# CHAPTER II

## REVIEW OF THE EARLIER WORKS
## AND
## THEORY OF INVESTIGATIONS

### II.1. Review of the Earlier Works

In supramolecular chemistry, host–guest chemistry describes complexes that are composed of two or more molecules or ions that are held together in unique structural relationships by forces other than those of full covalent bonds.[1,2] Host–guest chemistry encompasses the idea of molecular recognition and interactions through noncovalent bonding. Noncovalent bonding is critical in maintaining the 3D structure of large molecules, such as proteins and is involved in many biological processes in which large molecules bind specifically but transiently to one another.[3] There are four commonly mentioned types of non-covalent interactions: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions.[4]

The "host" component can be considered the larger molecule, and it encompasses the smaller, "guest", molecule. In biological systems, the analogous terms of host and guest are commonly referred to as enzyme and substrate respectively.

The thermodynamic benefits of host–guest chemistry are derived from the idea that there is a lower overall Gibbs energy due to the interaction between host and guest molecules. Chemists are exhaustively trying to measure the energy and thermodynamic properties of these non-covalent interactions found throughout supramolecular chemistry; and by doing so hope to gain further insight into the combinatorial outcome of these many, small, non-covalent forces that are used to generate an overall effect on the supramolecular structure.[2]

In order to rationally and confidently design synthetic systems that perform specific functions and tasks, it is very important to understand the thermodynamics of binding between host and guest. Chemists are focusing on the energy exchange of different binding interactions and trying to develop scientific experiments to quantify the fundamental origins of these non-covalent interactions by utilizing various techniques such as NMR spectroscopy, Raman spectroscopy, isothermal titration calorimetry, surface tension and UV-Vis Spectroscopy. The experimental data are quantified and explained through analysis of binding constants $K_a$, Gibbs energy $\Delta G^o$, Enthalpy $\Delta H^o$, and entropy $\Delta S^o$.[3]

D. S. Guo et al. described enzyme responsive supramolecular vesicle, which is an example of amphiphilic self-assembly.[5] It is very interesting for its applications in controlled delivery of Alzheimer's disease drugs. It is a smart drug delivery system based on the concept of supramolecular chemistry.

R. Sun et al. constructed a light-driven, supramolecular polymer by host–guest molecular recognition between calixarene and α-cyclodextrin based pseudorotaxane containing binaphthyl and azobenzene moieties.[6] It is a successful supramolecular polymerization by non-covalent host–guest molecular recognition.

G. Yu et al. developed a supramolecular aggregation, which is applied in cancer cell imaging.[7] Compared to other conventional fluorophores, tetraphenylethene based organic fluorogens show extraordinary aggregation-induced emission feature, which can hardly be accomplished without introduction of various driving forces due to the propeller-shaped structure and the dynamic rotation of the phenyl rings of it.

S. Angelos et al. presented dual-controlled nano-particle in which two different types of machines, i.e., nanoimpellers and nanovalves are brought together in and around mesoporous silica nanoparticle.[8] The molecular machines have been designed to work in such a way that the system functions as AND logic gate and provides sophisticated control of the contents of the pores.

L. Stricker et al. developed host–guest complexes with β-cyclodextrin, which act as light-responsive molecular switches.[9] The two cyclodextrin-based supramolecular

systems containing cyclodextrin vesicles and cyclodextrin-functionalized gold nanoparticles revealed excellent reversible, light-responsive aggregation and dispersion behavior.

P. Díez et al. constructed smart delivery systems, which consist of β-cyclodextrin-based supramolecular nanovalve.[10] The nanodevice behaves as an enzymatic logical OR operator, which is selectively fueled by the presence of D-glucose and ethyl butyrate.

E. Iglesias described formation of inclusion complexes of β-cyclodextrin with novocaine in aqueous solutions under various conditions by applying fluorescence, UV-vis spectroscopy and conductance study.[11] The 1:1 stoichiometry and binding constants were also explained by appropriate methods.

J. Liu et al. explained a supramolecular method for use in versatile gene delivery system made of β-cyclodextrin based polymer and adamantyl-terminated functional polymer by one step method.[12] They have done in vitro experiments, which revealed that the supramolecular assembly had good cytocompatibility and high transfection activity at high dose of DNA. Also, the supramolecular vector system exhibited about 60% silencing efficiency as a siRNA vector.

Z. Q. Shi et al. developed a facile method to construct reversible thermoresponsive switching for bacteria killing and detachment by host–guest self-assembly of β-cyclodextrin and adamantane.[13] The proposed method created a new route to extend the application of smart surfaces in the fields requiring long-term antimicrobial treatment.

W. Zhu et al. showed a simple and clean method to prepare cross-linked α-cyclodextrin nanoparticles with a low dispersion.[14] The nanoparticles were synthesized in water by cross-linking the inclusion complex of α-CD and poly-ethylene glycol. Anticancer drug cisplatin molecules were included in the cross-linked α-CD nanoparticles, and were used to evaluate the drug release behavior.

## II.2. Theory of Investigations

### II.2.1. Hydrophobic Interactions:

The tendency of nonpolar molecules in a polar solvent (usually water) to interact with one another is called the hydrophobic effect. The interactions between the nonpolar molecules are called hydrophobic interactions.

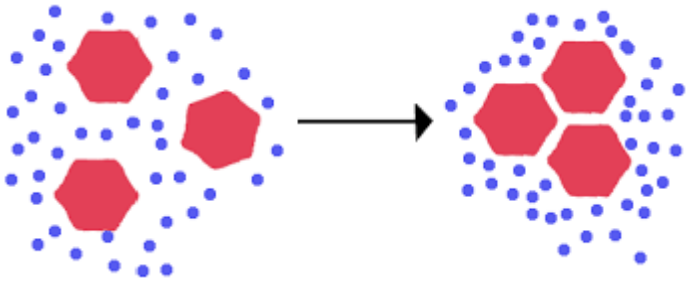

**Figure 1.** Hydrophobic molecules come closer in polar solvent.

Hydrophobic interactions can also be seen in the clustering of amphiphillic molecules such as phospholipids into bilayers and micelles. The hydrophobic areas of amphiphillic molecules cluster together to avoid the ordered "cage" of water molecules that would have been surround them and orient the hydrophillic ends as a shield like superficial structure that interacts harmoniously with the polar water molecules. Micelles occur when molecules having long hydrophobic chain form a hydrophobic core with the polar groups at the hydrophillic outer shell. Bilayers can be seen in cell membranes with hydrophillic outer and inner linings with the hydrophobic center.

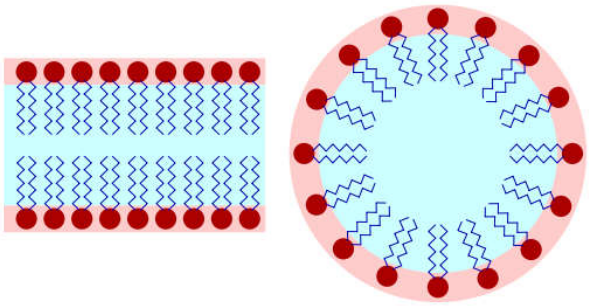

**Figure 2.** Structures of lipid bilayer and micelle.

## II.2.2. Van der Waals Forces:

The van der Waals forces are distance-dependent interactions between atoms or molecules. Van der Waals forces are relatively weak and quickly vanish at longer distances between interacting molecules.

The van der Waals forces play a fundamental role in various fields as supramolecular chemistry, structural biology, polymer science, nanotechnology, surface science, etc. Van der Waals forces also define many properties of organic compounds and molecular solids, including their solubility in polar and non-polar media.

The van der Waals forces include attraction and repulsions between atoms, molecules and surfaces. They differ from covalent and ionic bonding as they are caused by correlations in the fluctuating polarizations of the nearby particles.

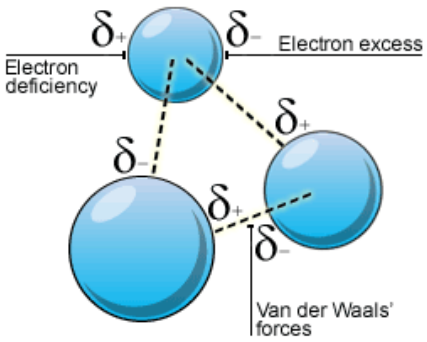


**Figure 3.** Strength of van der Waals forces is related to the size of atoms and molecules. The bigger the atom or molecule the higher is the van der Waals force.

## II.2.3. Hydrogen Bonds:

A hydrogen bond is the electrostatic attraction between two polar groups that occurs when a hydrogen atom covalently bonded to a highly electronegative atom such as nitrogen, oxygen or fluorine experiences the electrostatic attraction of another highly electronegative atom nearby to it.

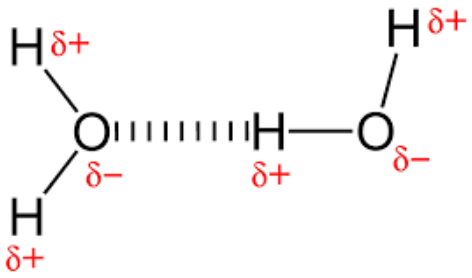


**Figure 4.** Hydrogen bonds between molecules of water.

Hydrogen bonds can occur between molecules (intermolecular) or within different parts of a single molecule (intramolecular). Depending on the nature of the donor and acceptor atoms which constitute the bond, the geometry and the energy of a hydrogen bond can differ between 1 and 40 kcal $mol^{-1}$. This makes them to some extent stronger than a van der Waals interaction and weaker than covalent or ionic bonds. This type of bond can occur in inorganic molecules such as water and in organic molecules like DNA and proteins.

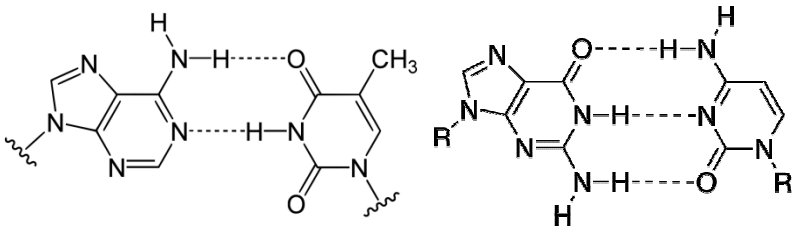


**Figure 5.** Hydrogen bonds between nucleic acid bases.

Intermolecular hydrogen bonding is responsible for the high boiling point of water compared to the other group 16 hydrides that have much weaker hydrogen bonds. Intramolecular hydrogen bonding is partly responsible for the secondary and tertiary structures of proteins and nucleic acids. It also plays an important role in the structure of polymers, both synthetic and natural.

### II.2.4. Electrostatic Forces:

Electrostatic force or Coulomb interaction is the attraction or repulsion of particles or objects because of their electric charge. The electrostatic force is one of the basic physical forces.

Two similar electric charges, as both positive or both negative, repel each other along a straight line between their centers, whereas two unlike charges, as one positive and one negative, attract each other along a straight line joining their centers.

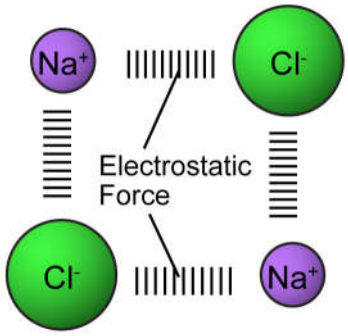


**Figure 6.** Electrostatic force between sodium and chloride ions.

### II.2.5. Ion-Dipolar Attractions:

An ion-dipole force is an attractive force that results from the electrostatic attraction between an ion and a neutral molecule that has a dipole.

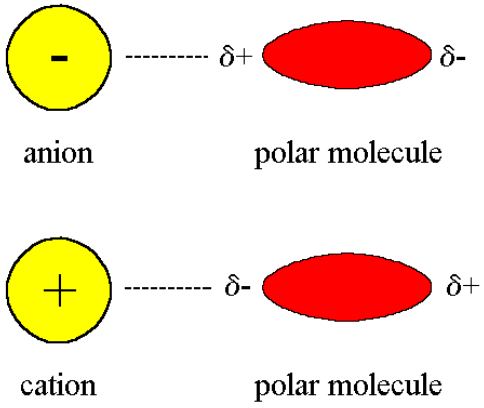


**Figure 7.** Examples of ion-dipolar attractions.

This type of attraction is most commonly found in solutions and especially important for solutions of ionic compounds in polar liquids. In this type of attraction a positive ion (cation) attracts the partially negative end of a neutral polar molecule or a negative ion (anion) attracts the partially positive end of a neutral polar molecule. Ion-

dipole attractions become stronger as either the charge on the ion increases or as the magnitude of the dipole of the polar molecule increases.

### II.2.6. Dipole-Dipole Attractions:

Dipole-dipole forces are attractive forces between the positive end of one polar molecule and the negative end of another polar molecule. Dipole-dipole forces have strengths that range from 5-20 kJ $mol^{-1}$. These are much weaker than ionic or covalent bonds and have a significant effect only when the molecules involved are close together.

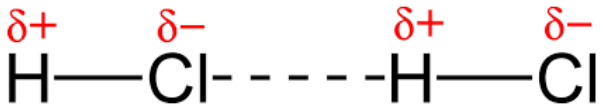


**Figure 8.** Example of a dipole-dipole attraction.

### II.2.7. $^{1}$H NMR Spectroscopy

Nuclear magnetic resonance (NMR) is a spectroscopic method that is very important to the chemists. Many nuclei may be studied by NMR techniques, but hydrogen and carbon are most commonly available. NMR gives information about the number of magnetically distinct atoms of the type being studied. When hydrogen nuclei is studied the number of each of the distinct types of hydrogen nuclei as well as information regarding the nature of the immediate environment of each type may be obtained.

**The Continuous-Wave (CW) Instrument**

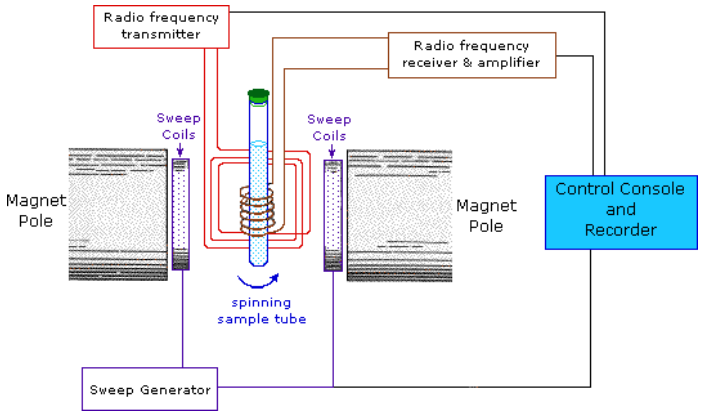


**Figure 9.** The basic elements of the classical nuclear magnetic resonance spectrometer.

**Chemical Equivalence**

All of the protons found in chemically identical environments within a molecule are chemically equivalent, and they often exhibit the same chemical shift. A molecule that has a set of protons those are chemically distinct from one another may give rise to different absorption peak, where the protons are said to be chemically non equivalent. Often, protons those are chemically equivalent are also magnetically equivalent. However, in some instances, protons those are chemically equivalent are not magnetically equivalent.

**Integrals and Integration**

The NMR spectrum not only distinguishes how many different types of protons are there in a molecule, but also reveals how many of each type are there within the molecule. In NMR spectrum, the area under each peak is proportional to the number of hydrogens generating that peak. The NMR spectrometer has the ability to electronically integrate the area under each peak. The integral does not give the absolute number of hydrogens, it gives the relative number of each type of hydrogen.

**Chemical Shift**

Different types of protons have signals at different positions and each type has a characteristic value, which is called the chemical shift. Important factors influencing chemical shift are electron density, electronegativity of neighboring groups and anisotropic induced magnetic field effects. Electron density shields a nucleus from the external field. A nucleus in the vicinity of an electronegative atom experiences reduced electron density and the nucleus is therefore deshielded. When the electronegative atom is removed further away the effect diminishes until it can be observed no longer. Anisotropic induced magnetic field effects are the result of a local induced magnetic field experienced by a nucleus resulting from circulating electrons that can either be paramagnetic when it is parallel to the applied field or diamagnetic when it is opposed to it.

In this research work, change in chemical shifts of the interacting protons has been observed, which is the indication of inclusion into cyclodextrin or supramolecular complexation in crown ethers.

### II.2.8. 2D ROESY

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.

### II.2.9. FTIR Spectroscopy

Molecules are excited to a higher energy state when they absorb infrared radiation. A molecule absorbs only selected frequencies of infrared radiation, which corresponds to the stretching and bending vibrational frequencies of the bonds in most covalent molecules. Not all the bonds in a molecule are capable of absorbing IR radiation, only those bonds that have a dipole moment that changes as a function of time are capable of absorbing IR radiation. The IR spectrum can be used for molecules as a fingerprint can be used for humans. By comparing IR spectra of two substances, one can establish whether

these are identical or not. Another and more important use of IR spectrum is to determine structural information about a molecule.

**Bond Properties and Absorption Trends**

The bond strength and the masses of the bonded atoms affect the IR absorption frequency. A diatomic molecule may be considered as two vibrating masses connected by a spring. The bond distance continuously changes, but an average bond distance can be defined.

When a bond vibrates like a harmonic oscillator, its energy of vibration periodically changes from kinetic to potential energy and back again. For a harmonic oscillator if the force constant of the spring is $K$ and the masses of the two bonded atoms are $m_1$ and $m_2$, the frequency of vibration is given by

$$\tilde{\nu} = \frac{1}{2\pi c}\sqrt{(\frac{K}{\mu})}$$

which is derived from Hooke's Law for vibrating springs. The reduced mass $\mu$ of the system is given by

$$\mu = \frac{m_1 m_2}{m_1 + m_1}$$

$K$ is a constant that varies from one bond to another.

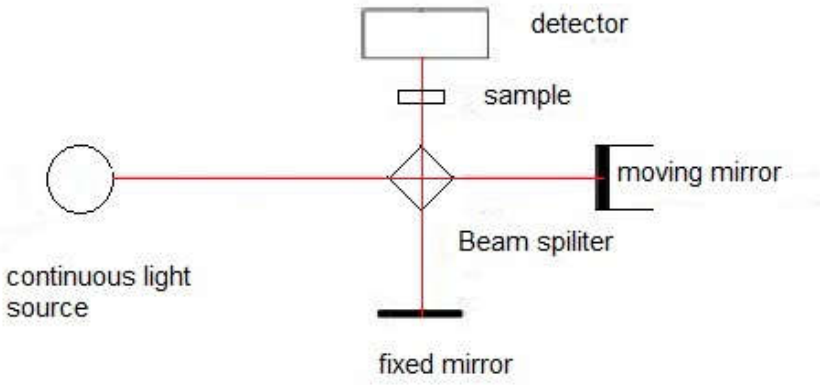


**Figure 10.** Schematic diagram of infrared spectrophotometer.

**The Infrared Spectrophotometer**

The instrument that determines the absorption spectrum for a compound is called infrared spectrophotometer. This instrument provides spectra of compounds in the range 4000 to 400 $cm^{-1}$.

**Preparation of Samples**

In this research work the samples were studied either in solid state or in solutions. The solid KBr pellet was prepared by mixing finely ground solid sample with powdered KBr and pressing the mixture under high pressure. The KBr pellet can be inserted into a holder in the spectrometer. The samples in solution were studied by putting the solution in a NaCl cuvette.

Inclusion phenomenon inside cyclodextrin molecule or complexation in solution may be satisfactorily illustrated by observing the shifts of stretching frequencies of the interacting atoms or groups by using FT-IR spectra, which was employed in this research work.

## II.2.10. UV-Visible Spectroscopy

When continuous radiation passes through a transparent material, a portion of the radiation may be absorbed. If that occurs, the residual radiation, when it is passed through a prism, yields a spectrum, called an absorption spectrum. In case of ultraviolet and visible spectroscopy, absorption of electromagnetic radiation results due to transitions between electronic energy levels.

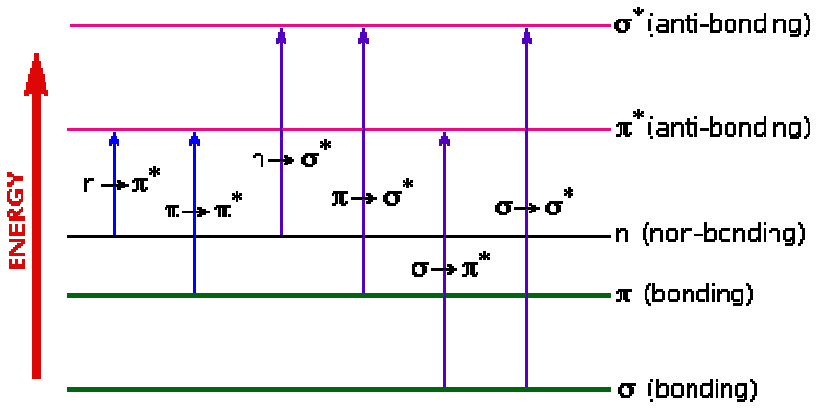


**Figure 11.** Electronic energy levels and transitions.

## Principles of Absorption Spectroscopy

The grater the number of molecules capable of absorbing light of a given wavelength, the grater is the extent of light absorption. Again, the more effectively a molecule absorbs light of a given wavelength, the grater is the extent of light absorption. From these guiding ideas, the following empirical expression, known as the Beer-Lambert Law may be formulated.

## Beer-Lambert Law

$A = \log (I_0/I) = \varepsilon cl$, for a given wavelength

$A$ = absorbance

$I_0$ = intensity of light incident upon sample cell

$I$ = intensity of light leaving sample cell

$c$ = molar concentration of solute

$l$ = length of sample cell (cm)

$\varepsilon$ = molar absorptivity

The term $\log (I_0/I)$ is known as absorbance. The molar absorptivity is a property of the molecule undergoing an electronic transition.

## Instrumentation

Instruments for measuring the absorption of UV or visible radiation are made up of the following components

1. Sources (UV and visible)
2. Wavelength selector (monochromator)
3. Sample containers
4. Detector
5. Signal processor and readout

*Sources of UV radiation:* The electrical excitation of deuterium at low pressure produces a continuous UV spectrum. Deuterium lamp emits radiation in the range 160 - 375 nm.

*Sources of visible radiation:* The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm.

*Wavelength selector (monochromator)*
All monochromators contain the following component parts

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

*Cuvettes:* The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are used for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses are used for the manufacture of cuvettes for use between 350 and 2000 nm.

*Detectors:* The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a photoemissive cathode, several dynodes and an anode. The resulting current is amplified and measured.

The linear photodiode array is an example of a multichannel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously.

Charge-Coupled Devices (CCDs) are similar to diode array detectors, but instead of diodes, they consist of an array of photocapacitors.

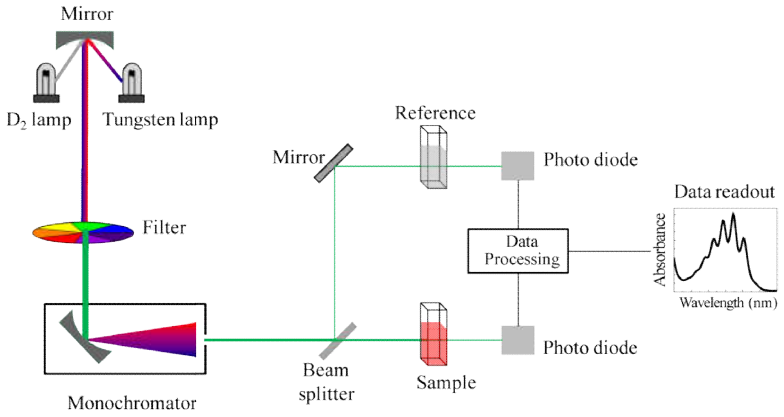


**Figure 12.** Schematic diagram of UV-Visible spectrometer.

In this thesis UV-visible spectroscopic studies have been performed basing upon the general principle of changes in molar extinction coefficient or molar absorptivity (Δε) of various guest molecules when complexed with cyclodextrin molecule, which is owing to the changes in the polarity of the environment of the chromophore of the guest molecules when these go from the polar aqueous environment to the apolar cavity of cyclodextrin.

## II.2.11. High Resolution Mass Spectrometry

Mass spectrometry is an analytical technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio. In simpler terms, a mass spectrum measures the masses within a sample.

### Instrumentation

The mass spectrometer has five components.

*Sample inlet:* The first component is the sample inlet, which brings the sample from the laboratory environment to the lower pressure of the mass spectrometer, which is in the range of a few millimeters of mercury.

*Ion source:* The next part is the ion source, where sample molecules are transformed into gas phase ions.

*Mass analyzer:* The mass analyzer separates the sample ions based on their mass-to-charge (*m/z*) ratio.

*Detector:* The ions are then counted by the detector.

*Data system:* The signal is recorded and processed by the data system, which is typically a computer. The output from the data system is the mass spectrum – a graph of the number of ions detected as a function of their *m/z* ratio.

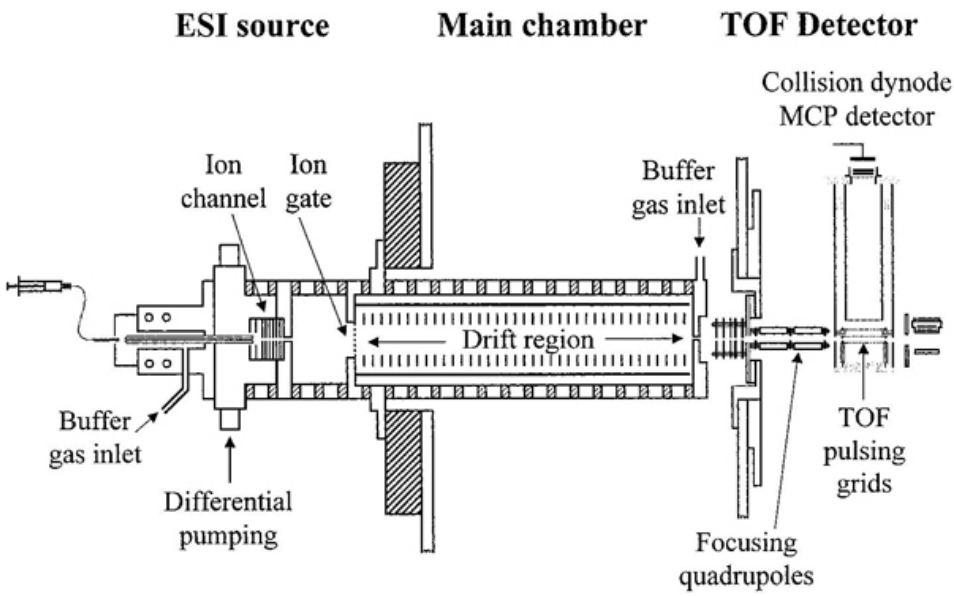


**Figure 13.** Schematic diagram of mass spectrometer.

**Ionization Methods**

The sample molecules must be converted to charged particles by the ion source before they can be analyzed and detected. Various ionization techniques include: electron ionization, chemical ionization, desorption ionization, electrospray ionization.

**Mass Analysis**

Once the sample has been ionized, the beam of ions is accelerated by an electric field and then passes into the mass analyzer, the region of the mass spectrometer where the ions are separated according to their *m/z* ratio. Various mass analyzers are: magnetic sector

mass analyzer, double-focusing mass analyzer, quadruple mass analyzer, time-of-flight mass analyzer.

In this thesis the inclusion complexes were analyzed by ESI-mass spectrometry. The spectra are shown and the observed peaks have been listed into tables in respective chapters with possible ions.

### II.2.12. Isothermal Titration Calorimetry

Isothermal titration calorimeters measure the heat change that occurs when two molecules interact. Heat is released or absorbed as a result of the redistribution and formation of non-covalent bonds when the interacting molecules go from the free to the bound state. ITC monitors these heat changes by measuring the differential power, applied to the cell heaters, required to maintain zero temperature difference between the reference and sample cells as the binding partners are mixed.

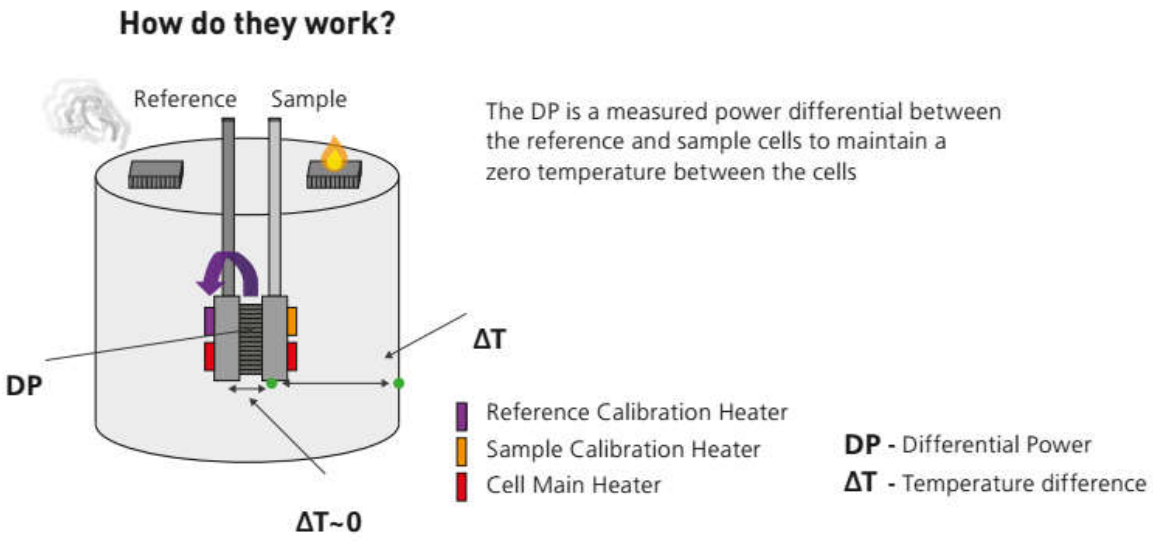


**Figure 14.** Scheme of working principle of isothermal titration calorimeters.

The reference cell usually contains water, while the sample cell contains one of the binding partners (the sample, often but not necessarily a macromolecule) and a stirring syringe which holds the other binding partner (the ligand).

The ligand is injected into the sample cell, typically in 0.5 to 2 µL aliquots, until the ligand concentration is two- to three-fold greater than the sample. Each injection of ligand results in a heat pulse that is integrated with respect to time and normalized for concentration to generate a titration curve of kcal/mol vs molar ratio (ligand/sample). The resulting isotherm is ftted to a binding model to generate the affnity ($K_D$), stoichiometry (n) and enthalpy of interaction (ΔH).

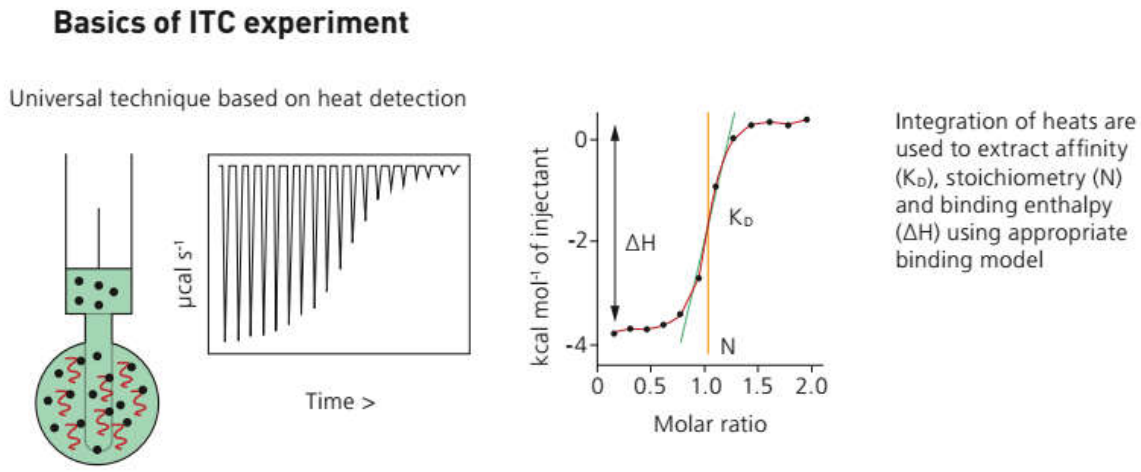


**Figure 15.** Basic principles of isothermal titration calorimeters.

In this thesis isothermal titration calorimetry was employed to find out the association constants at 298 K using a MicroCal VP-ITC (MicroCal, Inc., Northampton, MA, USA). First, the thermal equilibration was allowed at 298 K, which was followed by initial 120 s delay and the successive twenty five injections of the guest molecules to each cyclodextrin (duration of injection was 10 s having spacing of 180 s). Heat-burst curves were generated at each injection between micro cal $s^{-1}$ versus time in minute. The saturation curve for kcal/mol of the injectant against molar ratio was calculated by integration, using Origin 7.0 software to provide the heat associated with the injection. The association-affinity and thermodynamic properties of the binding phenomenon were found out by fitting the integrated heats of binding to the one site binding model to give the association constant (K), stoichiometry (N), binding enthalpy (ΔH) and the entropy (ΔS).

## II.2.13. Surface Tension Study

Surface tension is the elastic tendency of a fluid surface which makes it obtain the least possible surface area. Surface tension has the dimension of force per unit length or of energy per unit area. The two are equivalent, but when referring to energy per unit of area, it is common to use the term surface energy.

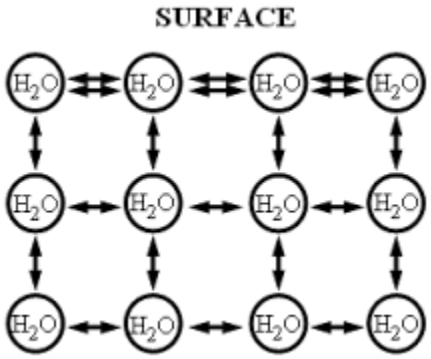


**Figure 16.** Surface tension occurs as the molecules at the surface form stronger bonds.

Surface tension measurement can be used to obtain valuable clues about the formation of inclusion complexes in cyclodextrins. It is found that surface tension of aqueous solutions of pure α and β-cyclodextrins don't show any remarkable change with increasing concentration and if the aqueous solutions of the guests show considerable variations, then surface tension study becomes an efficient tool to provide information about inclusion and also the stoichiometry of the formed inclusion complex.

The concentrations at which the inclusion occurred, i.e., the break point at the surface tension curve have been calculated by solving the equation of two straight lines and the values are given in tables of respective chapters.

## II.2.14. Conductivity Study

Conductivity is the ability of a solution, a metal or a gas to pass an electric current. In solutions the current is carried by cations and anions whereas in metals it is carried by electrons. How well a solution conducts electricity depends on a number of factors:

• Concentration

• Mobility of ions

• Valence of ions

• Temperature

All substances possess some degree of conductivity. In aqueous solutions the level of ionic strength varies from the low conductivity of ultra pure water to the high conductivity of concentrated chemical samples.

**How is conductivity measured?**

Conductivity may be measured by applying an alternating electrical current (I) to two electrodes immersed in a solution and measuring the resulting voltage (V). During this process, the cations migrate to the negative electrode, the anions to the positive electrode and the solution acts as an electrical conductor.

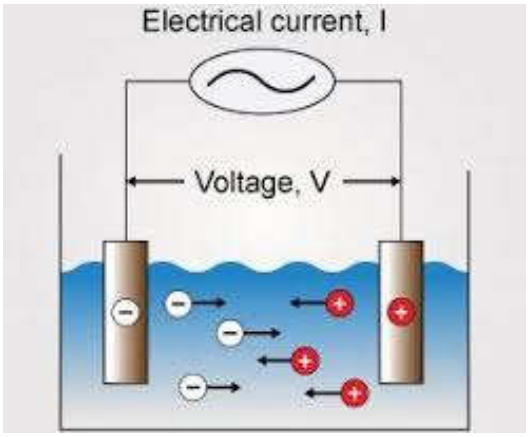


**Figure 17.** Migration of ions in solution.

**What is a conductive solution?**

Conductivity is typically measured in aqueous solutions of electrolytes. Electrolytes are substances containing ions, i.e. solutions of ionic salts or of compounds that ionize in solution. The ions formed in solution are responsible for carrying the electric current. Electrolytes include acids, bases and salts and can be either strong or weak. Most

conductive solutions measured are aqueous solutions, as water has the capability of stabilizing the ions formed by a process called solvation.

In this thesis conductivity (κ) of aqueous solutions of the guest molecules have been measured to get clue whether inclusion complexes have been formed while cyclodextrins being added to it. The studied guests showed considerable conductivity. As cyclodextrin was added to the aqueous solution, the κ was observed to show decreasing trend probably because of encapsulation of the guest molecules inside into the cavity of cyclodextrin. After a certain concentration of cyclodextrin a break was found in each of the conductivity curve indicating the formation of inclusion complexes. The values of κ and corresponding concentrations of cyclodextrins at each break have been shown in tables of respective chapters. The ratio of the concentrations of the guest and cyclodextrin at the break points were found to be approximately 1:1, suggesting the host-guest ratio to be 1:1.

In case of crown ether-ionic liquid complexation in solution conductivity measurement is highly beneficial as it provides data for very small changes in concentration of the free and complexed ions. The conductivity of a solution having IL with added CE gives important information about the formation and stability of CE-IL complex in the solution system. Thus, complexations of the studied ILs with CE are described by lowering of conductance, which becomes almost plateau after the CE/IL mole ratio passes the value of 1.0, clearly signifying the formation of sufficiently stable 1:1 CE-IL complex.

### II.2.15. pH Study

pH is a measure of the relative amount of hydrogen and hydroxide ions in an aqueous solution. In any collection of water molecules a very small number will have dissociated to form hydrogen ($H^+$) and hydroxide ($OH^-$) ions:

$$H_2O = H^+ + OH^-$$

The number of ions formed is small. In terms of molar concentrations, water at 25°C contains 1 $x10^{-7}$ moles per liter of hydrogen ions and the same concentration of hydroxide ions. In any aqueous solution, the concentration of hydrogen ions multiplied by the concentration of hydroxide ions is constant. Stated in equation form:

$$K_w = [H^+] [OH^-] \quad \text{(II.1)}$$

where, the brackets signify molar concentrations and $K_w$ is the dissociation constant for water. The value of $K_w$ depends on temperature. For example, at 25°C $K_w = 1.00 \times 10^{-14}$ and at 35°C $K_w = 1.47 \times 10^{-14}$. Acids and bases, when dissolved in water, simply alter the relative amounts of $H^+$ and $OH^-$ in solution. Acids increase the hydrogen ion concentration and because the product $[H^+]$ $[OH^-]$ must remain constant, acids decrease the hydroxide ion concentration. Bases have the opposite effect. They increase hydroxide ion concentration and decrease hydrogen ion concentration. For example, suppose an acid is added to water at 25°C and the acid raises the $H^+$ concentration to $1.0 \times 10^{-4}$ moles/liter. Because $[H^+]$ $[OH^-]$ must always equal $1.00 \times 10^{-14}$, $[OH^-]$ will be $1.0 \times 10^{-10}$ moles/liter. pH is another way of expressing the hydrogen ion concentration. pH is defined as follows:

$$pH = -\log [H^+] \quad \text{(II.2)}$$

Therefore, if the hydrogen ion concentration is $1.0 \times 10^{-4}$ moles/liter, the pH is 4.00. The term neutral is often used in discussions about acids, bases and pH. A neutral solution is one in which the hydrogen ion concentration exactly equals the hydroxide ion concentration. At 25°C, a neutral solution has pH 7.00. At 35°C, a neutral solution has pH 6.92. The common assertion that neutral solutions have pH 7 is not true. The statement is true only if the temperature is 25°C.

In this thesis pH of the aqueous solutions of amino acids, nucleosides, vitamins were measured to confirm the presence of ionic states of the molecules in solution.

### II.2.16. Density Study

The volumetric information includes 'Density' as a function of weight, volume and mole fraction and excess volumes of mixing. One of the well-recognized approaches to the study of molecular interactions in fluids is the use of thermodynamic methods. Thermodynamic properties are generally convenient parameters for interpreting molecular interactions in the solution phase. Fundamental properties such as enthalpy, entropy and Gibbs energy represent the macroscopic state of the system as an average of numerous microscopic states at a given temperature and pressure. An interpretation of

these macroscopic properties in terms of molecular phenomena is generally difficult. Sometimes higher derivatives of these properties can be interpreted more effectively in terms of molecular interactions. The volumetric information may be of immense importance in this regard. Various concepts regarding molecular processes in solutions like electrostriction, hydrophobic hydration, micellization and co-sphere overlap during molecular interactions have been derived and interpreted from the partial molar volume data of many compounds.

**Apparent Molar Volume**

The apparent molar volumes $\phi_V$ were determined from the solutions densities using the equation

$$\phi_V = M/\rho - 1000(\rho - \rho_o)/m\rho\rho_o \qquad \text{(II.3)}$$

where $M$ is the molar mass of the guest molecules, $m$ is the molality of the solution, $\rho$ and $\rho_0$ are the density of the solution and aqueous $\alpha$ and $\beta$-CD mixture respectively. The limiting apparent molar volumes $\phi_V^0$ were obtained by a least-square treatment to the plots of $\phi_V$ versus $\sqrt{m}$ using the Masson equation [15]

$$\phi_V = \phi_V^0 + S_V^* \cdot \sqrt{m} \qquad \text{(II.4)}$$

**Contributions of zwitter ionic group ($NH_3^+$), ($COO^-$); (CH), ($CH_2$) groups and end group to the limiting apparent molar volume ($\phi^0_V$) :**

The $\phi^0_V$ value of zwitterionic group, (CH), ($CH_2$) groups and end group of the amino acids were estimated from the following equations [16]

$$\phi^0_V = \phi^0_V(NH_3^+, COO^-) + \phi^0_V(CH) + n\,\phi^0_V(CH_2) + \phi^0_V(\text{end grp}) \qquad \text{(II.5)}$$

$$\phi^0_V(R) = \phi^0_V - \phi^0_V(NH_3^+, COO^-) - \phi^0_V(CH) \qquad \text{(II.6)}$$

$$\phi^0_V(CH) = \tfrac{1}{2}\,\phi^0_V(CH_2) \qquad \text{(II.7)}$$

**Hydration Number Estimated from Apparent Molar Volume**

The number of water molecules ($n_H$) hydrated the amino acids can be estimated from the value of measured standard partial molar volume. The values of $\phi_V^0$ of studied amino acids can be expressed as [17]

$$\phi_V^0(\text{amino acid}) = \phi_V^0(\text{int}) + \phi_V^0(\text{elect}) \qquad \text{(II.8)}$$

here, $\phi_V^0(\text{int})$ is intrinsic partial molar volumes of amino acids and $\phi_V^0(\text{elect})$ is electrostriction partial molar volume as a result of hydration of amino acids. The $\phi_V^0(\text{int})$ consists of two terms: van der Waals volume and volume due to packing effects. The values of $\phi_V^0(\text{int})$ for the amino acids were calculated from their crystal molar volume by using the following relationship,[17]

$$\phi_V^0(\text{int}) = (0.7 \,/\, 0.634)\ \phi_V^0(\text{cryst}) \qquad \text{(II.9)}$$

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 $\phi_V^0(\text{cryst})$ was calculated using the crystal densities of the amino acids represented by Berlin and Pallansch.[18] The hydration numbers is estimated using the relation

$$n_H = \phi_V^0(\text{elect}) \,/\, (V_e^0 - V_b^0) \qquad \text{(II.10)}$$

where $V_e^0$ is the molar volume of the electrostricted water and $V_b^0$ 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 $(V_e^0 - V_b^0)$. The value of $(V_e^0 - V_b^0)$ is calculated to be -3.0 or -3.3, at 298.15K respectively.

## II.2.17. Viscosity Study

As fundamental and important properties of liquids, viscosity and volume could also provide a lot of information on the structures and molecular interactions of liquid mixtures.

Viscosity and volume are different types of properties of one liquid, and there is a certain relationship between them. So by measuring and studying them together, relatively more realistic and comprehensive information could be expected to be gained. The relationship between them could also be studied. The viscometric information includes 'Viscosity' as a function of composition on the basis of weight, volume and mole fraction; comparison of experimental viscosities with those calculated with several equations and excess Gibbs energy of viscous flow. Viscosity, one of the most important transport properties is used for the determination of molecular interactions and studied extensively. Viscosity is not a thermodynamic quantity, but viscosity of a solution along with the thermodynamic property, $\phi^0_V$, i.e., the partial molar volume, gives a lot of information and insight regarding molecular interactions and the nature of structures in the solutions.

The experimental viscosity data for the studied systems are listed in respective chapters. The relative viscosity ($\eta_r$) has been analyzed using the Jones-Dole equation [19]

$$(\eta/\eta_o - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B\sqrt{m} \qquad \text{(II.11)}$$

where, $\eta_r = \eta/\eta_o$, $\eta$ and $\eta_0$ are the relative viscosities, the viscosities of the ternary solutions (guest + aqueous cyclodextrin) and binary aqueous mixture (aqueous cyclodextrin) and $m$ is the molality of the guest 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 reported.

The $B$-coefficients of zwitterionic group, (CH), ($CH_2$) groups and end group of the amino acids have been resolved as follows:

$$B = B\,(NH_3^+, COO^-) + B\,(CH) + n\,B\,(CH_2) + B\,(\text{end grp}) \qquad \text{(II.12)}$$

$$B\,(R) = B - B\,(NH_3^+, COO^-) - B\,(CH) \qquad \text{(II.13)}$$

$$B\,(CH) = \tfrac{1}{2}\,B\,(CH_2) \qquad \text{(II.14)}$$

## II.2.18. Refractive Index Study

Optical data, i.e., refractive index of solution mixtures provide interesting information about molecular interactions and structure of the solutions. 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.

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

Whenever light changes speed 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 speed 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:

$$\frac{V_A}{V_B} = \frac{\sin\theta_A}{\sin\theta_B} = \frac{n_B}{n_A} \qquad \text{(II.15)}$$

Thus, it is not necessary to measure the speed of light in a sample in order to determine its index of refraction. Instead, by measuring the angle of refraction, and knowing the index of refraction of the layer that is in contact with the sample, it is possible to determine the refractive index of the sample quite accurately.

Molar refractivity, was obtained from the Lorentz- Lorenz relation by using, $n_D$ experimental data according to the following expression [20]

$$R_M = \left\{(n_D^2\text{-}1)/(n_D^2+2)\right\}(M/\rho) \qquad \text{(II.16)}$$

where $R_M$, $n_D$, $M$ and $\rho$ are the molar refraction, the refractive index, the molar mass and the density of solution respectively.

The Limiting molar refraction ($R_M^0$) estimated from the following, [21]

$$R_M = R_M^0 + R_S\sqrt{m} \qquad \text{(II.17)}$$

## II.2.19. Ultrasonic Speed Study

In recent years, there has been considerably progress in the determination of thermodynamic, acoustic and transport properties of working liquids from ultrasonic speeds, density and viscosity measurement. The study of ultrasonic speeds and isentropic compressibilities of liquids, solutions and liquid mixtures provide useful information about molecular interactions, association and dissociation. Various parameters like molar isentropic and isothermal compressibilities, apparent molal compressibility, isentropic compressibility, deviation in isentropic compressibility from ideality, etc. can very well be evaluated and studied from the measurement of ultrasonic speeds and densities in solutions. Isentropic compressibilities play a vital role in characterization of binary and ternary liquid mixtures particularly in cases where partial molar volume data alone fail to provide an unequivocal interpretation of the interactions.

The isentropic compressibility ($\beta$) of a solution can be calculated from the Laplace's equation:

$$\beta = \frac{1}{u^2 \rho} \qquad \text{(II.18)}$$

where, $\rho$ is the solution density and $u$ is the ultrasonic speed in the solution. The determined isentropic compressibility ($\beta$) is adiabatic, not an isothermal one, because the local compressions occurring when the ultrasound passes through the solution are too rapid to allow an escape of the heat produced.

The apparent molal isentropic compressibility ($\phi_K$) of the solutions was calculated using the relation:

$$\phi_K = M\beta/\rho + 1000(\beta\rho_0 - \beta_0\rho)/m\rho\rho_0 \qquad \text{(II.19)}$$

$\beta_o$is the isentropic compressibility of the solvent mixture, $M$ is the molar mass of the solute and $m$ is the molality of the solution.

The limiting apparent isentropic compressibility $\phi_K^o$ may be obtained by extrapolating the plots of $\phi_K$ versus the square root of the molal concentration of the solutes by the computerized least- square method according to the equation. [22]

$$\phi_K = \phi_K^0 + S_K^* \sqrt{m} \qquad \text{(II.20)}$$

The limiting apparent molal isentropic compressibility ($\phi_K^o$) and the experimental slope $S_K^*$ can be interpreted in terms of solute-solvent and solute-solute interactions respectively. It is well established that the solutes causing electrostriction leads to the decrease in the compressibility of the solution. However, the poor fit of the solute molecules as well as the possibility of flexible hydrogen bond formation appear to be responsible for causing a more compressible environment.