CHAPTER-II

GENERAL INTRODUCTION (REVIEW OF THE EARLIER WORKS)

II.1. Host-Guest Chemistry-An Overview

Host-guest chemistry involves the idea of complementary binding between two or more molecules. An Inclusion complex is a well-defined discrete system in which a host molecule having convergent binding sites (i.e. donor atoms, sites for formation of hydrogen bonds and sizable cavity) and a guest having divergent binding sites (i.e. hydrogen bond acceptor atoms) are bound together by the means of the non-covalent interactions [29]. These binding interactions can be specific and directional, such as hydrogen bonding, π-π stacking interactions and non-directional such as electrostatics, dispersion and inductive forces, and hydrophobic or solvatophobic effects [30] [31].

Cyclodextrins belong to the family of cyclic oligosaccharides and are composed of α-(1,4) linked glucopyranose subunits. They have a cage-like supramolecular structure, which is quite similar to the structures formed from cryptands, calixarenes, cyclophanes, spherands and crown ethers. These compounds carry out chemical reactions where non-covalent interactions play a vital role between interacting molecules, ions or radicals. These reactions are mostly 'host-guest' type. Cyclodextrins are considered as the most important among all other supramolecular hosts mentioned above because of their inclusion complex forming capability, negligible cytotoxic effects and greater bioavailability. As a consequence of molecular complexation phenomena, inclusion complexes of CDs are widely used as optical sensors, electrochemical sensors, supramolecular catalysts, and in the pharmaceutical industry as anti-cancer agents [3].

There are three types of Cyclodextrins: α-cyclodextrin, β-Cyclodextrin and γ-cyclodextrin, referred to as first generation or parent cyclodextrins. α-cyclodextrin, β-Cyclodextrin and γ-cyclodextrin are composed of six, seven and eight α -(1,4)-linked glycosyl units, respectively [32]. β -Cyclodextrin is the most accessible, the lowest-priced and generally the most useful.

Cyclodextrin molecules possess truncated conical structure having secondary -OH groups extending from the wider rim and the primary –OH groups from the narrower rim. This gives rise to a hydrophilic outer surface, whereas the lipophilicity of the central cavity is comparable to an ethanolic solution. They have limited aqueous solubility because of having strong intermolecular hydrogen bonding in the crystal state [2]. Modification of the 2- or 3- hydroxyl group disrupts the hydrogen bonding occurring around the ring of the CD molecule. The

disruption enables more interactions of these hydroxyl groups with water molecules, which in turn results in altered solubility[33]. Cyclodextrins are insoluble in most of the organic solvents; they get soluble in some polar and aprotic solvents. Although in certain organic solvents, the solubility of cyclodextrins is higher than that of water, but complexation does not occur readily in non-aqueous solvents. The fact may be attributed to the increased affinity of the guest for the solvent compared to its affinity for water.

II.1.1 Inclusion Complex formation

Inclusion Complexation between the guest molecule and host molecules generally (such as cyclodextrins and crown ethers) proceeds through a non-covalent interaction between them. In these complexes (**Fig.II.1**.), a guest molecule is held within the hollow cavity of the host molecule.

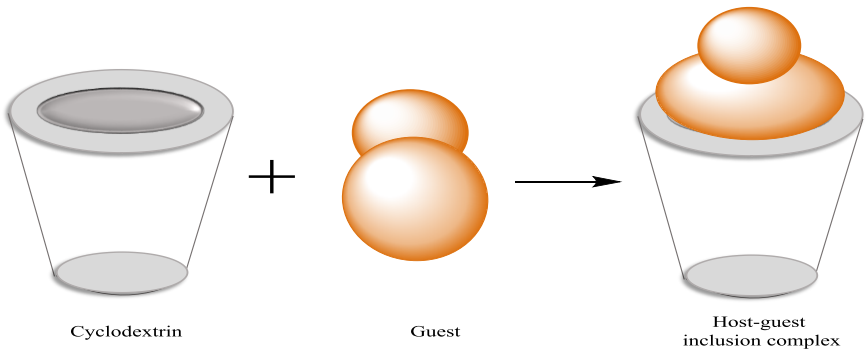
The lipophilic cavity of cyclodextrin molecules provides a suitable. microenvironment into which properly fitted non-polar moieties easily enter to form inclusion complexes [34]. During the formation of the inclusion complex, no bond breaking or bond-making process takes place [35].

The release of enthalpy-rich water molecules from the cavity plays the main driving force during complexation. Water molecules get displaced by more hydrophobic guest molecules present in the solution in order to attain an apolar–apolar association and minimize the strain caused within cyclodextrin ring and finally, it results in a more stable. lower energy state [36].

This complex formation procedure is often regarded as 'encapsulation' of the guest molecule, or at least the labile part of the molecule. Such encapsulation protects the drug molecule against environmental degradation and thus the rate of hydrolysis, oxidation, racemization and enzymatic decomposition get decreased [37]. In addition, cyclodextrins are capable of decreasing the photo degradation of various light-sensitive drugs.

Such inclusion process is dynamic in nature whereby the guest molecule continuously associates and dissociates from the host CD. The Binding strength depends on the extent of proper fitting of the 'host-guest' complex together and the nature of specific local interactions between surface atoms [2]. Complexes are formed either in solution or in the crystalline state and in most of the cases, water is the solvent of choice. Inclusion complexation can be achieved in a co-solvent system and in the presence of any non-aqueous solvent. Inclusion in cyclodextrins results in certain changes in physicochemical properties of guest molecules as they are temporarily encapsulated or caged within the host cavity. It gives rise to beneficial modifications of guest

molecules [38] such as solubility enhancement of completely insoluble guest molecules, stabilisation of labile guests against the environmental degradation, oxidation, hydrolysis, visible or UV light and heat, decrease in volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off flavours, unpleasant odours and controlled drug delivery and release [2]. Therefore, cyclodextrins are used in food [39], pharmaceuticals [40], cosmetics [41], environment protection [42], and the textile industry [43].



Scheme. II.1. Schematic representation for host-guest complexation by cyclodextrin.

II.1.2 The Stoichiometry

The stoichiometry of the inclusion complex formed is given by the number of G and H molecules contained in the supramolecular complex, the general representation being GnHm; the most studied and well-known stoichiometry is 1:1 (GH), implying the inclusion of a single guest molecule, but other stoichiometries like G1H2, G2H1, G2H2, G1H3, G3H1, etc., are also found in literature survey [44] .As the formation of the G1H2 complex can be the result of two successive equilibriums, the simultaneous presence of 1:1 and 1:2 complexes is also frequently mentioned [45-51] [52] [53].

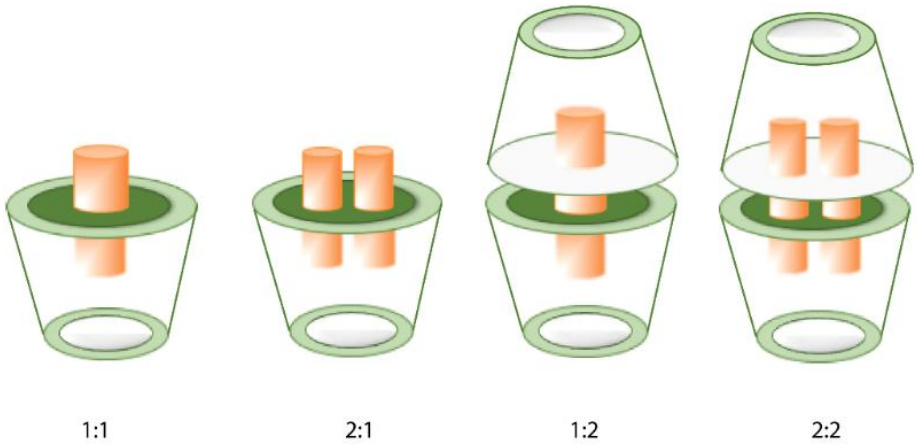


Fig.II.1. Schematic illustrations of stoichiometry of host-guest inclusion complex.

For 1:1 stoichiometric ratio, The association of the host CD and guest (G) molecules, and the dissociation of the formed CD/guest complex is governed by the following thermodynamic equilibrium,

$$H + G \rightleftharpoons H.G \quad (1)$$

One of the most reliable methods used for determining the stoichiometry of inclusion complexes is Job's method. It is also known as the 'Continuous Variation Method' (Job, 1928). The samples used in this experiment are prepared by mixing different volumes of the two solutions in such a way that the total concentration [H]+[G] remains constant and the molar fraction of the guest, XG varies in the range 0–1. The variation of the experimentally measured property i.e. the change of the absorbance of the guest during the addition of the host, ΔA, in presence of the host in respect with the value for the free guest is plotted vs. XG or XH. The value of XG for which the plot presents the maximum deviation gives the If ΔA v/s XH graph is plotted, then stoichiometry of the inclusion complex can be determined (R = 0.5 for 1:1 or 2:2 G:H complexes; R = 0.33 for 1:2 G: H complexes).

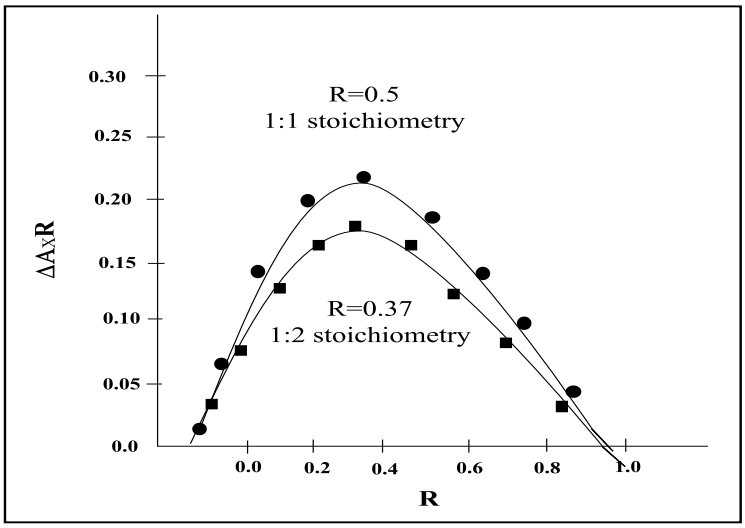


Fig. II.2. Job's plots for 1:1 and 1:2 stoichiometry.

II.1.2.1 Determination of both the stoichiometry and the association constant

The value of association constant determines the extent of host-guest interaction and from Job's method of continuous variation, the stoichiometry of the inclusion complex can be determined. Several stoichiometries are assumed and the experimental data are fitted to the corresponding linear or nonlinear models. The most frequently used equations are the Benesi-Hildebrand linear or double reciprocal equations.

Benesi-Hildebrand equation for 1:1stoichiometry is stated below

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[G]K_a} \cdot \frac{1}{[H]} + \frac{1}{\Delta\varepsilon[G]} \quad (2)$$

Where ΔA stands for difference in absorbance, Δε stands for change in molar extinction coefficient, K_a is the association constant, [H] and [G] represents concentration of host and guest respectively.

For Fluorescence studies, the equation becomes

$$\frac{1}{\Delta F} = \frac{1}{\Delta\varepsilon[G]K_a} \cdot \frac{1}{[H]} + \frac{1}{\Delta\varepsilon[G]} \quad (3)$$

Where, ΔF stands for difference in intensity.

Ii.1.3 Types Of Interactions

The thermodynamic properties of amino acids in aqueous solutions of drug molecule play a crucial role in the biological and industrial processes. The physicochemical properties can improve the understanding of the interactions taking place in the medium. The types of interactions are described below-

II.1.3.1 Hydrophobic Interactions

The term "hydrophobic interaction", defines the tendency of nonpolar groups to get associated in aqueous solution, thereby decreasing the extent of contact with neighbouring water molecules present in the solution. The enthalpy of formation of a hydrophobic bond is destabilizing ($\Delta H_o > 0$).

The water molecules become more ordered around exposed nonpolar solutes, which leads to an increase in entropy of the system and such entropy effect favours the formation of the hydrophobic interaction. Hydrophobic interactions are considered to be one of the most important effects in the stabilization of the conformation of proteins in aqueous solution.

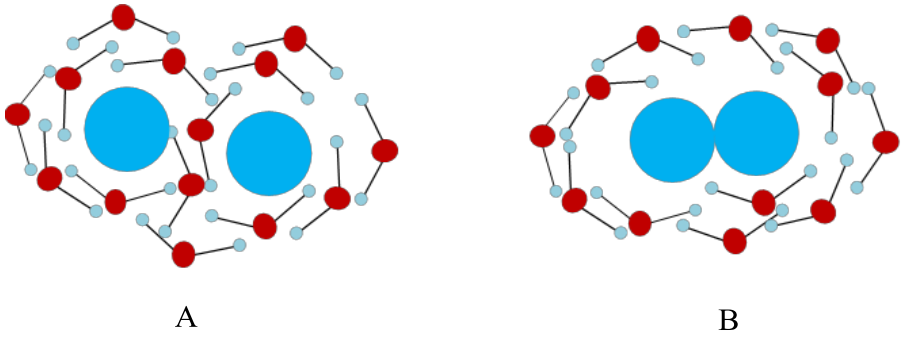


Fig. II.3. Hydrophobic-hydrophobic interaction: (A) no interaction between hydrophobic moieties (B) hydrophobic interaction takes place

II.1.3.2 Van der Waals force

The Van der Waals forces deal with the momentary attraction between molecules and atoms. Hydrophobic interactions occur mostly in aqueous solutions of both biological macromolecules and materials of low molecular weight. Since they originate from the atomic level, they are important in all aspects involving materials. They are not as strong as Coulomb or hydrogen bonding forces. The Van der Waals forces are held to be responsible for coagulation of colloids and coalescence of drops and bubbles.

Dipolar nature of Vander Waals force is described below-

(i). Dipole-dipole interaction

This type of interaction is observed in polar molecules. Attractions between dipoles are stronger than forces involving induced dipoles. The relative strength of the dipole-dipole interaction force depends upon the dipole moments. If the dipoles are fixed, the interaction energy decreases as a function of the dipoles, which means that the greater the dipole moment of the molecule, greater is this force of interaction. The solubility of one polar liquid in another polar liquid arises because of dipole-dipole interaction between unlike molecules.

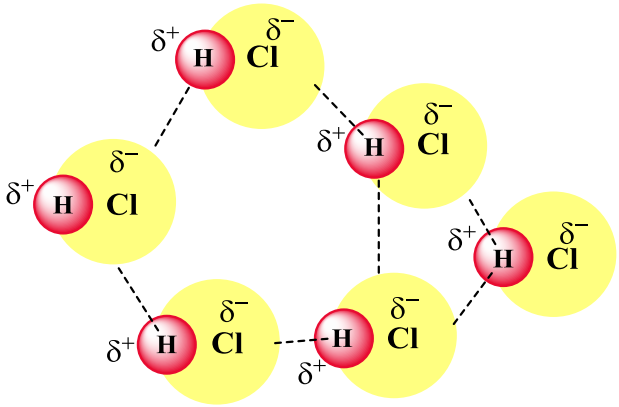


Fig. II.4. Dipole-dipole interaction

(ii). Ion-dipole interaction

These are the long-range interactions occurring between ions and partial charges in a polar molecule or dipole and are usually 50-200 kJ/mol in strength. Na^+ binds to six water molecules or to six oxygen in crown ether, it is an example of such interaction.

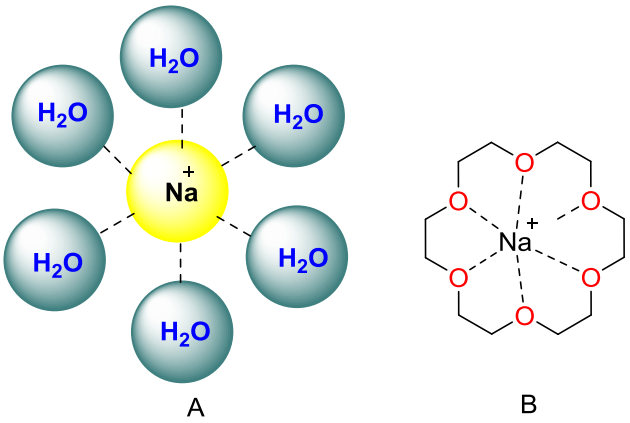


Fig. II.5. Ion-dipole interaction operates when (A) Na^+ is surrounded by six molecules of water and (B) Na^+ binds to the oxygen atoms of 18C6.

(iii). Dipole-induced dipole interactions

The electrical field of the dipole is able to induce a dipole moment in an adjacent molecule (polar or nonpolar). Then the induced dipoles electrostatically interact with the polarizing dipole. Since these forces need both a polar molecule (and ion) and a non-polar molecule, such occurrence only happens between induced dipoles and permanent dipoles.

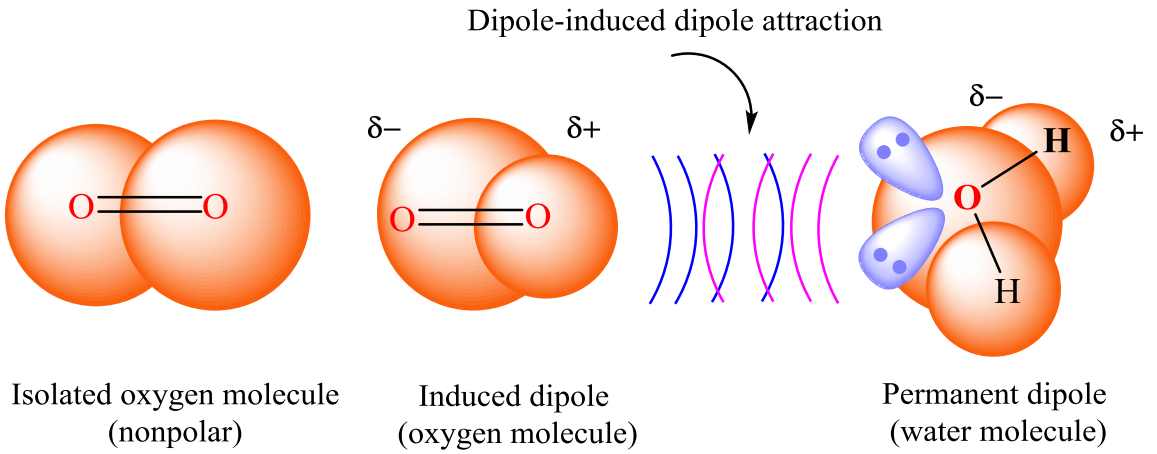


Fig. II.6. Dipole-induced dipole interaction

(iv). Instantaneous Dipole-induced dipole interactions

The uncharged molecules which contain nonpolar bonds can interact electrostatically. Dipoles can appear transiently when electrons in a nonpolar bond get unevenly distributed due to the random motion of electrons. These transient dipoles are known as instantaneous dipoles. Instantaneous dipoles can be induced if a nonpolar bond comes into closer vicinity with a polar bond or charged atom (**Fig. II.7.**); such dipoles are called induced dipoles. The electrostatic attractions between instantaneous dipoles are known to be Instantaneous Dipole-induced dipole interactions.

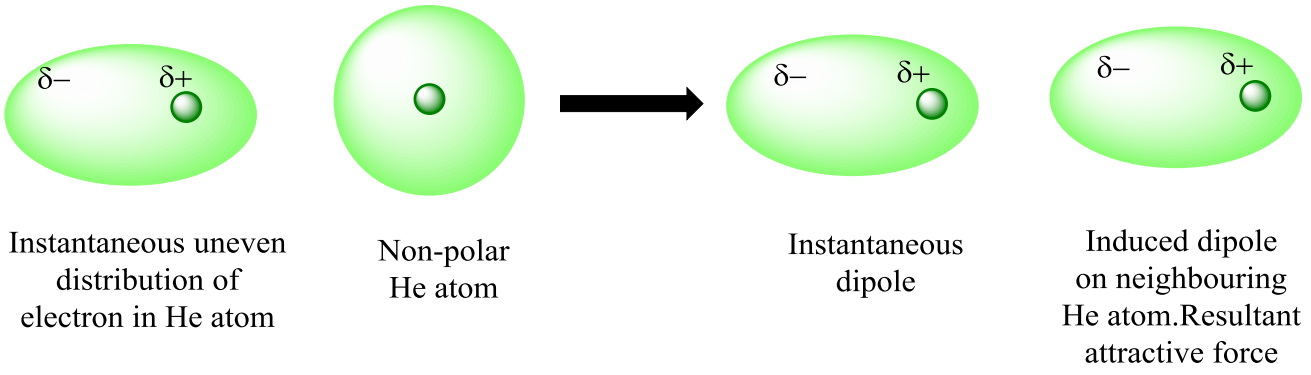


Fig. II.7. Instantaneous Dipole-induced dipole interactions.

II.1.3.3 Hydrogen bonds

Moore T S and Winmill T F [54] the first proposed the concept of hydrogen bond in molecular interaction studies. Hydrogen bonding is considered as a coulombic force of attraction between

H atom from a molecular fragment A–H in which A is more electronegative than H, and an atom or a group of atoms in the same or a different molecule [55] [37].

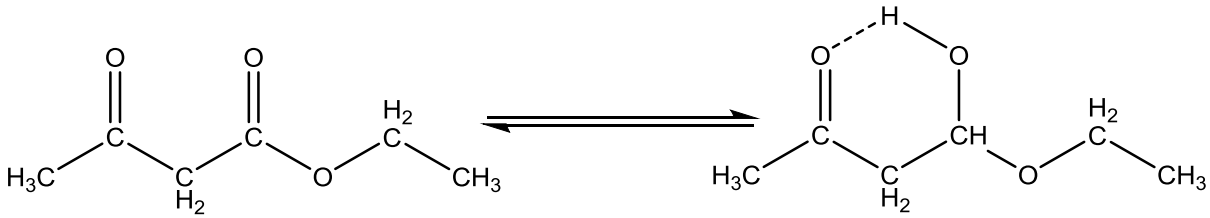
A typical hydrogen bond is represented as A–H•••B–D, where the three dots denote the bond. A–H represents the hydrogen bond donor. B is the acceptor and B is bonded to D. In some specific cases A and B are the same and A-H and B-H bond distances are the same as well leading to symmetric hydrogen bonds. The H•••B bond strength increases with the increase in electronegativity of A.

The length of the A–H bond usually increases on hydrogen bond formation which leads to a red shift in the infrared A–H stretching frequency and there occurs an increase in the infrared absorption cross-section for the A–H stretching vibration. The greater the lengthening of the A–H bond in A–H•••B, the stronger is the H•••B bond. The A–H•••B–D hydrogen bond leads to a characteristic shift in NMR signals because of the pronounced proton deshielding for H in A–H, through hydrogen bond spin-spin couplings between A and B. [56]. Hydrogen bonding is of two types- (a) Intra molecular hydrogen bond and (b)Inter hydrogen bonding interaction [57].

(a) Intra molecular hydrogen bonding

Hydrogen bonding occuring within the same molecules is termed as **'intramolecular hydrogen bonding'**. It results in chelating or ring formation.

Examples: The Enol form of Ethyl acetoacetate gets stabilised through intramolecular hydrogen bonding.



Keto form Enol form

Fig. II.8. Intra molecular hydrogen bonding in Ethyl acetoacetate.

(b) Intermolecular hydrogen bonding

Hydrogen bonding occuring between two or more similar or different molecules is named as '**inter molecular hydrogen bonding'**. The molecular association is direct evidence of such kind of hydrogen bonding.

Examples: Water, Alcohols, Amines and acids

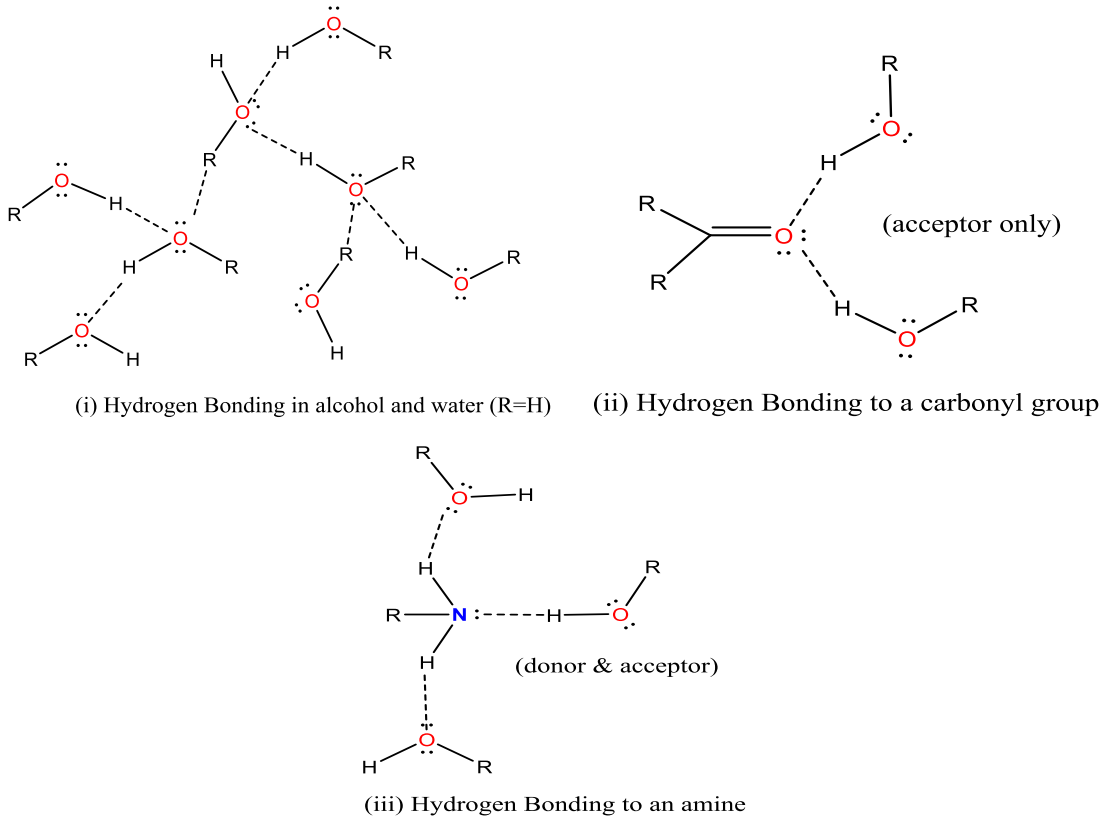


Fig. II.9. Inter molecular hydrogen bonding in (i) water and alcohols (ii) carbonyls and (iii) amines

Inter molecular hydrogen bonds start to break down with the increase in dilution, where as intra molecular hydrogen bonds do not get affected [58] [59] [60, 61] [62]. The hydrogen bonding exchange interaction does not affect any change in electron density in other parts of molecules participating in hydrogen bond formation. In proton acceptor, molecule charge redistribution arises due to the polarization interaction. The electron rearrangement influences charge distribution in another part of molecules [62].

II.4 METHODS OF DETECTING THE INCLUSION PROCESS

Structural characterization is of particular significance for supramolecular host-guest complexes, which are the basis of most CD applications in medicine, catalysis or in food chemistry, separation and sensor technology. NMR spectroscopy has become the most important method for structural elucidation of inclusion complexes. There are a few alternatives to NMR in the study of inclusion complexes such as fluorescence, UV-visible spectroscopy studies

play a major role in measuring complexation energetics. FTIR, High-Resolution Mass Spectroscopy (HRMS) is used in characterizing solid inclusion complexes.

II.4.1. FTIR Spectra of solid inclusion complexes

Infrared (IR) radiation refers to extensively to the part of the electromagnetic spectrum between visible and microwave region. Infrared (IR) radiation having frequencies less than about 100 cm^{-1} is absorbed and converted by a molecule into the energy of molecular rotation. Such absorption is quantized, but the vibrational spectra appear as bands rather than as lines as a single vibrational energy change is accompanied by a number of rotational energy changes. The vibrational rotational bands occur between 4000 cm^{-1} and 400 cm^{-1}. The frequency or the wavelength of absorption is dependent upon the relative masses of the atoms involved, the force constants of the bonds and also the geometry of the atoms. The bond positions in IR spectra are represented as wavenumbers ($\bar{\upsilon}$), whose unit is cm^{-1}.

IR absorption positions are generally presented as either wave numbers ($\bar{\upsilon}$) or wavelengths (l). Wave number defines the number of waves per unit length i.e. wave numbers is directly proportional to frequency, as well as the energy of the IR absorption. The unit of the wave number is cm^{-1}(reciprocal centimetre). Wavelengths are inversely proportional to frequencies and their associated energy. The stretching frequencies can be assigned by applying Hooke's law-

$$\bar{\upsilon} = \frac{1}{2\pi c}\sqrt{\frac{f}{M_x M_y / M_x + M_y}} \qquad (4)$$

$\bar{\upsilon}$= Vibrational frequency (cm^{-1})

c= velocity of light (cm/s)

f= force constant of the bond (dyne/cm). It is a measure of bond stiffness.

M_x & M_y = Mass (g) of atom x and atom y respectively. For single bond, the approximate value of f is 5×10^5 dyne cm^{-1}. For double and triple bond the values of 'f' will be two and three times respectively. In the IR spectrum, wavelength or wavenumber taken as the x-axis and absorption intensity or per cent transmittance as the y-axis.

II.4.2. NMR Spectroscopy

Nuclear Magnetic Resonance spectroscopy (NMR) is considered to be one of the most useful techniques to study interactions of cyclodextrins and crown ethers with guest compounds. This technique provides information about the exact orientation of the guest molecule inside the cavity. The other e physicochemical parameters and stoichiometry of the inclusion complexes can also be evaluated with the help of NMR spectroscopy.

By observing the difference in the chemical shifts of protons between the free guest and host species and the suggested complex, it can be easily concluded whether the inclusion complex has formed or not. The stability of the inclusion complex and the orientation of the drug molecule can be investigated NMR studies. Demarco and Thakkar, were the first to notice a change in chemical shift of CD protons (specifically H3 and H5) in the presence of guest molecules and from this observation they concluded that inclusion had taken place.

Greatbanks & Pickford [63] also observed that when $\Delta\delta$ H3 > $\Delta\delta$ H5, there occurs partial inclusion of the guest inside the hollow cavity of host and when $\Delta\delta$ H3 < $\Delta\delta$ H5, a total inclusion takes place.

II.4.2.1. 2D ROESY

The implementation of two-dimensional NMR spectroscopy has widely influenced the potential power of NMR as a tool for the structure elucidation of larger molecules.

It's main advantages are:

• Splitting of signals into two orthogonal dimensions and

• the orientation of the guest molecule inside the host cavity can be easily understood.

Rotating Frame Nuclear Over Overhause Effect Spectroscopy is very effective for determining which signals arise from protons in close vicinity to each other in space, even in their non-bonded condition. Nuclear Overhauser Effect (NOE) has well-accepted application in structure determination. With high field NMR spectrometer, the detection of NOE by NOESY sometimes becomes quite difficult for molecules having molecule weight in the order of 1000 to 2000, as the NOE effect changes its sign depending on the time required for molecular correlation. When molecules show motional correlation time near the condition $\omega_0\tau_c = 1$, where ω_0 denotes Larmor frequency and τ_c stands for correlation time, no NOE will be observed. When $\omega_0\tau_c > 1$ (in the case of macromolecule) the NOE reaches -1 and specificity is lost due to spin diffusion. The ROESY

experiment with spin lock in the rotating frame is specifically suitable. for overcoming these difficulties since ROESY enhancement is always positive and increases uniformly with $\omega_0\tau_c$. NOE peaks appear in a positive phase and diagonal peaks have a negative phase in the ROESY spectrum. A ROESY spectrum proceeds through space correlations via spin-spin relaxation. ROESY is capable of detecting chemical and conformational exchange.

II.4.3. OPTICAL SPECTROSCOPY

II.4.3.1 UV-Visible spectroscopy

Absorption of visible and ultraviolet (UV) radiation is directly related to the excitation of electrons, in both atomic and molecular level. Since the energy levels of matter are quantized, only light having a precise amount of energy, which can cause transitions from lower energy levels to the higher ones will be absorbed. The larger the gap between the energy levels, the greater the energy needed to promote the electron from lower energy level to the higher one; This promotion of electron results in light having a higher frequency, and hence as a result of this, the wavelength is shorter and gets absorbed. The possible electronic transitions that light might cause are –

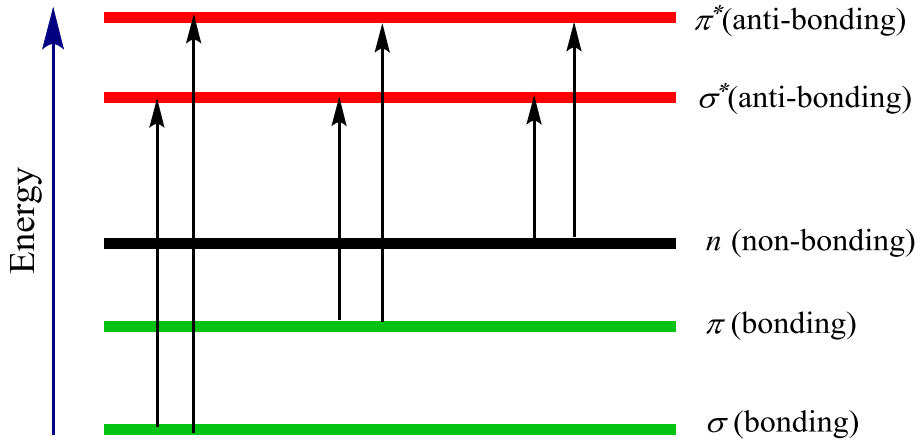


Fig. II.10. Different types of Electronic Transitions in UV-Visible region.

II.4.3.2. Different types of Electronic Transitions

Transitions between electronic states are divided into the following categories:

π ⟶ π* transitions: For molecules consisting ofπ bonds (such as alkenes, alkynes, aromatics, acryl compounds, nitriles) light promote electrons from a π bonding molecular orbital to a π

anti-bonding molecular orbital. This is called a π → π^*transition (high extinction coefficient). Groups of atoms involved in π bonding are usually called 'chromophores'.

n **→π* transitions:** Lone pair electrons on oxygen and nitrogen atoms get promoted from their non-bonding molecular orbital to a π anti-bonding molecular orbital. Such transition is known as

n **→π*** transition. It requires less energy than **π → *π****transitions and the transition probability is also low.

n **→ б* transition:** Saturated compounds which contain lone-pairs (such as water, ammonia, hydrogen disulfide only have ***n*** **→ б*** and **б → б*** transition in the UV-visible range.

б → б* transition: Bonding electrons undergo such type of transition. The promotion of electrons from **б** to **б*** requires **a** large amount of energy, 2-3 times more than other transitions. Conventional UV spectroscopy can not detect such transitions.

After absorbing light of specific wavelength all molecules undergo electronic transition, but for most of the cases, very high energy radiation (in the vacuum ultraviolet, <200 nm) is needed. Therefore, the absorption of light in the UV-visible region will only result in the following transitions:

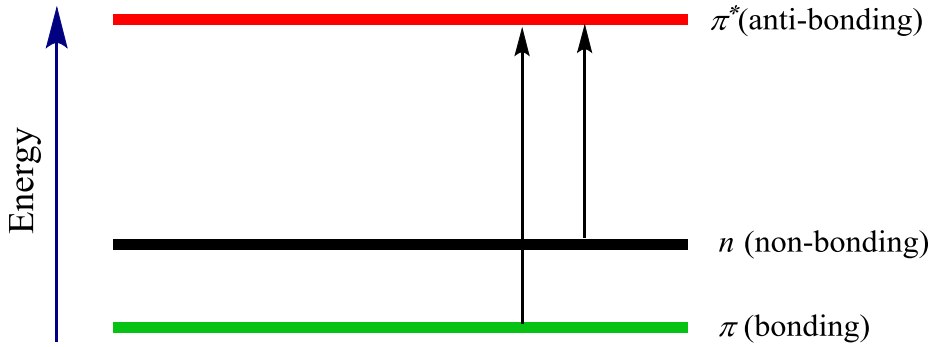


Fig. II.11. Electronic Transitions in UV-Visible region

II.4.3.3. Beer-Lambert Law

According to the Beer-Lambert Law, the **absorbance** is directly **proportional** to the **concentration** of the solute in a solution .

The Beer-Lambert Law can be expressed in the form of the following equation:

$$A = \varepsilon c l \quad (5)$$

A = absorbance

l = optical path length (cm)

c = concentration of solution (mol dm^{-3})

ε = molar extinction coefficient, which is constant for a particular substance at a particular wavelength (dm^3 mol^{-1} cm^{-1})

If the Beer-Lambert Law is obeyed, then the **absorbance versus concentration curve** should be linear. This graph is known as a **calibration graph**.

II.4.4. Fluorescence Spectroscopy

Fluorescence is a spectrochemical method of analysis, in which the molecules of the analyte get excited by irradiation of specific wavelength and emit radiation of a different wavelength. The emission spectrum gives information for both qualitative and quantitative analysis.

II.4.4.1. Phenomena of fluorescence

Luminescence is considered as the emission of light from any substance and happens from electronically excited states. According to the convention, luminescence can be splitted into two categories depending on the nature of the excited state (i) fluorescence and (ii) phosphorescence,

In excited singlet states, the electron in the excited orbital gets paired to the second electron of opposite spin (by opposite spin) in the ground-state orbital. As a result of this, return to the ground state is a spin-allowed transition and takes place rapidly by the emission of a photon. The emission rates of fluorescence are 10^8 s^{-1}, so that a typical fluorescence lifetime is near 10 ns (10 x 10^{-9} s). The lifetime (τ) of a fluorophore is the average time between its excitation and emission. Fluorescence often occurs from aromatic molecules. A significant characteristic of fluorescence is high sensitivity detection.

II.4.4.2. Steady-state Fluorescence:

Fluorescence measurements can be mainly divided into two types: steady-state and time-resolved. Steady-state is performed with constant illumination and the sample is illuminated with a continuous beam of light, and the intensity (or emission spectrum) is recorded. Because

of the ns timescale of fluorescence, most measurements are steady-state measurements. When the sample is first exposed to light, a steady state is reached almost instantaneously. Fluorescence spectral data are conventionally presented as emission spectra. A fluorescence emission spectrum is actually a plot of the fluorescence intensity versus wavelength (nanometers) or wavenumber (cm^{-1}). Emission spectra depend on the chemical structure of the fluorophore and the solvent in which it is dissolved. The intensity and shape of the spectra depends upon the following factors-

(i) Excitation wavelength

(ii) The concentration of the solvent used

(iii) The path length of the cuvette

(iv) Self-absorption of the sample

The directly recorded emission spectra represent the rate of photon emission over specific wavelength interval regulated by the slit widths and dispersion of the emission monochromator. Similarly, the excitation spectrum represents the relative emission of the fluorophore at each excitation wavelength.

II.4.5. Conductance Study

The conductivity study not only confirms the formation of a host-guest inclusion complex but also gives the stoichiometry of the assembly [64]. With the successive addition of cyclodextrin in the solution of the guest molecule, the conductivity of the guest molecules decreases on a regular basis. This type of observation is in good agreement with the formation of inclusion complexes. The insertion of the guest molecule inside the cavity of the CD molecule decreases the number of the free guest molecule, resulting in the reduction in conductivity of the solution. The curves having a noticeable break suggest the formation of host-guest inclusion with a stoichiometry of 1: 1.

II.4.6. HRMS STUDY

The molecular mass obtained from mass spectrograph should be almost equal to the calculated average mass (average atomic weight of each element present in the molecule or the monoisotopic mass calculated). The mass measured by mass spectrometry depends entirely on the resolution of the analyzer. But, if the instrument fails to resolve the isotopes, the different

peaks in the isotopic cluster merge to form a single peak that spreads over several masses. Thus, the mass measured by the instrument corresponds to the average mass.

The first step in the mass spectrometry involves the production of ions of the compound in the gaseous phase by electron ionization:

$M + e^- \longrightarrow M^{\bullet +} + 2e^-$

This molecular ion undergoes fragmentations as it is a radical cation having an odd number of electrons, which can fragment to give either a radical or an ion having an even number of electrons, or it can give rise to a molecule and a new radical cation. All these ions are separated in the mass spectrometer depending upon their mass-to-charge ratio. The plots are obtained in the form of their proportion to their abundance. Thus a mass spectrum of any specific molecule is detected. The result is obtained as a plot of ion abundance versus mass-to-charge ratio[65] [66] [67]. The x-axis of the mass spectrum represents the mass-to-charge ratio (m/z value), where m denotes the relative mass and z is the charge number m/z denotes a dimensionless quantity.

On the other hand, if the resolution is high enough to distinguish the different peaks in the isotopic clusters, the mass obtained directs to the calculated monoisotopic mass. Furthermore, high-resolution mass spectrometry (HRMS) gives rise to very narrow peaks with greater accuracy. High resolving power allows an increase in the selectivity of detection in both cases of compounds found in literature and unknown target compounds.

II.4.7. Raman Spectroscopy:

Raman spectroscopy is a scattering method, which is based on Raman Effect, i.e., frequency of scattered radiation is different from the frequency of incident radiation (monochromatic). The basis of Raman Spectroscopy is the inelastic scattering of incident radiation via its interaction with the vibrating molecules present around it. It probes the molecular vibrations [68, 69].

In Raman spectroscopy, the sample is illuminated with a monochromatic laser beam. This beam interacts with the molecules of sample and scattered light is originated. The scattered light is used to construct a Raman spectrum. Raman spectra arise because of the inelastic collision between incident monochromatic beam and molecules of the sample. When a monochromatic beam strikes at the sample, it gets scattered in all possible directions after its interaction with sample molecules. If this scattered radiation has a frequency equal to the frequency of incident radiation then it is known as **Rayleigh scattering**. Only a small fraction of scattered radiation

has a different frequency from the frequency of the incident radiation. It gives rise to **Raman scattering**. If the frequency of incident radiation is higher than the frequency of scattered radiation, Stokes lines appear in the Raman spectrum. And if the frequency of incident radiation is lower than that of the scattered radiation, anti-Stokes lines are observed in the Raman spectrum.

A Raman spectrum is represented as an intensity-versus wavelength shift. Raman spectra are recorded over a range of 4000–10 cm^{-1} [70]. However, Raman active normal vibrational modes of organic molecules occur in the range of 4000– 400 cm^{-1} [69]. For hydrogen bonding studies the vibrational spectroscopy such as Raman spectroscopy is one of the most reliable methods. Hydrogen bonding shifts show the frequency half band width in the Raman spectra and the molecular band intensity along with vibration are also observed. Raman spectroscopy is believed to be a reliable and non-destructive technique for both the qualitative and quantitative analysis of a variety of drugs in their solid and solution phase.

II.4.7. Scanning Electron Microscope (SEM) Study

The Scanning Electron Microscope is used to study the surface morphology of specimens. Secondary electrons get emitted from the surface on irradiating the specimen with a fine electron beam (electron probe). Two-dimensional scanning of the electron probes over the surface gives an idea about the topography of the surface.

II.5. TECHNIQUES USED TO INVESTIGATE THE DIVERSE PHYSICOCHEMICAL PARAMETERS IN AQUEOUS SOLUTION

The study of molecular interactions plays a significant role understand the phenomenon related to molecular aggregates. Molecular interactions provide detailed interpretation about the fundamental problems concerned with the mechanism of biochemical catalysis and pave the paths of chemical reactions. Molecular interactions are of utmost importance in elucidating the morphology and properties of compounds as well as energy transfer in phase transitions and enzymes etc. So the study of molecular interactions has a wide range of application in the field of engineering, chemistry, biology, physics, and the interfaces of these subjects.

From the fundamental thermodynamic properties such as density, viscosity and conductivity study, we can derive a number of parameters like partial molar volume, A and B coefficient, viscosity deviation, association constant etc. These derived thermodynamic parameters are proved to be informative to understand the extent of molecular interactions, hydrogen bonding

phenomenon, which based on polarity and size of molecules in a solution phase in specific composition and over a fixed temperature range. Compositional dependence of thermodynamic properties helps to determine the nature and magnitude of molecular aggregation resulting from interactions. The investigations on Physico-chemical properties of solute and solvent mixture provide applicative information of physical nature and strength of molecular interaction.

II.5.1. Density

The apparent molar volume is defined as the sum of the geometric volume of the solute molecule and changes in volume in the presence of a solvent [71]. The apparent molar volumes of the solutes can be calculated by using the following relation [72].

$$\phi_V = \frac{M}{\rho_0} - \frac{1000(\rho - \rho_0)}{c\rho_0} \tag{6}$$

or

$$\phi_V = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0} \tag{7}$$

Where M denotes the molar mass of the solute, c is the molarity; m is the molality of the solution; ρ_0 and ρ are the densities of the solvent and the solution respectively. Limiting apparent molar volume or partial molar volume (ϕ_V^0)the experimental slopes (S_V^*)is obtained by employing the least square fitting method to the equation stated below (Masson equation) [73].

According to Masson

$$\phi_V = \phi_V^0 + S_V^* \sqrt{c} \tag{8}$$

Where, S_V^* is the experimental slope. It indicates the nature of the solute-solute interaction. ϕ_V^0 refers to the extent of solute-solvent interactions The temperature dependence of in various solvents can be expressed by the general equation as follows:

$$\phi_V^0 = a_0 + a_1 T + a_2 T^2 + \cdots\cdots \tag{9}$$

Where, a0, a_1, a_2 are the empirical coefficients of a specific electrolyte and T is the temperature in Kelvin.

The limiting apparent molar expansibilities (ϕ_E^0) can be calculated by the following equation:

$$\phi_E^0 = \left(\delta\phi_V^0/\delta T\right)_P = a_1 + 2a_2T \tag{10}$$

The limiting apparent molar expansibilities (ϕ_E^0) change in magnitude with the change of temperature. It determines the structure-making or breaking tendency of any solute. Helper [74] developed a technique to investigate the sign of ϕ_E^0 in terms of long-range structure-making and breaking capacity of the solutes in the mixed solvent systems. The general thermodynamic expression is given below:

$$\left(\delta\phi_E^0/\delta T\right)_P = \left(\delta^2\phi_V^0/\delta T^2\right)_P = 2a_2 \tag{11}$$

If the sign of $\left(\delta\phi_E^0/\delta T\right)_P$ is positive or small negative the electrolyte acts as structure maker and if the sign becomes negative, it is a structure breaker.

II.5.2. Viscosity

Jones and Dole [75] suggested an empirical equation quantitatively correlating the relative viscosities of the electrolytes with molar concentrations (*c*):

$$\frac{\eta}{\eta_o} = \eta_r = 1 + A\sqrt{c} + Bc \tag{12}$$

The above equation can be rearranged as:

$$\frac{\eta_r - 1}{\sqrt{c}} = A + B\sqrt{c} \tag{13}$$

Where A and B are constants specific to ion-ion and ion-solvent interactions. The equation is applicable equally to aqueous and non-aqueous solvent systems where there is no ionic association and has been used extensively. The term A√c, originally ascribed to Grüneisen effect, arose from the long-range coulombic forces between the ions. At higher concentrations the extended Jones-Dole equation, involving an additional coefficient *D*, originally used by Kaminsky [76], has been used by several workers [77] and is given below:

$$\frac{\eta}{\eta_o} = \eta_r = 1 + A\sqrt{c} + Bc + Dc^2 \tag{14}$$

The coefficient D cannot be evaluated properly and the significance of the constant is also not always meaningful.

The plots of against √c for the electrolytes should give the value of A-coefficient. There are certain cases, where the values come out to be negative or considerably scatter and also a deviation from linearity occur. A-coefficient should be zero for non-electrolytes. According to Jones and Dole, the A-coefficient probably represents the stiffening effect on the solution of the electric forces between the ions, which tend to maintain a space-lattice structure [75] [78]. The sign of the B-coefficient may be either positive or negative which depends on the ions and the solvent. The B–coefficients are obtained as slopes of the straight lines using the least square method and intercepts are equal to the A-coefficient.

II.6.5 Conductance

One of the most precise and direct techniques available to determine the extent of the dissociation constants of electrolytes in aqueous, mixed and non-aqueous solvents is the "*conductimetric method*." Conductance data in conjunction with viscosity measurements gives much information regarding ion-ion and ion-solvent interaction.

II.6.5.1. Dissolved Ions Conduct Electricity

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 [79], 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:

$$\Lambda = \Lambda_o - S\sqrt{c} \tag{15}$$

Where,

$$S = \alpha\Lambda_o + \beta \tag{16}$$

$$\alpha = \frac{(z^2)k}{3(2+\sqrt{2})\varepsilon_r kT\sqrt{c}} = \frac{82.406\times10^4 z^3}{(\varepsilon_r T)^{\frac{3}{2}}} \tag{17}$$

$$\beta = \frac{z^2 eFk}{3\pi\eta\sqrt{c}} = \frac{82.487 z^3}{\eta\sqrt{\varepsilon_r T}} \tag{18}$$

Pitts (1953) and Fuoss and Onsager (1957) individually worked out the solution of the problem of electrolytic conductance accounting for both long-range and short-range interactions. The conductance values at infinite dilution are different for two different theory (Fuoss-Onsager theory and Pitt's theory) and the derivation of the Fuoss-Onsager equation was questioned [80] [81]. Fuoss and Hsia [82]further modified the original Fuoss-Onsager equation.

The results of conductance theories can be expressed in a general form:

$$\Lambda = \frac{\Lambda_o - \alpha\Lambda_o\sqrt{c}}{(1+\kappa\alpha)}\left(\frac{1+\kappa\alpha}{\sqrt{2}}\right) - \frac{\beta\sqrt{c}}{(1+\kappa\alpha)} + G(\kappa\alpha) \tag{19}$$

Where $G(\kappa\alpha)$ is a complicated function of the variable. The simplified form:

$$\Lambda = \Lambda_o - S\sqrt{c} + Ec\ln c + J_1 c + J_2\sqrt[3]{c} \tag{20}$$

$$\Lambda = \Lambda_o - S\sqrt{c} + Ec\ln c + J_1 c + J_2\sqrt[3]{c} - F\Lambda c \tag{21}$$

Where,

$$Fc = \frac{4\pi R^3 N_A}{3} \tag{22}$$

II.6.5.2. Ionic Association

The plot of Λ against $\sqrt{c}$ (limiting Onsager equation) explains the rate of dissociation or association of electrolytes. The electrolyte is considered to be completely dissociated when positive deviation happens ($\Lambda_{o\,exp} \rangle \Lambda_{o\,theo}$) but if negative deviation ($\Lambda_{o\,exp} \langle \Lambda_{o\,theo}$) takes place, the electrolyte is supposed to be associated. Conductance measurements help us to determine the values of the ion-pair association constant, K_A for the process:

$$M^{z+} + A^{z-} = MA \tag{23}$$

$$K_A = \frac{(1-\alpha)}{\alpha^2 c\gamma_{\pm}^2} \tag{24}$$

$$\alpha = 1 - \alpha^2 K_A c\gamma_{\pm}^2 \tag{25}$$

Where $\gamma_{\pm}$ denotes the mean activity coefficient of the free ions at concentration.

For strongly associated electrolytes, the constant K_A and Λ_o are determined using Fuoss-Kraus equation [83]or Shedlovsky's equation [84]

$$\frac{T(z)}{\Lambda} = \frac{1}{\Lambda_o} + \frac{K_A}{\Lambda_o^2} \cdot \frac{c\gamma_{\pm}^2 \Lambda}{T(z)} \tag{26}$$

Where $T(z) = F(z)$ (Fuoss-Kraus method) and $1/T(z)=S(z)$ (Shedlovsky's method).

$$F(z) = 1 - z(1 - z(1 - \ldots)^{-\frac{1}{2}})^{-\frac{1}{2}} \tag{27}$$

$$\frac{1}{T(z)} = S(z) = 1 + z + \frac{z^2}{2} + \frac{z^3}{8} + \ldots\ldots \tag{28}$$

A plot of $T(z)/\Lambda$ against $c\gamma_{\pm}^2 \Lambda / T(z)$ is a straight line, where $1/\Lambda_o$ is the intercept and K_A / Λ_o^2 is the slope.

The Fuoss-Hsia [82] conductance equation for associated electrolytes is given by:

$$\Lambda = \Lambda_o - S\sqrt{\alpha c} + E(\alpha c)\ln(\alpha c) + J_1(\alpha c) - J_2(\alpha c)^{\frac{3}{2}} - K_A \Lambda \gamma_{\pm}^2(\alpha c) \tag{29}$$

The conductance of symmetrical electrolytes in dilute solutions are stated below

$$\Lambda = \alpha(\Lambda_o - S\sqrt{\alpha c} + E(\alpha c)\ln(\alpha c) + J_1 R(\alpha c) - J_2 R(\alpha c)^{\frac{3}{2}} \tag{30}$$

$$\frac{(1-\alpha)}{\alpha^2 c\gamma_{\pm}^2} = K_A \tag{31}$$

$$\ln \gamma_{\pm} = \frac{-k\sqrt{q}}{(1 + kR\sqrt{\alpha c})} \tag{32}$$

The conductance parameters are obtained from the least square treatment after setting

$$R = q = \frac{e^2}{2\varepsilon kT} \text{ (Bjerrum's critical distance)} \tag{33}$$

According to Justice the method of fixing the *J*-coefficient by setting, $R = q$ clearly permits a better value of K_A to be obtained. Since the equation (30) is a series expansion truncated at the $c^{3/2}$ term, it would be preferable that the resulting errors be absorbed as must as possible by J2 rather than by K_A, whose theoretical interest is greater as it contains the information concerning

short-range cation-anion interaction. From the experimental values of the association constant K_A, one can use two methods in order to determine the distance of closest approach, 'a', of two free ions to form an ion-pair. The following equation has been proposed by Fuoss [85]

$$K_A = \frac{4\pi N_A \alpha^3}{3000} \exp\left(\frac{e^2}{\alpha \varepsilon kT}\right) \tag{34}$$

In some cases, the magnitude of K_A was too small to permit a calculation of a. The distance parameter was finally determined from the more general equation due to Bjerrum [86].

$$K_A = \frac{4\pi N_A \alpha}{1000} \int_{r=a}^{r=q} r^2 \exp\left(\frac{z^2 e^2}{r \varepsilon kT}\right) dr \tag{35}$$

II.6.5.3. Extension of Fuoss Conductance Equation

Fuoss introduced a slight modification to his model to minimise a boundary condition error [87, 88] He proposed that the ion pairs (ion approaching with their Gurney co-sphere) are divided into two categories- contact pairs (having no contribution to conductance) and the solvent separated ion pairs (which can only contribute to the net transfer of charge). In 1978 Lee-Wheaton [89] introduced a new conductance equation, which effectively diminished such boundary errors based on the Gurney co-sphere model. The conductance data were evaluated with the help of the Lee-Wheaton conductance equation [90]

$$\Lambda = \alpha_i \left[\begin{array}{l} \Lambda_o \left\{1 + C_1 \beta\kappa + C_2 (\beta\kappa)^2 + C_3 (\beta\kappa)^3\right\} \\ -\dfrac{\rho\kappa}{1+\kappa R}\left\{1 + C_4 \beta\kappa + C_5 (\beta\kappa)^2 + \dfrac{\kappa R}{12}\right\} \end{array} \right] \tag{36}$$

The mass action law association [91]is

$$K_{A,c} = \frac{(1-\alpha_i)\gamma_A}{\alpha_i^2 c_i \gamma_\pm^2} \tag{37}$$

and the equation for the mean ionic activity coefficient:

$$\gamma_\pm = \exp\left[-\frac{q\kappa}{1+\kappa R}\right] \tag{38}$$

The standard deviation (σ_A) was calculated by the following equation:

$$\sigma_A^2 = \sum_{i=1}^{n} \frac{[\Lambda_{i(calc)} - \Lambda_{i(obs)}]^2}{n-m} \tag{39}$$

Where n is the number of experimental points and m is the number of fitting parameters. The conductance data were analyzed by fixing the distance of closest approach R with two parameter fit (m=2). For the electrolytes with no significant minima observed in the versus R curves, the R values were arbitrarily preset at the centre to centre distance of solvent-separated pair:

$$R = a + d \tag{40}$$

Where, i.e., the sum of the crystallographic radii of the cation and anion and d is the average distance corresponding to the side of a cell occupied by a solvent molecule. The definitions of d and related terms are described in the literature [92]. R was generally varied by a step 0.1 Å.

II.6.5.4. Limiting Ionic Conductance

The limiting ionic conductance of an electrolyte can be easily determined from the theoretical equations and experimental observations. At infinite dilutions, the motion of an ion is limited solely by the interactions with the surroundings solvent molecules as the ions are infinitely apart. Under these conditions, the validity of Kohlrausch's law of independent migration of ions is undeniable. Thus:

$$\Lambda_0 = \lambda_o^+ + \lambda_o^- \tag{41}$$

At present, limiting ionic conductance is the only function which can be divided into ionic components using experimentally determined transport number of ions, i.e.

$$\lambda_o^+ = t_+ \Lambda_0 \quad \text{and} \quad \lambda_o^- = t_- \Lambda_0 \tag{42}$$

II.6.5.5. Stokes' Law and Walden's Rule

According to Stokes' law, for a spherical ion of the radius R_i with movements in a solvent of dielectric field, the limiting conductance λ_o^i can be represented as

$$\lambda_o^i = \frac{|z_i e| eF}{6\pi\eta_o R_i} = \frac{0.819|z_i|}{\eta_o R_i} \tag{43}$$

$$\lambda_o^i \eta_o = \frac{0.819 z_i}{R_i} = constant \tag{44}$$

This is known as the Walden rule [93]

II.6.5.6. Thermodynamics of Ion-Pair Formation

The standard Gibbs energy changes (ΔG^o) for the ion- association process can be calculated from the equation

$$\Delta G^o = -RT \ln K_A \tag{45}$$

The standard enthalpy change ΔH^o and standard entropy change ΔS^o can be evaluated from the following set of equations

$$\Delta H^o = -T^2 \left[\frac{d(\Delta G^o / T)}{dT} \right]_P \tag{46}$$

$$\Delta S^o = -T^2 \left[\frac{d(\Delta G^o)}{dT} \right]_P \tag{47}$$

The values are fitted with the help of the following Polynomial equation

$$\Delta G^o = c_0 + c_1(298.15 - T) + c_2(298.15 - T)^2 \tag{48}$$

c_0, c_1 and c_2 are the empirical coefficients. The standard values of the coefficients at 298.15 K can be calculated using the equations stated below

$$\Delta G^o_{298.15} = c_0 \tag{49}$$

$$\Delta S^o_{298.15} = c_1 \tag{50}$$

$$\Delta H^o_{298.15} = c_o + 298.15 c_1 \tag{51}$$

The standard entropy of ion-association of electrolytes is dependent upon the following factors

- the size and shape of the ions,
- charge density on the ions,
- electrostatic interaction of the solvent molecules around the ions

ΔG^o can also be calculated from the following equation

$$\Delta G^o = N_A W_{\pm} \tag{52}$$

$$K_A = \left(\frac{4\pi N_A}{1000} \right) \int_a^R r^2 \exp\left(\frac{2q}{r} - \frac{W_{\pm}}{kT} \right) dr \tag{53}$$

2q/r denotes Columbic part of the interionic mean force potential and $W_{\pm}$ is its non-columbic part [92].

II.6.6 Refractive Index

Molar refractivity was obtained from the Lorentz-Lorenz relation [94] [95] by using the following equation

$$R_M = \frac{(n_D^2 - 1)}{(n_D^2 + 2)}\left(\frac{M}{\rho}\right) \tag{54}$$

Where R_M , n_D , M and ρ indicate the molar refraction, the refractive index, the molar mass and the density of solution respectively.