newton's second law of motion

$Hev = \frac{mv2}{r}$ (II.30)

Squaring both sides,

$H^2e^2v^2 = \frac{m2v4}{r2}$

$H^2e^2 = m2v2/r2$

But $\frac{1}{2}mv^2 = eV$

Therefore $mv^2 = 2eV$

Putting the value of mv^2 in equation

$H^2e^2 = m.2eV/r^2$

$H^2e = 2mV/r^2$

$m/e = H^2r^2/2V$

From the above equation it is clear in a given magnetic field strength and accelerating voltage, the ions of m/e value will follow a circular path of radious r. the ions of various m/e values reach the collector, amplified and recorded. The mass spectrum can be recorded either by

(i) Changing H at constant V or

(ii) Changing V at constant H

When magnetic field is varied, the method is called magnetic scanning. It is called electric voltage scanning when potential is varied at constant field strength H.

The mass spectrum of the parent ion gives the molecular mass of the sample. In the mass spectrum it is important to locate the molecular ion at the high mass regeion of the spectrum. The stability of the parent ion decides the relative abundance. The peak intensity of the molecular ion differs from one compound to another.

Some important features of the parent ion peak are as follows:

a) The molecular ion peak in aromatic compounds is relatively much intense due to the presence of π electron system.

b) Conjugated olefins show more intense molecular ion peak as compared to the corresponding non conjugated olefins with the same number of unsaturation. Conjugated olefins are more stable than the corresponding non conjugated olefins.

c) Unsaturated compounds give more intense peaks than saturated or cyclic compounds.

d) The relative abundance of the saturated hydrocarbon is more than the corresponding branched chain compound with the same number of carbon atoms.

e) The substituent groups like –OH, -OR, -NH_2 etc which lower the ionization potential increase the relative abundance in case of aromatic compounds. Also the groups like –NO_2,-CN etc which increase the ionization potential decrease the relative abundance in case of aromatic compounds.

f) Absence of molecular ion peak in the mass spectrum suggests that the compound under investigation is highly branched or tertiary alcohols.

g) In case of chloro or bromo compounds, isotope peaks are also formed along with the molecular ion peak.

Metastable peaks can be easily determined in the mass spectrum. Some features of them are

a) They do not necessarily occur at the integral m/e values.

b) These are much broader than normal peaks

c) These are of relatively low abundance.

ESI-mass spectrometric analyses were used to detect the formation of IC synthesized by the method mentioned earlier in the solid state. The observed peaks are analysed which confirms that both α and β-CD forms inclusion complexes with guest molecules in the solid state. The stoichiometric ratio of host CD and the guest is 1:1.

II.4.11. Infra-Red Spectra Study

Infra- red spectrum is an important study that gives sufficient information about the structure of the molecule. Unlike UV-Spectrum, it provides a large number of absorption bands from which a wealth of information can be derived about the structure of an organic compound. The absorption of IR radiations causes the various bonds of a molecule to stretch and bend with respect to one another. The most important region for an organic chemist is 2.5 μ to 15μ in which molecular vibrations can be detected and measured in IR spectrum.

The absorptions of IR radiations can be expressed either in terms of wavelength or wave number. Band intensity is either expressed in terms of absorbance (A) or transmittance (T).

$A = \log 1/T$

This technique can be employed to establish the identity of two compounds or to determine the structure of new compound.

The absorption of IR- radiations causes an excitation of molecule from a lower to a higher vibrational level. Each vibrational level is associated with a number of closely spaced rotational levels. The IR-spectra is considered as vibrational-rotational spectra. All the bons in a molecule are not able to absorb infra-red energy but only those bonds which are accompanied by a change of dipole moment will absorb in this region. These vibrational transitions are called infra-red active transitions. They are responsible for absorption of energy in this region. For example, vibrational transitions of C=O, N-H, O-H etc bands are accompanied by a change of dipole moment and thus absorbs in the IR-region. Transitions in carbon-carbon bonds in symmetrical alkenes and alkynes are not accompanied by the change in dipole moment and hence do not absorb in the IR region. Since the absorption in the IR region is quantized, a molecule of the organic compound will show a number of peaks in the IR region.

In the infra-red spectroscopy, the absorbed energy brings predominant changes in the vibrational energy which depends upon

a) Masses of the atoms present in a molecule

b) Strength of the bonds

c) The arrangement of the atoms within the molecule.

Two types of fundamental vibrations are-a) stretching and b) bending.

Types of stretching vibration:

(i)Symmetric Stretching (ii) Assymetric stretching

Types of bending vibration:

(i)Scissoring (ii) Rocking (iii) Wagging (IV) Twisting

The infra-red light is absorbed when the oscillating dipole moment interacts with the oscillating electric vector of an IR beam. For this interaction or absorption to occur, it is important that the dipole moment at one extreme of the vibration must be different from the dipole moment at the other extreme of the vibration in a molecule. For IR absorption, the vibrations should not be centro-Symmetric. Only those vibrations are IR active which are not centro-symmetric. As most of the functional groups in organic chemistry are not centro-symmetric, this technique is informative to organic chemists. The IR spectrum of a molecule results due to transitions between two different vibrational energy levels. The vibrational energy of a chemical bond is quantized.

Selection Rules:

a) If a molecule has a centre of symmetry, then the vibrations are centrosymmetric and are inactive in the Infra-Red.

b) The vibrations which are not centrosymmetric are active in Infra-red.

Factors influencing vibrational frequencies:

Electronic effects: electronic effects include inductive, mesomeric and field effects etc. under the influence of these effects the bond strength changes and as a the absorption frequency shifts from the normal value. The +I effect causes the wave number of equation to decrease whereas the groups which have –I effect increase the wave number of absorption. Conjugation also lowers the absorption frequency.

Hydrogen Bonding: hydrogen bonding brings about remarkable downward frequency shifts. Stronger the hydrogen bonding, greater is the absorption shift towards lower wave number than normal value. Two types of hydrogen bonding

can be readily distinguished in infra-red technique. Intermolecular hydrogen bonds give rise to broad bands whereas bands arising from intramolecular hydrogen bonds are sharp and well defined. Intermolecular hydrogen bonds are concentration dependent.

The formation of inclusion complex of various guest molecules with α and β-CD is supported by FT-IR study. There are many changes in the FT-IR spectra of solid inclusion complexes due to the loss of bending and vibrating peaks of the guest molecule after complexation. The IR spectra of the guest are charecterised by various distinct peaks of different functional groups. Broad characteristic peaks of –OH at about 3412.10 cm^{1} and 3349.84 cm^{1} are present in the spectrum for α and β-CD. But if we examine the IR-spectra of the inclusion complex, it has been found that many peaks of the guest are either absent or shifted. The reason may be that after inclusion in the cavity of CD the environment of the guest is changed. The –O-H frequency of both α and β-CD are shifted to lower region probably due to involvement of the –O-H groups of the host molecules in hydrogen bonding with the guest molecule. No additional peaks are recognized in the solid inclusion complexes which mean no chemical reaction occurred between the guest molecule and CD.

II.4.12. ^{1}H-NMR :

The nucleus of a hydrogen atom behaves as a spinning bar magnet because it possesses both electric and magnetic spin. Nuclear magnetic resonance involves the interaction between an oscillating magnetic field of electromagnetic radiation and the magnetic energy of the hydrogen nucleus or some other type of nuclei when these are placed in an external static magnetic field. The sample absorbs electromagnetic radiation in radiowave region at different frequencies since absorption depends upon the type of protons or certain nuclei contained in the sample.

It has been found that

$\omega = \gamma H_{\circ}$

ω= angular precessional velocity, γ= gyromagnetic ratio, $H_{\circ}$ = applied field in gauss

According to the fundamental NMR equation which correlates electromagnetic frequencies with the magnetic field,

$\gamma H_{\circ} = 2\pi\upsilon$

υ= frequency of electromagnetic radiation.

Therefore, $\omega = 2\pi\upsilon$

The precessional frequency may be defined as the number of revolutions per second made by the magnetic moment vector of the nucleus around the external field $H_{\circ}$. All nuclei carry a charge. So they will possess spin angular momentum. The moment of the spin angular momentum is quantized. The spin quantum number I is associated with mass number and atomic number of nuclei. The circulation of the nuclear charge generates a magnetic moment along the axis. The intrinsic magnitude of the generated dipole is expressed in terms of magnetic moment μ.

If a proton is placed in a magnetic field, then it starts precessing at a certain frequency in the radiowave region and thus will be capable of taking up one of the two orientations with respect to the axis of the external field.

a) Alignment with the field

b) Alignment against the field.

If a proton is precessing in the aligned orientation, it can pass into the opposed orientation by absorbing energy. The transition from one energy state to the other is called flipping of the proton. The transition between the two energy states can be brought about by the absorption of a quantum of electromagnetic radiation in the radiowave region with energy $h\upsilon$.

Relaxation Process:

(i) **Spin-Spin Relaxation:** it is due to the mutual exchange of spins by two precessing nuclei which are in close proximity to each other. Each precessing nucleus is associated with a magnetic vector component rotating in a plane perpendicular to the field. The spread of energy among the nuclei concerned results in line broadening which makes nmr spectra of solids comparatively more interesting.

(ii) **Spin-Lattice Relaxation:** it involves the transfer of energy from the nucleus from higher energy state to molecular lattice. The energy is transformed to the components of lattice as the additional translational, vibrational and rotational energy. The total energy of the system remains the same. An efficient relaxation process involves a short time and results in the broadening of absorption peaks.

(iii) **Quadrupole Relaxation:** it is a prominent relaxation process for nuclei having $I > ½$. The nuclei due to anisotropic interaction between non spherical, electrically quadruple nuclei and the electric field gradients at the nucleus caused by electric environments possess an asymmetric positive charge distribution on the nuclei. Hence these nuclei exhibit electric quadruple moments and relax rapidly and display very broad signals.

Chemical shift:

When a molecule is placed in a magnetic field, its electron is caused to circulate and thus they produce secondary magnetic field or induced magnetic field. Rotation of electrons about the proton itself generates a way that at the proton, it opposes the applied field. Thus the field felt by the proton, is diminished and the proton is said to be shielded. But if the induced field reinforces the applied field, the proton feels a higher field strength and thus the proton is said to be deshielded. Shielding shifts the proton upfield and deshieleding shifts the absorption downfield to get effective field strength necessary for absorption. Such shifts in the position of nmr absorptions is called chemical shift. For

measuring chemical shift of various protons in a molecule, tetramethyl silane is taken as a reference. The difference in the absorption position of the proton with respect to TMS signal is called chemical shift (δ value).

Factors influencing chemical shift

(i) Inductive effect

(ii) Van Der Waal's deshielding

(iii) Anisotropic effects

(iv) Hydrogen bonding

II.4.13. 2D ROESY NMR:

Rotating-frame Overhauser Effect SpectroscopY is an experiment in which homonuclear NOE effects are measured under spin-locked conditions. ROESY is especially suited for molecules with motional correlation times. In such cases the laboratory-frame NOE is nearly zero, but the rotating-frame NOE (or ROE) is always positive and increases monotonically for increasing values of motional correlation times. In ROESY the mixing time is the spin-lock period. During this time spin exchange occurs among spin-locked magnetization components of different nuclei. Different spectral density functions are relevant for ROESY than for NOESY and these cause the ROE's to be positive for all values of motional correlation time.

2D ROESY spectroscopy provides conclusive evidence about the spatial proximity of the interacting atoms of the host and the guest by observing the intermolecular dipolar cross-correlations. Two protons which are situated within 0.4 nm in space may produce a Nuclear Overhauser Effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or rotating-frame NOE spectroscopy (ROESY). As the structural features of α and β-CD described earlier, the inclusion phenomenon into the CD cavity may be proved by the appearance of NOE cross-peaks between the H3 or H5 protons of CD and the interacting protons of the

guest recognizing their spatial proximity. For establishing this, 2D ROESY were obtained of the inclusion complexes in D_2O. The observed cross-peaks signify the insertion of the guest molecules inside the cyclodextrin cavities.