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 formed from two or more molecules or ions that are bound together in distinctive structural relationships by forces other than those of covalent bonds.[1,2] Host-guest chemistry encompasses the concept of molecular interactions and recognition via non-covalent bonding. Non-covalent bonding is crucial in maintaining the three-dimensional framework of big molecules such as nucleic acids and proteins, and is involved in various biological processes where big molecules specifically but transiently bind to one another.[3] Such non-covalent interactions are classified commonly into four types : van der Waals forces, ionic bonds, hydrophobic interactions and hydrogen bonds.[4]

Supramolecular chemistry has demonstrated important concepts which include molecular recognition, molecular folding, molecular self-assembly, mechanically-interlocked molecular architectures, host-guest chemistry and dynamic covalent chemistry. The study of non-covalent forces is essential for understanding several biological processes from cell structure. Supramolecular research is often inspired by biological systems. The larger molecule can be considered the “host” component, which binds the smaller “guest” molecule. The analogous terms of guest and host in biological systems are generally referred to as substrate and enzyme respectively.

The thermodynamic advantages of host–guest chemistry stem from the fact that the interaction between guest and host molecules results in a lower overall Gibb’s free energy.

Chemists are working tirelessly to determine the thermodynamic properties and energy of the non-covalent forces involved in supramolecular chemistry, in order to gain a

better understanding of the combinatorial outcome of these numerous small non-covalent interactions that are used to generate an overall effect on the supramolecular structure.[2]

In order to develop synthetic systems that perform particular functions, it is necessary to understand the thermodynamics of binding between guest and host. Chemists are focusing on the exchange of energy involved in various binding interactions and making an effort to design scientific experiments for quantifying the fundamental basis of these non-covalent interactions by using different techniques such as UV-Visible spectroscopy, differential scanning calorimetry, surface tension and NMR spectroscopy. The experimentally obtained data are quantified and elucidated through analysis of enthalpy $\Delta H°$, entropy $\Delta S°$, Gibb's energy $\Delta G°$ and binding constant K_a.[3]

B. Tian *et al.* described the generation of cyclodextrin (CD) polymers and summarized their potential applications in diagnosis and cancer treatment, drug delivery, food, biodegradable capsules and so on.[5]

Y. X. Sun *et al.* constructed a facile and targeted gene delivery system, PEI-CD/AdGRGDS, by conjugating adamantyl peptide (AdGRGDS) and β-cyclodextrin (β-CD) modified polyethylenimine (PEI-CD) based on host–guest interaction. This gene delivery system exhibited better DNA binding ability and showed excellent ability in compacting DNA into uniform spherical nanoparticles.[6]

For waste water treatment, Alsbaiee *et al.* successfully developed a mesoporous β-CD polymer with high-surface-area. It could rapidly remove the mixture of organic micropollutants through adsorption with rate constants 15-200 times higher than those of non-porous β-CD adsorbent materials and activated carbons.[7]

G. Liu *et al.* presented the development of novel CD-based supramolecular hydrogels and their applications in the field of biomedical science.[8]

S. Goswami *et al.* investigated the formation of inclusion complexes of a painkiller Isoxicam, belonging to the oxicam group of non-steroidal anti-inflammatory drugs (NSAIDs) and its copper complex with the four cyclodextrins such as HP-γ-CD, HP-β-CD, γ-CD and β-CD.[9]

W. Su *et al.* developed an inclusion complex, tetrandrine-hydroxypropyl-β-cyclodextrin (TET-HP-β-CD), which showed to alleviate inflammation and pulmonary fibrosis via inhalation administration.[10] This revealed that TET-HP-β-CD act as an attractive candidate for treating pulmonary fibrosis.

D. Patra *et al.* constructed self-powered micropumps using host-guest molecular recognition between *trans*-azobenzene and α- and β-CD.[11] Both surface coatings and hydrogels based on host-guest partners were employed as scaffolds for the construction of micropumps. These soft dual stimuli-responsive micropumps can be activated either by introducing guest molecules or by UV light. Also, the recharging of these micropumps could be done via reversible host-guest interaction.

Wang *et al.* developed β-CD covalent organic framework (COF) through the condensation reaction between terephthalaldehyde and heptakis(6-amino-6-deoxy)-β-CD at room temperature.[12] This β-CD COF was exploited for constructing chiral stationary phase on capillary electrochromatography in order to separate chiral drugs.

R. Sun *et al.* successfully constructed a light-driven, supramolecular polymer by molecular recognition between calixarene and α-CD based pseudorotaxane containing azobenzene and binaphthyl moieties.[13] It is a successful supramolecular polymerization via non-covalent host-guest molecular recognition.

Guo *et al.* described chemosensor applications based on novel host-dye complexes formed of calixarenes as hosts and lucigenin (LCG) as the fluorescence guest.[14] These sensing systems were used to set-up enzyme assays for choline oxidase and acetylcholinesterase, which enables enzymatic inhibitors screening as well as to determine specifically absolute concentrations of both choline and acetylcholine.

Wang *et al.* fabricated a new nanosupramolecular binary vesicle from *p*-sulfonatocalix[4]arene and asymmetric viologen.[15] It is responsive to the multiple external stimuli such as redox, temperature and host-guest inclusion. These external stimuli function as an effectual key that actuates the efficient release of the entrapped substrates.

Cheignon *et al.* reported the surface decoration of $La_{0.9}Tb_{0.1}F_3$ nanoparticles by calixarenes,[16] which results in the photosensitization of Tb ions from the $La_{0.9}Tb_{0.1}F_3$

nanoparticles. The interactions of these calixarene capped nanoparticles with rhodamine 6G and paraquat was interestingly observed to be stronger than for the parent systems which lacks capping of the calixarene to the nanoparticles surface.

Y. X. Wang *et al.* constructed coassemblies using amphiphilic calixarene and two drugs, mitoxantrone.HCl and irinotecan.HCl. The anchoring of the surface of these coassemblies with targeting ligands, biotin–pyridinium and hyaluronic acid–pyridinium, results in the enhanced anticancer activities of the drugs.[17]

II.2. Theory of Investigations

II.2.1. Hydrophobic Interactions :

In a polar solvent (usually water), the nonpolar molecules tends to interact with one another. This tendency of nonpolar molecules is termed as hydrophobic effect. The interactions that exist between the nonpolar molecules are called hydrophobic interactions. The word hydrophobic signifies “water-hating”, and it describes the partitioning of nonpolar molecules and water molecules, which increases hydrogen bonding between water molecules and reduces the contact area between nonpolar molecules and water molecules. Thermodynamically, free energy change of water molecules which surrounds a solute is the hydrophobic effect. Hydrophobicity is indicated by a positive change in free energy of the surrounding solvent, whereas hydrophilicity is implied by a negative change in free energy.

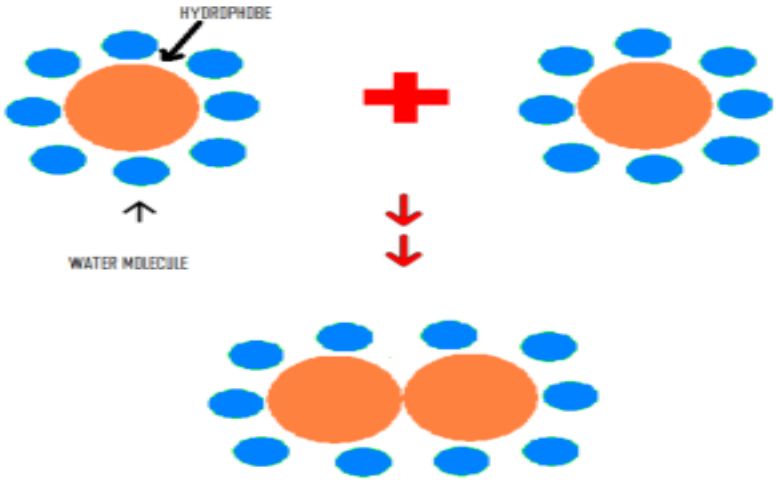

Figure 1. Hydrophobic molecules come closer in polar solvent.

The hydrophobic effect is responsible for various biological effects, including small molecule-protein associations, cell membrane and vesicle formation, protein folding and introduction of membrane proteins into the nonpolar environment of lipid. Applying hydrophobicity concept, the two non-interacting molecules can be made to undergo reactions by means of minimizing the intermolecular distance adding water to the system.

II.2.2. Van der Waals Forces :

The van der Waals forces are relatively weak interactions between molecules or atoms that are distance-dependent. These forces between interacting molecules vanishes quickly at longer distances.

The van der Waals forces plays a crucial role in diverse fields such as polymer science, surface science, nanotechnology, supramolecular chemistry, structural biology, etc. They also define several properties of molecular solids and organic compounds, including their solubility in non-polar and polar media.

The van der Waals forces include repulsions and attractions between molecules, atoms and surfaces. They differ from ionic and covalent bonding as they are caused by temporarily fluctuating polarizations of the adjacent molecules or atoms.

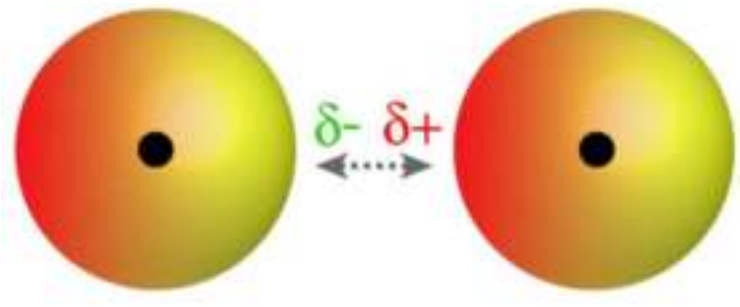

Figure 2. Van der Waals forces acting between molecules.

II.2.3. Hydrogen Bonds :

A hydrogen bond is the electrostatic force of attraction between a H-atom covalently bonded to a more electronegative atom such as fluorine (F), oxygen (O) or nitrogen (N), and another highly electronegative atom adjacent to the H-atom.

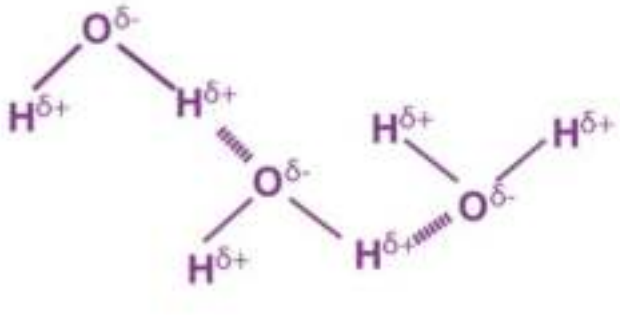

Figure 3. Hydrogen bonds between water molecules.

Hydrogen bonds are of two distinct types, namely, intermolecular hydrogen bond (occur between separate molecules) and intramolecular hydrogen bond (occur within one single molecule). Depending on the nature, geometry and environment of the specific acceptor and donor atoms, the energy of a hydrogen bond can vary from 1-40 kcal/mol. This makes hydrogen bond relatively stronger compared to a van der Waals interaction, and weaker than completely ionic or covalent bonds. This type of bond can exist in organic molecules such as proteins and DNA, and in inorganic molecules like water.

Intermolecular hydrogen bonding is accountable for the water's high boiling point (100°C) compared to the hydrides of group-16 possessing much weaker hydrogen bonds.

Intramolecular hydrogen bonding is somewhat accountable for the tertiary and secondary structures of nucleic acids and proteins. It also plays a significant role in the structure of both natural and synthetic polymers.

II.2.4. Electrostatic Forces :

Electrostatic force, also called the Coulomb interaction or Coulomb force, is the repulsive or attractive force between the electrically charged particles. It is one of the fundamental physical forces.

Two like charges repel one another along a straight line joining their centres, whereas two unlike charges attract one another along a straight line between their centres.

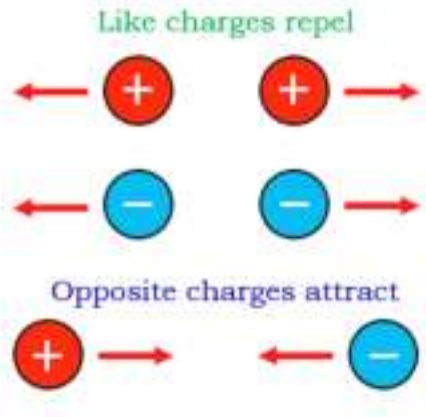

Figure 4. Electrostatic force working between charged species.

II.2.5. Ion-Dipolar Attractions :

An ion-dipolar interaction is the electrostatic attraction between an ion and a dipolar molecule. It is commonly involved in the solutions of ionic compounds in polar solvents. In this type of attraction an anion attracts the counter positive part of a neutral polar molecule or a cation attracts the counter negative part of a neutral polar molecule. Ion-dipole attractions gets stronger with the increase in the magnitude of the dipole of the polar molecule or the increase in the charge of an ion.

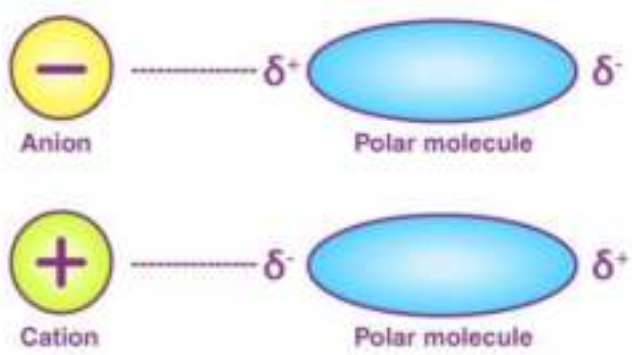

Figure 5. Examples of ion-dipolar attractions.

II.2.6. Dipole-Dipole Attractions :

Dipole-dipole forces are defined as the attractive forces that result between the negative side of one polar molecule and the positive side of another polar molecule. The strengths of dipole-dipole forces range from 5-20 kJ/mol. These forces are much weaker compared to covalent or ionic bonds and possess a pronounced effect only when the molecules involved are close together.

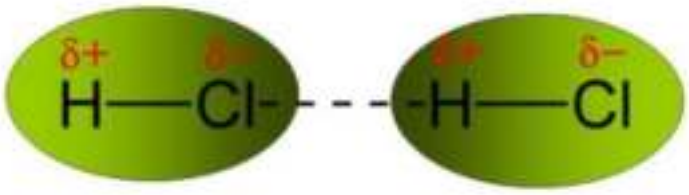

Figure 6. Example of a dipole-dipole attraction.

II.2.7. ^{1}H NMR Spectroscopy :

Nuclear magnetic resonance (NMR) is one of the very important spectroscopic methods to the chemists. NMR techniques may be used to study many nuclei, but the most commonly available are carbon and hydrogen. It provides information about the number of magnetically distinct type of atoms being studied. When studying hydrogen nuclei, the number of each of the distinct types of hydrogen nuclei and information concerning the nature of their immediate environment may be obtained.

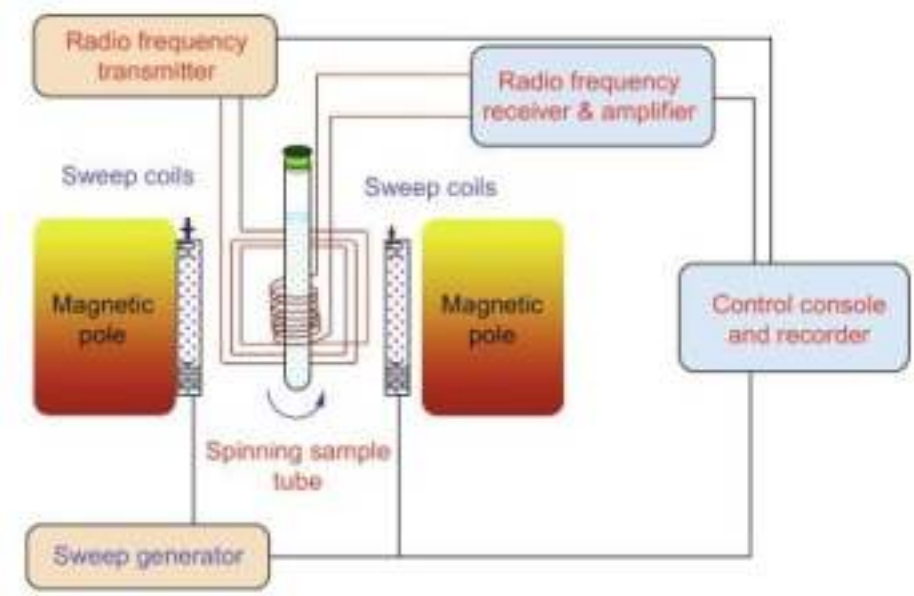

Figure 7. Schematic illustration of an NMR instrument.

Chemical Equivalence

A set of protons within a molecule present in chemically identical environments are said to be chemically equivalent. These chemically equivalent protons usually show same chemical shift. A molecule possessing set of protons which are chemically distinct from each other may produce different absorption peak, and such protons are chemically nonequivalent. Often, those protons which are chemically equivalent are also magnetically equivalent. But in some instances, chemically equivalent protons may not be magnetically equivalent.

Integrals and Integration

The NMR spectrum provides not only the number of different types of protons present in a molecule, but also reveals the number of each type within the molecule. The area under each peak in NMR spectrum is proportional to the number of protons giving rise to that peak. The area under each peak can be integrated electronically by the NMR spectrometer. The integral provides the relative number of each type of proton rather than the absolute number of protons.

Chemical Shift

In ^{1}H NMR spectrum, protons with different magnetic environments show signals at different positions and each type of proton possess a characteristic value, which is called the chemical shift. Although the signals position in the spectrum may alter with the external magnetic field strength, a signal for TMS (tetramethylsilane) is used to report as a reference signal in a ^{1}H NMR. The major factors that influence chemical shift are anisotropic induced magnetic field effects, electron density and electronegativity of neighboring groups. Electron density around a nucleus generates a local magnetic field which shields a nucleus from the external magnetic field. A nucleus near to an electronegative atom experiences reduced electron density resulting in the deshielding of a nucleus. Magnetic anisotropic effect is caused by a local induced magnetic field experienced by a nucleus as a result of circulating electrons, which can be paramagnetic when aligned to an external field or diamagnetic when opposed to it.

In this research work, chemical shift changes of both the guest and host protons have been observed, which indicates the encapsulation of guest into cyclodextrin or calixarene.

II.2.8. FT-IR Spectroscopy :

When molecules absorb infrared (IR) radiation, they are excited to a higher energy level. Only selected infrared frequencies are absorbed by a molecule, which corresponds to the bonds bending and stretching vibrational frequencies in most covalent molecule. All of the bonds in a molecule does not have a capability to absorb infrared radiation, only those bonds with a varying dipole moment can absorb infrared radiation. The infrared spectrum can be used to identify molecules in the same way that a fingerprint can be used to identify humans. One can determine whether two substances are identical or not by comparing their infrared spectra. Another useful application of infrared spectrum is to establish a molecule's structural information.

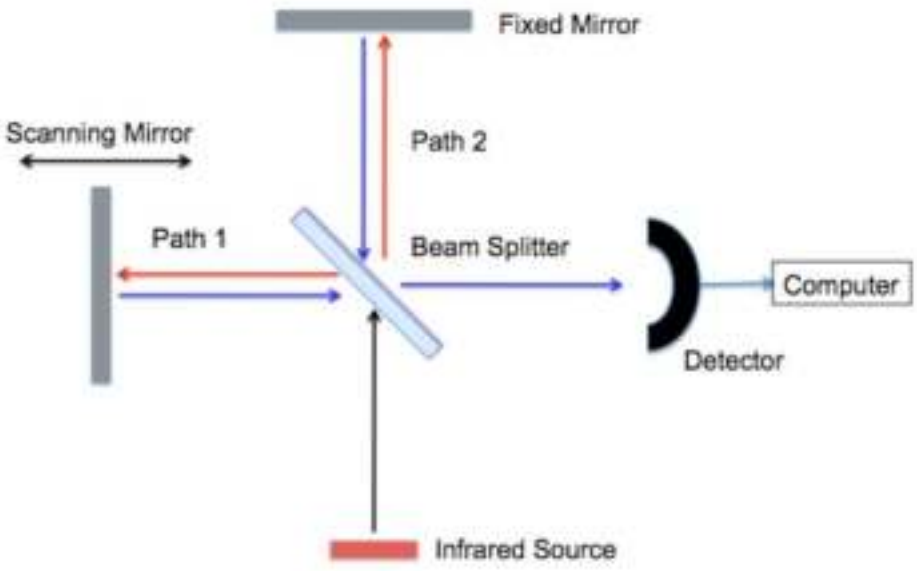

Figure 8. A schematic diagram of an interferometer used in a Fourier Transform Infrared (FT-IR) Spectrophotometer

The IR absorption frequency is affected by the masses of the bonded atoms and the bond strength. The bond of a diatomic molecule may be considered as a spring, which vibrates like a simple harmonic oscillator. If the two bonded atoms have masses m_1, m_2 and the force constant of the spring is K, the vibrational frequency according to the Hooke's law is given by

$$\bar{\upsilon} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

where K indicates the bond strength, and μ is the reduced mass of the system which is given by

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

The Infrared Spectrophotometer

An infrared spectrophotometer is an instrument used to determine the absorption spectrum of a compound. This instrument gives spectra of compounds over the frequency range 4000-400 cm^{-1}.

Preparation of Samples

The FT-IR spectra were recorded in the spectrophotometer by loading solid KBr pellet into a sample holder. KBr pellet was composed by mixing minute amount of solid sample with dry KBr powder and then pressing this powdered mixture under high pressure.

In this research work, the inclusion of a guest into a host molecule has been demonstrated satisfactorily by observing the shifts of vibrational frequencies of the interacting groups.

II.2.9. UV-Visible Spectroscopy :

A portion of continuous radiation may be absorbed when it passes through a transparent material. Then the residual radiation when passed through a prism, gives an absorption spectrum. In ultraviolet and visible spectroscopy, absorption of electromagnetic radiation occurs as a result of transitions between different electronic energy levels. The four types of possible transitions are $\sigma - \sigma *$, $n - \sigma *$, $\pi - \pi *$ and $n - \pi *$.

The extent of light absorption increases as the number of molecules capable of absorbing light of a given wavelength increases. Again, the greater the number of molecules efficiently absorbing light of a given wavelength, greater is the degree of light absorption. Based on these concepts, Beer-Lambert law may be given by the following empirical equation,

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

A = Absorbance

I_o = Intensity of incident light

I = Intensity of light leaving sample

ε = Molar absorptivity

c = Molar concentration sample solution

l = length of sample cell (cm)

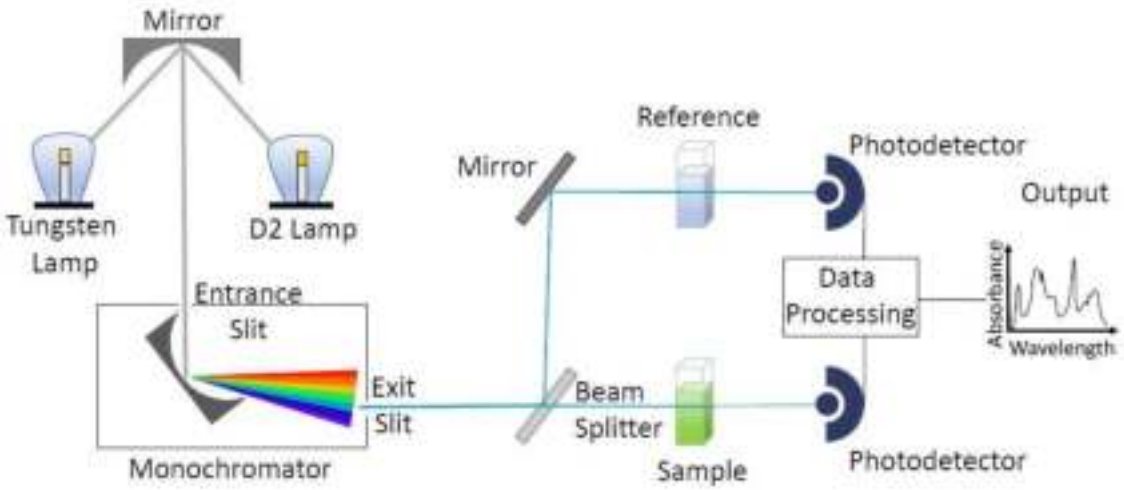

Figure 9. Schematic diagram of UV-Visible spectrophotometer.

In this thesis, binding affinity of various guests with cyclodextrin and calixarene have been calculated by fitting UV-Visible spectroscopic data to the Benesi-Hildebrand equation. The spontaneity of the inclusion process were also determined from the binding affinity data obtained from UV-Visible spectroscopy.

II.2.10. Fluorescence Spectroscopy :

Fluorescence spectra is an emission spectra which arises due to the transition of an excited molecule from the singlet excited electronic state to the singlet ground electronic state. Each electronic state have various vibrational states. In fluorescence, the molecules first absorbs a photon and excited from its ground electronic state to one of the vibrational levels of the excited electronic state. The excited molecule undergoes collisions with other molecules and loses vibrational energy until it reaches the lowest vibrational level from the excited electronic state. The molecule then emits a photon and returns to one of the vibrational levels of the ground electronic state showing fluorescence (Figure 10). Since the ground state possess various vibrational levels, the photons emitted during fluorescence will have different frequencies and the analysis of these frequencies along with their relative intensities helps in determining the structure of the different vibrational levels.

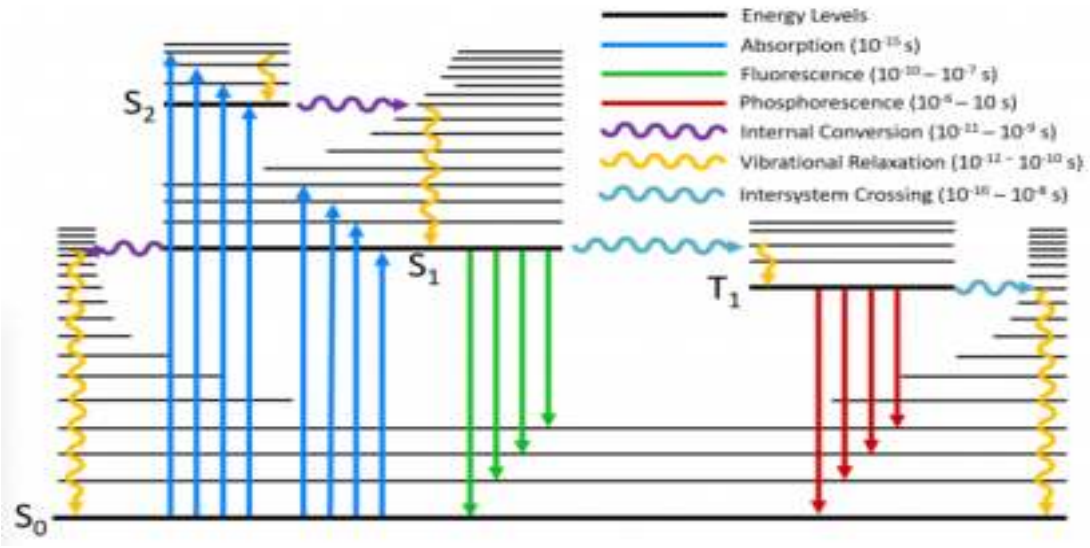

Figure 10. Jablonski Diagram.

In this thesis, fluorescence study was employed to further verify the binding affinity evaluated from UV-Visible spectroscopic study. The obtained fluorescence data were fitted to the modified Benesi-Hildebrand equation for determining binding affinity of the inclusion complexes of indole-3-methanol and cyclodextrins.

II.2.11. Mass Spectrometry :

Mass spectrometry is an analytical method used to determine the mass-to-charge ratio (m/z) of one or more molecules present in a sample. It is used in many fields to measure the masses of molecules, and to define their chemical structure or identity. In mass spectrometry, a sample is bombarded by a highly energized beam of electrons which causes the molecules of a sample to split up into positively charged fragments or simply become positively charged without undergoing fragmentation. A specific type of ion has a specific m/z ratio. The m/z ratio indicates the molecular mass for most of the ions, as the ions mostly possess a single charge. A molecular ion or a parent ion is formed, when an electron from a molecule is removed.

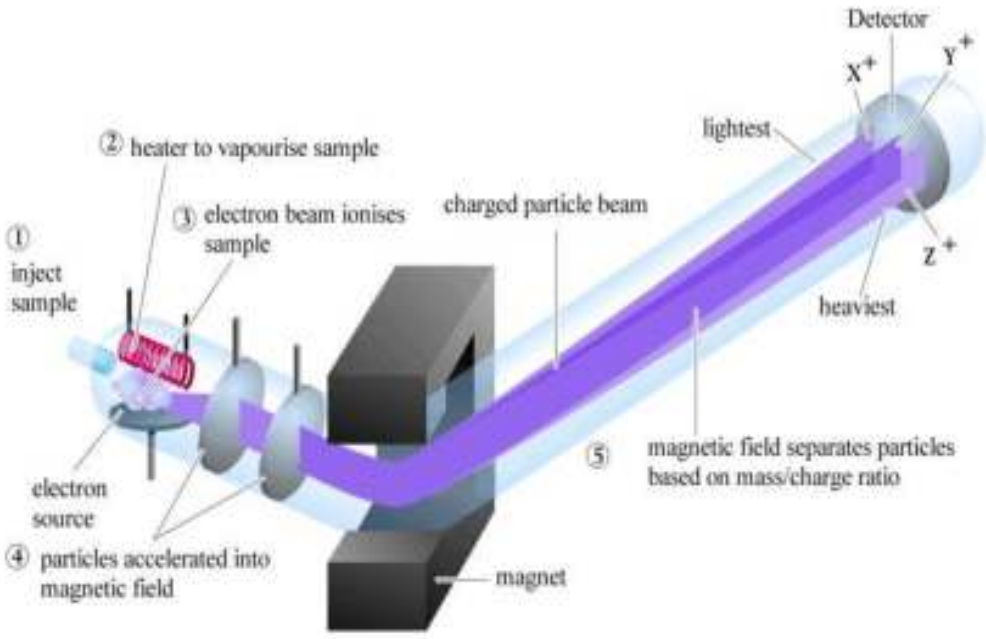

Figure 11. Diagrammatic representation of working principle of mass spectrometer.

$$M + e^- \text{ ----------> } M^+ + 2e^-$$

The m/z value for a parent ion is the molecular mass of the compound. The parent ion peak sometimes represents the base peak that can be simply identified but mostly, molecular ion peak does not emerge as a base peak and has often a low abundance. During the inclusion process there is neither formation nor breaking of a bond, hence, the m/z ratio of inclusion complex should be equal to the combined molecular masses of guest and host molecules, or it may add with proton or certain impurities such as sodium. Therefore, mass spectrometric analysis can be employed to confirm the inclusion complex formation and to estimate its stoichiometry.

II.2.12. Differential Scanning Calorimetry :

Differential scanning calorimetry (DSC) is a powerful thermoanalytical tool in which the heat change in a sample is measured with the temperature variation. It allows us to detect the existence of impurities or crystal structure changes by observing the variation in melting point, which registers a peak other than the melting temperature in the DSC thermograms. Thermal stability of the sample can also be elucidated from the thermodynamic parameters obtained from DSC experiments.

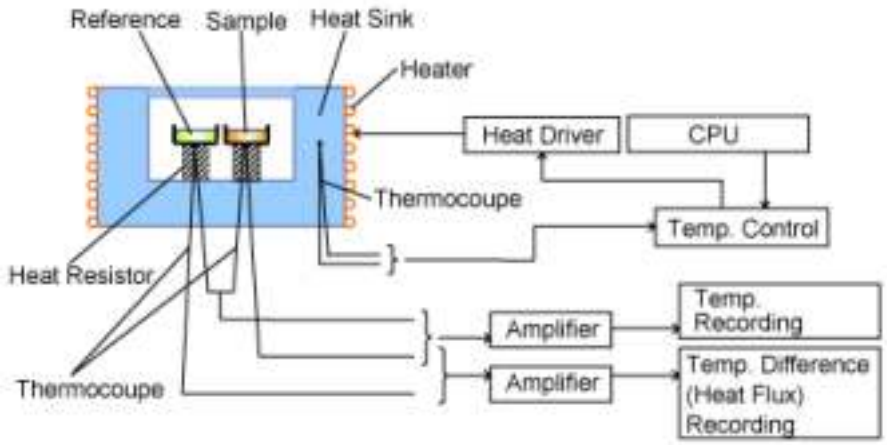

Figure 12. Block diagram of Heat Flux DSC.

DSC instrument is of two types : Heat flux DSC and Power Compensation DSC. Heat flux type includes the reference and sample holder, heater, heat resistor and heat sink. The heater provides heat to the reference and the sample via a heat resistor and a heat sink. The sample has lower heat capacity than the heat sink. The exothermic or endothermic phenomena of sample is compensated by heat sink. It helps to maintain the same difference of temperature between the reference and sample. The difference in the quantity of heat supplied to the reference and the sample is proportional to the difference of temperature of both the holders. Calibration of the standard material enables quantitative measurement of unknown sample.

In this thesis, we studied the physicochemical state of the guest after encapsulation into the cavity of cyclodextrin and calixarene. Generally, the endothermic peak of the guest in DSC thermograms either shifts to a different temperature or it may reduce on complexation with cyclodextrin and calixarene, which suggests a change in crystal lattice, sublimation point or melting point owing to inclusion complexation.

II.2.13. Thermogravimetric Analysis :

Thermogravimetric analysis (TGA) is a type of thermal analysis method that measures the sample mass as the temperature changes. Many materials exhibit mass change as a function of temperature as they undergo degradation followed by loss of volatile components. This phenomenon can furnish more information about materials under test.

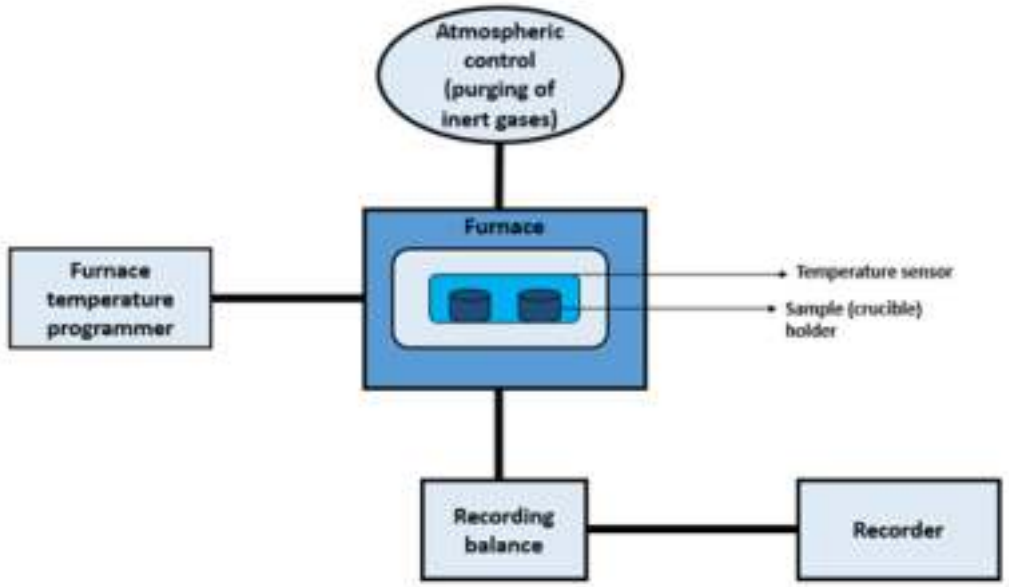

Figure 13. Schematic diagram of TGA instrument.

TGA is carried out by subjecting a sample in a chosen atmosphere to a precise temperature program. Measuring the mass of a sample at regular intervals throughout the temperature profile gives a plot of mass vs temperature yielding thermogravimetric curve. This characteristic curve indicates the thermal decomposition of a sample.

In this research work, TGA has been used to study and compare the thermal stability of inclusion complex with the free guest.

II.2.14. Powder X-Ray Diffraction :

Powder X-ray diffraction (PXRD) is a rapid analytical method particularly employed for determining phase of a crystalline material and can give information on dimensions of unit cell. The material, which is to be to be analyzed, is finely ground, homogenized and average bulk composition is measured.

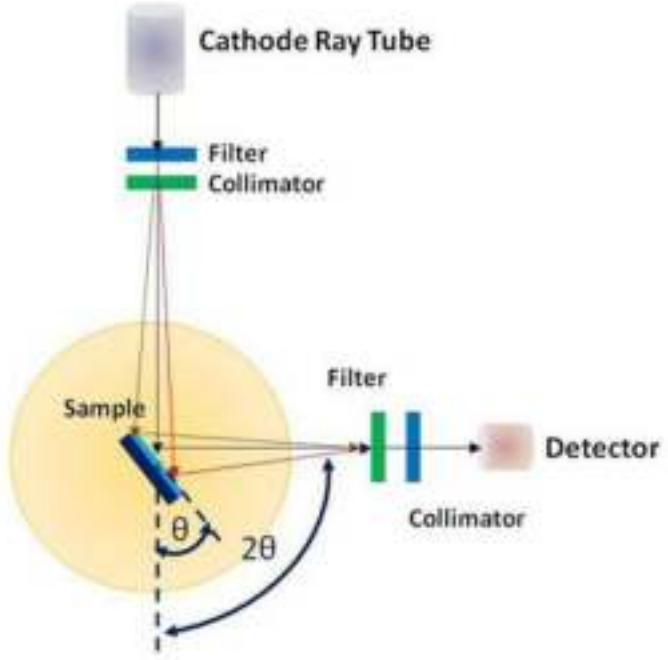

Figure 14. Diagrammatic representation of working principle of PXRD.

X-ray diffraction technique is currently widely used for studying the atomic spacing and crystal structures. For X-ray wavelengths, crystalline solids behave as 3-D diffraction gratings, analogous to the spacing of planes in a crystal lattice. The emergence of constructive interference on interaction of X-rays with a crystalline sample is the basis of X-ray diffraction. Diffraction occurs when X-ray of wavelength comparable to the space between two atoms is scattered by a periodic array of atoms, producing constructive interference at specific angles. The incident X-rays interact with the sample and give rise to constructive interference pattern when conditions satisfy Bragg's law -

$$n\lambda = 2d\,sin\theta$$

where θ = glancing angle, d = inter-planar spacing, λ = wavelength of incident X-ray, n = diffraction order.

After interaction of X-rays with the sample, diffracted X-rays are collected, identified, processed and counted. Data was assembled by scanning the sample over a range of 2θ angles.

II.2.15. Scanning Electron Microscopy :

In scanning electron microscope (SEM), a focused beam of high-energy electrons is applied to scan the surface of a sample to produce high resolution images.

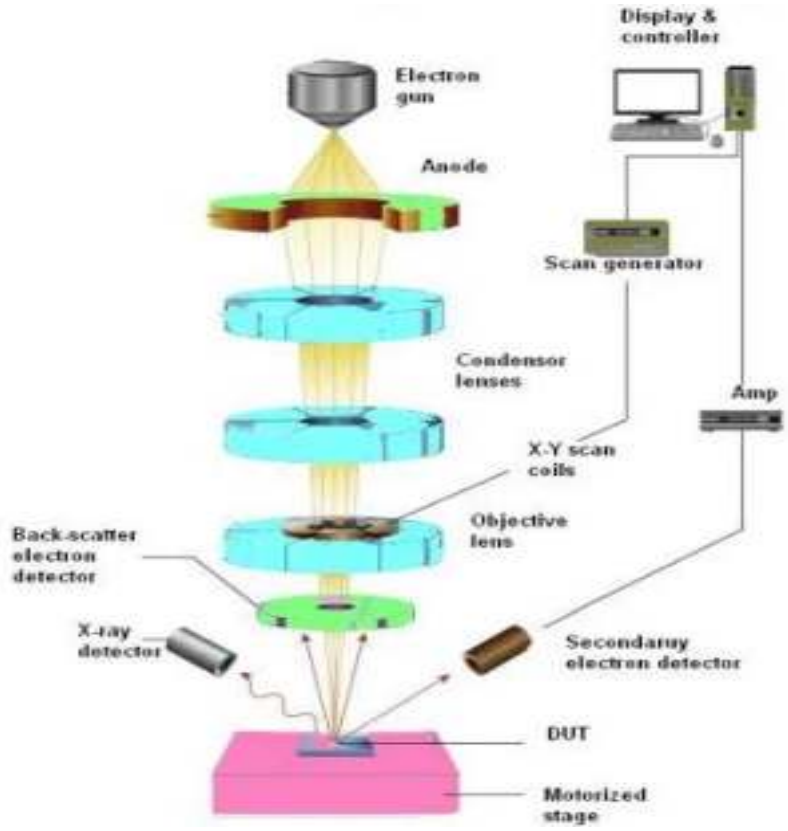

Figure 15. Schematic diagram of scanning electron microscope.

The high-energy electrons interact with atoms present in the sample, giving rise to a variety of signals containing information about the chemical composition, crystalline structure, surface topography and orientation of materials constituting the sample. The interaction of electrons with the sample produces secondary electrons, characteristic X-rays and backscattered electrons. One or more detectors collect these signals to create images of the sample surface. SEM can image areas ranging in width from 1 cm to 5 microns in scanning mode.

II.2.16. Surface Tension Study :

Surface tension is the elastic tendency of liquid surfaces to contract to the smallest possible area. It has the dimension of force per unit length, or of energy per unit area.

Although the two are equivalent, when citing to energy per unit of area, the term surface energy is commonly used.

The measurement of surface tension can provide valuable indication regarding the formation of inclusion complexes in cyclodextrins. It is found that aqueous solutions of β-CD and HP-β-CD don't show any significant variation in surface tension with increasing concentration, while that of the guests display considerable changes, therefore, surface tension analysis becomes an effective tool for providing information about encapsulation as well as the stoichiometry of the inclusion complex.

The concentrations of CD and guest have been obtained from a single break point in the plot of surface tension vs CD concentration, which suggested a 1:1 stoichiometry of the inclusion complexes.

II.2.17. Molecular Docking Study :

Molecular docking is a method for predicting the preferred orientation of a ligand to the binding site of a target when both ligand and target are bound together to form a stable adduct. The binding affinity or the strength of association between a ligand and a target can be predicted based on their preferred orientation. Using the scoring function, various possible adduct conformations obtained from molecular docking are ranked and grouped together. Based on docking score, the most favourable and lowest energy docked conformer is chosen.

Molecular docking is the most common method used in structure-based drug design, as it has the potential to predict the binding-conformation of a ligand to the appropriate binding site of a target. Characterisation of the binding behaviour plays a significant role in rational drug design as well as to elucidate fundamental biochemical processes.

In this thesis, molecular docking studies have been performed for predicting possible binding mode as well as binding free energy of different guests with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and p-sulfonatothiacalix[4]arene using MOE2015 software, automated docking program AutoDock 4.2 and PyRx software.

II.2.18. Antibacterial Activity Analysis :

Microbial infections are one among the chief clinical threats that may be associated to morbidity and mortality due to the microbial resistance to the existing antimicrobial therapeutics. Research and development of novel antibacterial agents from several sources help in tackling microbial resistance. The goal of screening the antibacterial activity is to detect the possibility of drug resistance among commonly known pathogens and also to ensure sensitivity to chosen drugs against particular infections. Briefly, antibacterial assay can be used for the discovery of antimicrobial agents/drugs, comparative analysis of antimicrobial agents/drugs, epidemiology and prognosis of health outcome. Numerous laboratory methods can be used to assess the *in vitro* antibacterial activity of a compound, agar well diffusion method being one among them. Agar well diffusion is a qualitative assay which is simple and easier to perform. However, agar well diffusion method is suitable only for diffusive test materials. Further, the experimental results procured by following the agar well diffusion technique is affected by the following factors; well diameter, concentration of the antibacterial agent placed into the well, agar type and its concentration, thickness and the pH of the medium, the microorganism assessed and the incubation time period. Numerous pathogenic microorganisms are used as indicator strain in the agar well diffusion method namely, Gram negative bacteria such as *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, and Gram positive bacteria like *Bacillus amyloliquefaciens*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Bacillus subtilis*.

In this present work, the antibacterial activity of indole-3-methanol, ticlopidine and their inclusion complexes has been assessed considering commonly known pathogens, which includes Gram-negative bacteria *Salmonella sp.*, *Shigella sp.*, *Proteus vulgaris* and *Pseudomonas aeruginosa* sp., and Gram-positive bacteria *Bacillus amyloliquefaciens* and *Bacillus subtilis* by agar well diffusion technique.

II.2.19. Cell Viability Assay :

Cell viability or cytotoxicity assays are a measure of cellular or metabolic alterations that are associated with viable or non-viable changes. These assays can either detect

structural changes like the loss of membrane integrity upon cell death or physiological and biochemical activities that indicate whether the cell is living or dead. The most common means to measure cytotoxicity is to assess cell membrane integrity. Compounds that possess cytotoxic effects often disrupt the integrity of the cell membrane. Dyes like trypan blue and propidium iodide are generally excluded from the healthy cells' interiors; however, if the cell membrane is damaged, they can freely pass it and stain intracellular components. Similarly, the flow of chemicals ordinarily sequestered inside cells to the outside can be used to assess the cell membrane integrity. Cytotoxicity can be studied using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) or the MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. MTT assay is one among the most widely used assays to assess cell viability and differentiation. The basis of MTT assay, a colorimetric assay is the ability of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase to convert the MTT dye to water insoluble formazan, which yields a purple color. The purple colored insoluble formazan thus yielded is solubilized using solubilization solutions such as acidified ethanol solution or dimethyl sulfoxide or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid to form a colored product, whose quantification is done by measuring its absorbance at a range of 500-600 nm wavelength using a spectrophotometer.

Thus, cytotoxicity assays finds use in screening newly developed drugs to assess their cytotoxic effects, if any, in order to get rid of such unwanted side effects of the drugs. Also, cytotoxicity assay is done to look for cytotoxic compounds, which may be used to develop a therapeutic that targets cancer cells. Cell lines often used in cytotoxicity assays include WRL-68 cell, HeLa cells, Chinese hamster ovary (CHO) cells, ACHN cell, corneal epithelial cells, canine renal cells, lung fibroblasts and microorganisms.

In this thesis, cytotoxicity study of indole-3-methanol, ticlopidine and their inclusion complexes towards normal liver cell line WRL-68 and human kidney cancer cell line ACHN has been assessed by MTT assay.

II.2.20. Reactive Oxygen Species Generation Study :

Reactive oxygen species (ROS) are extremely reactive, oxygen-containing, short-lived molecules that participate in cell signaling. ROS are involved in a number of biological activities, which depends on the concentration of ROS. In case of tumor cells, ROS plays a dual role in either suppressing or promoting tumor formation. However, the role is concentration dependent. At low to moderate concentrations, ROS acts as a secondary messenger and mediates cell proliferation and differentiation. However, the exceeding rise in the levels of R0S has deleterious effects on proteins, nucleic acids, lipids, membranes and organelles, thereby blocking cell cycle and finally leading to cytotoxicity and apoptosis (cell death). Numerous therapeutic drugs for cancer is based on the induction of apoptosis by the generation of signal molecules like ROS. Thus, DNA metabolism, DNA replication-related enzymes and the apoptotic pathway serves as the chief target for developing cancer chemotherapeutics.

In this study, ROS production in kidney cancer cell line (ACHN) treated with TCP and TCP-β-CD complex was studied. Generation of intracellular ROS was determined using a fluorescent probe, 2, 7-dichlorodihydrofluorescein diacetate (DCFDA). In the presence of free oxygen radicals, DCFDA undergoes oxidation to 2, 7-dichlrofluorescein (DCF), which fluoresces and can thus be visualized using a fluorescent microscope.