CHAPTER I

INTRODUCTION

This chapter gives a background on supramolecular and solution chemistry. The chapter mainly focuses on total overview of cyclodextrin-based supramolecular assembly, its wide range of applications especially in drug delivery and different biological applications and various molecular interactions taking place between ionic liquid and amino acids in aqueous medium. The general features of cyclodextrin-based inclusion complexes, their characterization and the importance of their hybrids are discussed along with the main objectives of the thesis. Finally, the chapter briefs about the motivation of our research work, scope, objectives and applications.

NECESSITY OF THE RESEARCH WORK

I.1. Objective, Scope and Applications of the Research Work

Research is conducted to contribute to science through the systematic collection, interpretation and evaluation of data. Research creates new knowledge and understanding. The world is developing through the research undertaken by humans. Our environment is made up of matter and living organisms, so most of the research work is confined to the group exploring the interactions between the matter and the living organisms surrounding it.

The term supramolecular chemistry was first introduced by Jean-Marie Lehn who received Nobel Prize for his extensive work in this field in 1987 along with Pedersen and Cram [1]. It is an emerging field in chemistry, also in material science, which can be defined as the chemistry of molecular assemblies as well as the chemistry of non-covalent bonds. The fundamental of supramolecular chemistry was first described in 1894 when the *lock and key principle* was recognized by Fischer [2]. In the 1930s, the discovery of the aggregation of molecules via intermolecular interactions led to the coining of the term 'supramolecule'. During the 1950s, extensive works on cyclodextrins by Cramer; in the 1960s on the host-guest complexes of crown ether compounds by Pederson and Cram on spherands, cavitands; and recently box-like container molecules discovered by Stoddart, speed up research in the field of supramolecular chemistry [3,4].

In recent years, supramolecular chemistry has been divided into three broad categories; (a) host-guest chemistry (b) clathrates and (c) self-assembly depending on

size and shape. In host-guest chemistry, host molecules also known as cavitands are molecular entities that possess permanent intramolecular cavities (e.g., cyclodextrins, calixarenes, and cucurbiturils) to encapsulate guest molecules [5]. Clathrates are lattice-structured complexes when two or more host molecules cause a gap in between, thus generating an extra molecular cavity. Self-assembly is another supramolecular entity where two molecules, neither belong to the typical descriptions of host nor the guest.

One unique benefit of supramolecular complexes is their ability to clarify the presence and bounds of binding energy additivity, which is given in most applications, such as sensible medication design. An additional benefit is that several interactions typically contribute to supramolecular complexes, and a single association step already pays the loss of translation entropy for each intermolecular relationship. The van der Waals forces of contact, hydrogen bonding interactions, π-π stacking, electrostatic interactions, and hydrophobic interactions are among the non-covalent forces that stabilize the inclusion complexes [6]. Molecular self-assembly, molecular recognition, host-guest chemistry, molecular machineries, and dynamic covalent chemistry are just a few of the topics covered in the broad subject of supramolecular chemistry.

Figure 1: Schematic illustration of host-guest supramolecular inclusion complex

Supramolecular Host-Guest chemistry concerns about non-covalent binding or complexation between a host and a guest as depicted in **Figure 1**. The host is commonly defined as a large molecule or aggregate such as an enzyme or synthetic cyclic compound that possesses a sizeable, pre-organized central hole or cavity such as CDs, calix[n]arenes, crown ethers, etc. [7,8]. The guest may be an organic or inorganic cation, a simple inorganic anion, an ion pair, or a more complicated organic molecule such as an anticancer drug [9,10]. Our mother nature is also full of natural host-guest systems

including antigen-antibody, DNA-ligand, enzyme-substrate, and protein-carbohydrate complexes.

The advancement of supramolecular chemistry has been aided by macrocycle–based host-guest chemistry [11]. The macrocyclic hosts include cyclodextrins, calixarenes, crown ethers, cucurbiturils, and other macrocycles [12,13]. Because of their amphiphilic nature, cyclodextrins (CDs) are especially intriguing in this context [14]. Amphiphiles are of interest because they can self-assemble in aqueous systems to produce well-defined structures including micelles, vesicles, nanorods, nanotubes, and sheets that can be used in drug delivery, nanodevices, and cell imaging, among other disciplines [15,16]. Since they greatly enhance the assemblies' conductance, electrical, fluorescence, catalytic, and thermal qualities, cyclodextrin-modified nanoparticles have drawn a lot of attention in recent years. This has improved the assemblies' potential uses as drug delivery systems and nanosensors [17, 18]. As a result, a wide range of complex probes have been created for use in the production of molecular switches, supramolecular polymers, chemosensors, transmembrane channels, logic gates based on molecules, and other intriguing host-guest systems [19–20].

Cyclodextrins (CDs) are derived from starch and contain six (α-CD), seven (β-CD), eight (γ-CD), or more (α-1,4)-linked α-d-glucopyranose units. They are nontoxic nanocarriers and are also known as cyclic oligosaccharides [21]. The outer surface of CDs is hydrophilic in nature whereas internal cavities of cyclodextrins are relatively hydrophobic. For both polar and nonpolar guests including small molecules and different drug molecules, CDs can act as hosts [22]. Due to the binding properties of CDs, it has the stability to form stable inclusion complexes with several biologically active compounds, and for health products ranging from drugs to food compounds [23,24]. The host molecules in this case are α-cyclodextrin and β-cyclodextrin, which have six and seven glucopyranose units, respectively. These are chosen because of their high inclusion efficiency, small size, affordability, and minimal toxicity [25]. The pharmaceutical, food, and cosmetics sectors, as well as tissue engineering and biomedical devices, have all discovered extensive uses for CDs [26]. To extend the light, air, and thermal stability of various bioactive molecules, enzymes, medications, volatile organic compounds, flavors, essential oils, flavonoids, vitamins, and other compounds, as well as to improve their water solubility, bioavailability, and side effect protection, inclusion complexation is used within the non-polar cavity of CDs [27].

In supramolecular chemistry, if macrocycles are termed as pillars, then calixarenes are regarded as the third pillar after the well-investigated cyclodextrins and crown ethers. Calixarenes are viewed as one of the most significant classes of macrocyclic host compounds [28]. Calixarenes are the condensation product of Phenol and aldehyde as many aromatic compounds can be derived from phenol, resorcinol, or pyrogallol [29,30]. Because of the flexible hydrophobic cavity, they can make rim modifications to incorporate specific guest molecules such as drugs, vitamins, and metal ions [31,32]. The *p*-sulfonatothiacalix[4]arene (TSC4X) which is a derivative of calixarene, is composed of phenolic groups linked by the 'S' atom at the 2 and 6 positions. The sulfonated calixarenes from the calixarene family are less toxic, sufficiently soluble in water & and possess high stability [33-35]. It has been explored that, the TSC4X compound finds application in the field of smart materials, drug delivery, chemical sensors, and molecular recognition owing to their nontoxic behavior and high selectivity and affinity for various kinds of guests in aqueous medium [36,37]. π-π stacking, electrostatic and hydrophobic interactions provide stability when various guest molecules are complexed with TSC4X [38]. Hence, the fundamental investigations involving the interactions of sulfonated calixarenes with different types of guests are important for their advanced applications.

Pharmacologists nowadays are very concerned with the stability and controlled release of pharmaceuticals. Examining whether medication molecules may be encapsulated within the cyclodextrin molecule is crucial for protecting them from environmental impacts and mitigating side effects for their regulated release. Therefore, to accomplish this goal, research has been done on the complex formation of bioactive guest molecules with the host molecule, α-cyclodextrin (α-CD), such as mephenesin (MEP) and 1-butyl-2,3-dimethylimidazolium tetrafluoroborate [Bdmim]BF_4 ionic liquid (IL).

In this thesis, the encapsulation of MEP into α-CD cavity has been investigated. One of the most important drugs from the family of glycerol ether is MEP or (3-(2-methylphenoxy)propane-1,2-diol). It is also regarded as a blockbuster drug and is known as centrally acting skeletal muscle relaxant [39]. It is a colorless, odorless, crystalline solid soluble in ethyl alcohol and propylene glycol [40]. The specific action of MEP on spinal interneurons owes to the abolition of polysynaptic reflex contractions and unaltered monosynaptic knee-jerk reflexes [41,42]. Pharmacological investigations of the properties of mephenesin have disclosed that this compound possesses both muscle paralyzing and anticonvulsant properties [43]. Mephenesin has insignificant local

anaesthetic action in vivo but has a prominent local anaesthetic action in vitro and local infiltration is also observed [44-46]. Furthermore, the cytotoxicity of mephenesin and its inclusion complex was tested for its in vitro anticancer activity against the human renal adenocarcinoma cell line (ACHN).

Recently, the field of supramolecular chemistry has received a lot of attention, particularly with regard to host-guest interactions. Cyclodextrin (CD) is the most prominent possible host among them all [47]. Ionic liquids are generally organic salts, mainly containing organic cations and polyatomic inorganic anions and are liquids at temperatures below 100 degrees Celsius. The low vapor pressure of ionic liquids is one of their most important properties [48]. Furthermore, by careful selection of anionic and cationic components, their physical and chemical properties can be tuned. ILs are called a safer alternative to volatile organic solvents for the environment. Ionic liquids [IL] have been developed as a more environmentally friendly alternative to volatile organic solvents [49]. Although widely used as a solvent, IL is currently being used in a variety of industries including electrochemistry, catalysis, spectroscopy, and more. Ionic liquids are commonly referred to by various other names also, such as molten salts, synthetic solvents, neoteric solvents [50]. Inorganic anions and organic cations make up the majority of ionic liquids. Ideally, the cation should be asymmetric, i.e. the alkyl groups should be different to be liquid at room temperature. The polarity and hydrophilic/hydrophobic nature of ionic liquids can be attuned by selecting the right combination of anions and cations. Ionic liquids have earned the title of "designer solvent" [51] due to these types of properties. In this article, 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate [Bdmim]BF_4 is currently gaining attention in production due to its wide recyclability and ease of solubility at room temperature, thus making it a green solvent. Complexes including CD-IL have many industrial applications for the stability against various atmospheric hazards and have many applications without any type of chemical modification [52-55]. We explored the formation of host-guest inclusion (IC) complexes of IL[Bdmim]BF_4 with α-CD, specifically for formation, stabilization, transport, and controlled release without chemical modification by various reliable techniques such as ^{1}H NMR, FT-IR, UV-visible spectroscopy, DSC, SEM, and XRD analysis.

Ionic liquids are generally synthesized from imidazolium-based cations and highly fluorinated anions, irrespective of that they are also obtained from salts, sugars, amino acids, and biomolecules that exist in nature, many of them used as pharmaceutical additives [56]. Ionic liquids are used as designer solvents in many inorganic and biocatalytic reactions [57] and 'green' alternatives for volatile organic solvents. They can

be used as heat transfer fluids in processing biomass, as conductive liquids in batteries, solar cells and also in analytical equipment. They make up electrolytes in lithium-ion batteries, super capacitors and metal plating baths [58,59]. Benzyltributylammonium chloride (BTBACl) and Benzyltriethylammonium chloride (BTEACl) are water soluble and in many biphasic organic transitions such as in the agrochemicals, polymer and pharmaceutical industries, can be used as phase transfer catalyst. Density, viscosity, conductance, refractive index, surface tension etc. are the important parameters to measure the physicochemical properties of ionic liquids [60].

Amino acids, the structure block of proteins are often taken as the model compounds to study the consequence of additives on proteins, as the physicochemical properties and interactions of proteins cannot be measured directly due to their complex conformation and configuration [61]. Due to their central role in biochemistry, amino acids are important in nutrition and are commonly used in food technology and industry. They are quite helpful in understanding the various interactions in solutions. L-phenylalanine, $C_9H_{11}NO_2$ is an essential amino acid. It is the only form of phenylalanine found in proteins. Major dietary sources of L-phenylalanine include meat, fish, eggs, cheese, and milk. Phenylalanine is most commonly used for a skin disorder that causes white patches to develop on the skin (vitiligo). L-tryptophan, $C_{11}H_{12}N_2O_2$ is an essential amino acid that is necessary for making proteins. It is naturally found in red meat, poultry, eggs, and dairy. L-tryptophan is important for many organs in the body. L-tryptophan is not made by the body and must be consumed from the diet [62, 63].

The solute–solvent interaction between BTBACl, L-Phenylalanine and L-Tryptophan in aqueous media plays a significant role in the optimization of several important biotechnological processes. L-phenylalanine and L-tryptophan (two solute molecules) interact with an ionic liquid (Benzyltributylammonium chloride) in an aqueous medium. Based on the different parameters such as apparent molar volume, viscosity B-coefficient, molar refraction, molar conductance at different temperatures and different concentrations, the molecular level interactions have been explained from density, viscosity, refractive index and conductance measurements. Using the Masson equation, the experimental slopes and the limiting apparent molar volumes are obtained which explain the solute-solute and solute-solvent interactions. Hepler's technique and dB/dT values have been used to examine the structure-making and structure-breaking nature of the solutes in the solvents. Viscosity parameters, A and B obtained from the Jones-Doles equation explained the solute-solute and solute-solvent interactions in the

solution. Lorentz-Lorenz equation has been used to calculate the molar refraction. The behavior of many other bio-molecules can be explained by considering amino acids as a model and the mechanism has been extended to elucidate the behaviour of other (biological) systems.

Vitamin C (Ascorbic acid) $C_6H_8O_6$, with molar mass 176.13 g/mol is white to pale-yellow water-soluble vitamin. Our body does not store it but it is supplied from citrus fruits, broccoli and tomatoes for the growth and repair of tissues in all parts of our body. It is in a class of medications called antioxidants. It is needed by the body to help heal wounds, to enhance the absorption of iron from plant foods and to support the immune system. It helps the body to make collagen, an important protein used to make skin, cartilage, tendons, ligaments and blood vessels. It plays an important role in several metabolic functions including the activation of the vitamin B, folic acid, the conversion of cholesterol to bile acids and the conversion of the amino acid, tryptophan to the neurotransmitter, serotonin. Humans, unlike most animals, are unable to synthesize Vitamin C endogeneously, so it is an essential dietary component [64].

An attempt has been made to provide an interpretation of solute-solvent and solute-solute interactions prevailing in Vitamin C (Ascorbic acid) in 0.001m, 0.003m and 0.005m aqueous Ionic liquid systems of Benzyl tributyl ammonium chloride (BTBACl) and Benzyltriethylammonium chloride (BTEACl) at 298.15K, 308.15K and 318.15 K through density, viscosity, conductance and surface tension measurements. The determination of the behavior of ion associations and solvation processes that associate in solution is achieved from conductivity measurements.

I.2. Choice of Biologically Active Molecule, Host Molecules, Ionic Liquids, Food Preservatives and Solvents Used in the Research Work

Below is a list of the names of the host molecules, solvent molecules, amino acid molecules, ionic liquids, and biologically active molecules-

Biologically Active Molecules:

- Mephenesin
- Ascorbic acid

Host Molecule:

- α-cyclodextrin

Ionic Liquids:

- Benzyltributylammoniun chloride
- Benzyltriethylammonium chloride
- 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate

Amino Acids:

- L-phenylalanine
- L-tryptophan

Solvents:

- Water
- Ethanol

I.3. Methods of Investigations Used in the Research Work

Name of the Investigation Methods are listed below:

- UV-Vis Spectroscopy
- Differential Scanning Calorimetry (DSC)
- Powder X-Ray Diffraction (PXRD)
- Scanning Electron Microscopy (SEM)
- FTIR Spectroscopy
- Thermogravimetric Analysis (TGA)
- ^{1}H-NMR Spectroscopy
- Surface Tension Study
- Conductivity Study
- Density Study
- Viscosity Study
- Refractive Index Study
- Antimicrobial Activity
- MTT Assay

CHAPTER II

This chapter primarily contains a preview of previous literature. Further, this chapter explores the theory of investigations that are carried out during experiments.

REVIEW OF THE EARLIER WORKS AND THEORY OF INVESTIGATION

II.1. Review of the Earlier Works

Host-guest chemistry, as used in supramolecular chemistry, refers to complexes made up of two or more molecules or ions that are joined in unique structural relationships by forces other than covalent bonds [1,2]. The idea of molecular interactions and recognition through non-covalent bonding is included in host-guest chemistry. Non-covalent bonding plays a crucial role in preserving the three-dimensional structure of large molecules, such proteins and nucleic acids, and is involved in a variety of biological processes where large molecules temporarily but selectively link with one another. Four common categories are used to classify these non-covalent interactions: hydrophobic interactions, ionic bonds, van der Waals forces, and hydrogen bonds.

Molecular self-assembly, molecular folding, molecular recognition, host-guest chemistry, mechanically-interlocked molecular structures, and dynamic covalent chemistry are among the key ideas developed by supramolecular chemistry. Understanding the various biological processes that depend on non-covalent interactions for structure and function is essential. Supramolecular research frequently draws inspiration from biological systems. It is possible to think of the "host" component as the bigger molecule, which contains the smaller "guest" molecule. The corresponding concepts of host and guest in biological systems are often referred to as substrate and enzyme, respectively.

In order to better understand the combinatorial result of these numerous, tiny, non-covalent forces that are combined to produce an overall effect on the supramolecular structure, chemists are making a comprehensive effort to measure the energy and thermodynamic properties of these non-covalent interactions found throughout supramolecular chemistry.

The interaction between the host and guest molecules results in a decreased total Gibbs free energy, which underlies the thermodynamic benefits of host-guest chemistry.

It is crucial to comprehend the thermodynamics of host-guest binding in order to develop artificial systems that carry out specific tasks. The exchange of energy involved in different binding interactions is the focus of chemists, who are working to develop scientific experiments using a variety of techniques, including UV-visible spectroscopy, NMR spectroscopy, differential scanning calorimetry, thermogravimetric analysis, and surface tension, to quantify the fundamental roots of these non-covalent interactions [3]. In order to quantify and clarify the experimentally acquired data, the binding constant (K_a), enthalpy (ΔH^0), entropy (ΔS^0), and Gibb's free energy (ΔG^0) are analyzed.

According to S. Giuffrida et al. a host-guest inclusion combination involving α-cyclodextrin and platinum acetylacetonate in a water solution can be readily exposed to visible light in order to produce tiny and stable platinum nanoparticles in a single step. The most noteworthy benefits provided by this synthetic process are the exclusive control of the reaction by an external trigger, the elimination of the unwanted reaction products without modifying the sample, and the lack of ionic repulsions between the metal nanoparticles [4].

Inclusion complexes of cyclodextrins with nonpolar pharmaceuticals, as reported by V. Crupi et al. are a subject of current interest in pharmaceutical science because they improve the chemical stability, bioavailability, and aqueous solubility of weakly water-soluble medicines [5].

A host-guest interaction-based conjugation of β-cyclodextrin modified polyethylenimine (PEI-CD) and adamantyl peptide (AdGRGDS) resulted in the simple and targeted gene delivery system described by Y. X. Sun et al. The PEI-CD/AdGRGDS gene delivery system demonstrated good DNA binding capability and good ability to compact DNA into uniform spherical nanoparticles thanks to the rational design between AdGRGDS and PEI-CD [6].

β-cyclodextrin barrels were introduced by W. C. E. Schofield et al. These barrels can be attached to solid surfaces by the Williamson ether synthesis reaction, which involves the deposition of an intermediate poly(4-vinylbenzyl chloride) linker layer by pulsed plasma. The process of loading and releasing scent molecules involves the

development of a host-guest inclusion complex with β-cyclodextrin linked to the surface [7].

The interaction of the painkiller Isoxicam, which is a member of the oxicam group of nonsteroidal anti-inflammatory medicines (NSAIDs), and its copper complex with several cyclodextrins (β-CD, γ-CD, HP-β-CD, and HP-γ-CD) has been studied in both the solid and solution states, as reported by S. Goswami et al. [8].

According to M. Gangopadhyay et al., there are notable variations in the formation of inclusion complexes between a recently synthesized triphenylamine derivative and two distinct macrocyclic hosts, cucurbit[7]uril and β-cyclodextrin [9].

A supramolecular method for creating self-powered micropumps was introduced by D. Patra et al. It relies on the molecular recognition of "host-guest" between transazobenzene and R- and β-cyclodextrin. The micropumps were designed using hydrogels and surface coatings based on host-guest pairs as scaffolds. These soft micropumps respond to two different stimuli and can be activated by adding guest molecules or light. Furthermore, reversible host-guest interaction can be used to recharge the micropumps [10].

The controlled self-assembly of multiple-responsive SAP is explained by Z. Du et al. [11]. This is based on the selective host-guest inclusion of β-cyclodextrin with a modified poly(ethylene glycol) that has an azobenzene block, a poly(ethylene glycol)methyl ether chain, a ferrocene end group, and a C11 alkyl chain.

A supramolecular method to increase the fluorescence intensity of coumarin dye by interacting with the relatively new host cucurbit[7]uril (CB[7]) was presented by M. Gupta et al. [12]. The virtually nonfluorescent coumarin was converted into a highly fluorescent entity in water upon addition of the nonfluorescent host CB[7].

Through molecular recognition between calixarene and α-CD based pseudorotaxane including azobenzene and binaphthyl moieties, R. Sun et al. successfully synthesized a light-driven, supramolecular polymer [13]. Through non-covalent host-guest molecule recognition, supramolecular polymerization has been accomplished.

Novel host-dye complexes consisting of calixarenes as hosts and lucigenin (LCG) as the fluorescent guest were the basis for the chemosensor applications that Guo et al. reported [14]. The choline oxidase and acetylcholinesterase enzyme assays were set up

using these sensing devices, allowing for the screening of enzymatic inhibitors and the precise determination of the absolute quantities 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 co-assemblies using amphiphilic calixarene and two drugs, mitoxantrone.HCl and irinotecan.HCl. The anchoring of the surface of these co-assemblies 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. van der Waals Forces

The distance-dependent van der Waals forces are relatively weak interactions between molecules or atoms. When the distances between interacting molecules increase, the van der Waals force becomes comparatively small and eventually disappears. van der Waals force magnitude is dependent on molecule surface area, and van der Waals force magnitude increases with increasing molecule surface area.

van der Waals force plays a crucial role in a wide range of disciplines, including surface science, supramolecular chemistry, polymer science, structural biology, and nanotechnology. It also clarifies the creation of molecular solids, certain characteristics of organic compounds, and the mechanism underlying the solvolysis of a solute molecule in both polar and non-polar environments. The London dispersion force between immediately generated dipoles and all intermolecular forces are frequently included in the definition of the word "van der Waals force."

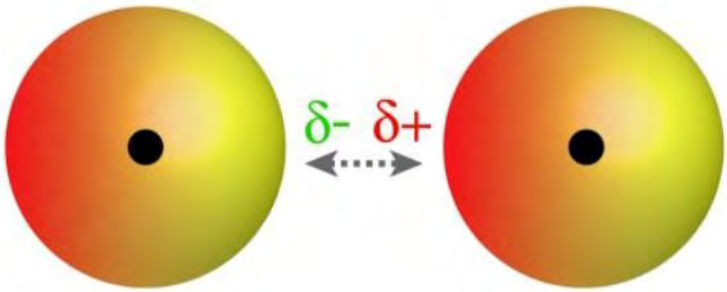

Figure 1: van der Waals forces acting between molecules

The van der Waals contact distance is the distance at which the force between atoms becomes repulsive as opposed to attracting as they approach one another; the phenomenon is developed by the electron clouds of the atoms restraining one another.

II.2.2. Hydrogen Bonds

A hydrogen bond occurs when a lone pair on an electron-rich donor atom, such as an atom of nitrogen (N), oxygen (O), or fluorine (F), and hydrogen (H) atoms, partially interacts with other molecules to form a bond between them.

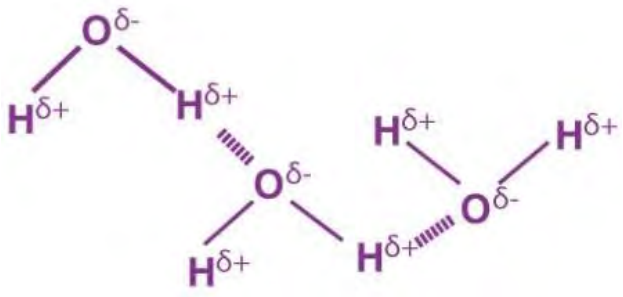

Figure 2: Hydrogen bonding in water molecules

Intermolecular hydrogen bonds, which are bonding interactions between two different molecules, and intramolecular hydrogen bonds, which are bonding interactions between sections of the same molecule, are the two different forms of hydrogen bonds. Depending on their geometry, the makeup of the donor and acceptor atoms that make up the bond, and the surrounding conditions, the bonding energy can range from 1 to 40 kcal/mol.

They become somewhat more robust than a van der Waals contact as a result, but they still fall short of fully covalent or ionic connections. Both organic molecules like DNA and proteins and inorganic ones like water are known to contain this kind of connection. Water's boiling point is raised to 1000°C by hydrogen bonding between its molecules, which also distinguishes it from other Group-16 hydrides. Moreover, proteins and nucleic

acids' secondary and tertiary structures result from intramolecular hydrogen bonding. It is also essential to the structure of natural and manmade polymers.

II.2.3. Hydrophobic Interactions

The phrase "hydrophobic effect" refers to the propensity for nonpolar compounds to aggregate in an aqueous solution while disregarding water molecules. The term "water-hating" refers to the process of separating water molecules from nonpolar substances. This optimizes the hydrogen bonding interaction between water molecules, hence reducing the contact area between water and nonpolar molecules. The hydrophobic effect is the thermodynamic shift in the free energy of water molecules surrounding a solute. Hydrophobicity is shown by a positive free energy change in the surrounding solvent, while hydrophilicity is implied by a negative free energy change.

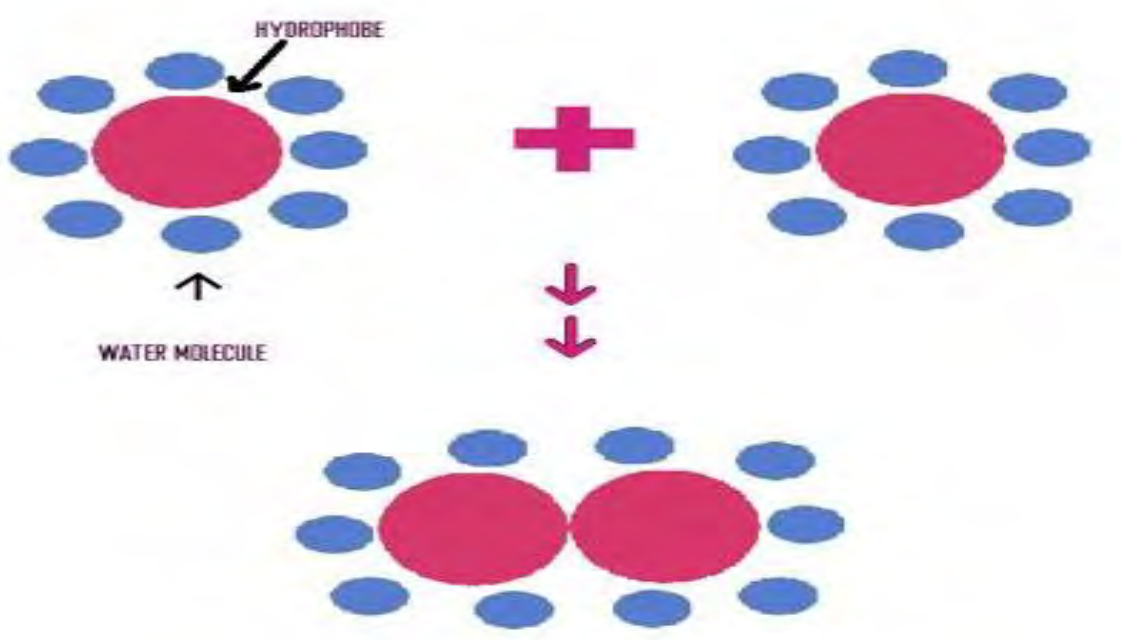

Figure 3: Hydrophobic interaction brings the interacting molecules closer

Numerous biological processes, including the creation of cell membranes and vesicles, the insertion of membrane proteins into nonpolar lipid environments, protein folding, and the interactions between proteins and small molecules, are regulated by the hydrophobic effect. By decreasing the intermolecular distance and adding water to the mixture, we can occasionally induce a response between two non-interacting molecules by using the hydrophobicity idea.

II.2.4. Electrostatic Forces

The electrostatic force, which is defined as the force between two stationary, electrically charged particles, is governed by Coulomb's law. Electrostatic force, also known as Coulomb force, is the term used to describe the electrical force that exists between two charged things at rest. The strength of the electrostatic force between stationary charges is always described by Coulomb's law.

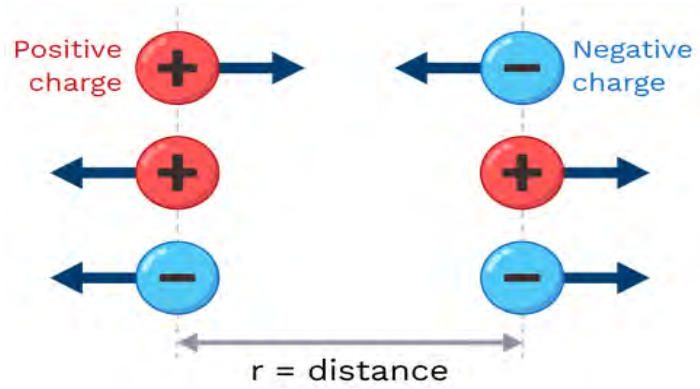

Figure 4: Electrostatic force working between charged species

The electrostatic force acts in the bond direction and is directly proportional to the product of the charge magnitudes and inversely proportional to the square of the distance between them. While opposite charges attract one another, the same charges repel one another.

II.2.5. Dipole-Dipole Attractions

The attraction interactions between the positive ends of two polar molecules and their respective negative ends are known as dipole-dipole forces. The intensities of dipole-dipole forces vary from 5 kJ to 20 kJ per mole. Its strength is weaker than that of ionic or covalent bonds, therefore it only really matters when the molecules are near to one another.

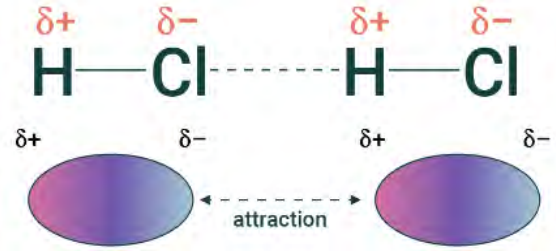

Figure 5: Dipole-Dipole attraction between the polar molecules

II.2.6. Ion-Dipole Interaction

The term "ion-dipolar interaction" refers to an electrostatic interaction between a charged ion and a dipolar molecule. It is essentially an attracting force that is shown to be dominant in solutions, namely in ionic compounds that dissolve in polar liquids. The anti-part of a polar molecule is attracted to a cation or an anion. Ion-dipole attractions grow stronger as the polar molecule's dipole magnitude increases or as the ion's charge increases. In many chemical situations, these interactions become critical difficulties.

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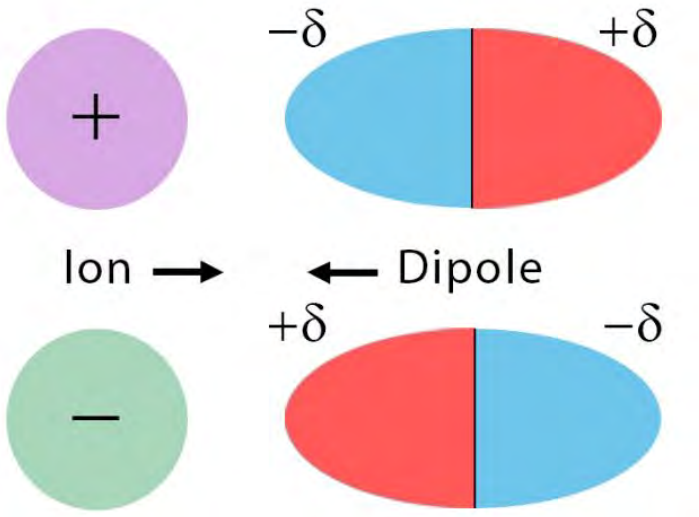

Figure 6: Ion–Dipolar attraction between the ion and polar molecules

II.2.7. Solute-Solvent Interactions

The study of a homogenous mixture made up of two or more components is known as solution chemistry. The term "solute" refers to a substance that is present in minute quantities and dissolves into a larger quantity of another substance, which is known as the solvent and determines the final phase of the solution. One of the most important aspects of solution chemistry during solution preparation is the solute-solvent interaction. A solute can dissolve in a certain solvent molecule when there is a strong

solute-solvent interaction. One of the crucial variables is a solution's concentration, which expresses how much solute is contained in a specific volume of solvent or solution. An aqueous solution is one in which water serves as the solvent.

II.2.8. ^{1}H-NMR Spectroscopy

Nuclear magnetic resonance spectroscopy, or NMR spectroscopy, is the most reliable spectroscopic technique for analyzing an organic molecule out of all of them. By separating many hydrogen atoms with distinct magnetic environments, it gives us information on the local magnetic environment surrounding the atomic nucleus. In addition, it provides us with information on the quantity of hydrogen atoms in each of the many magnetically distinct settings.

The sample is put in a magnetic field, and sensitive radio receivers pick up the nuclear magnetic resonance (NMR) signal that is created when radio waves excite the sample's nuclei. Details about a molecule's electronic structure and functional groups can be accessed by altering the resonance frequency due to the variations in the intramolecular magnetic field surrounding an atom. NMR spectroscopy is the most common method for identifying monomolecular organic compounds in modern organic chemistry practice because the fields are distinct or highly typical of particular molecules. In a similar vein, proteins and other complicated compounds are identified by biochemists using NMR. NMR spectroscopy not only identifies molecules but also offers comprehensive details about their dynamics, structure, reaction state, and chemical environment. Proton and carbon-13 NMR spectroscopy are the two most popular forms of NMR spectroscopy; nevertheless, they are only applicable to samples containing spin-wielding nuclei.

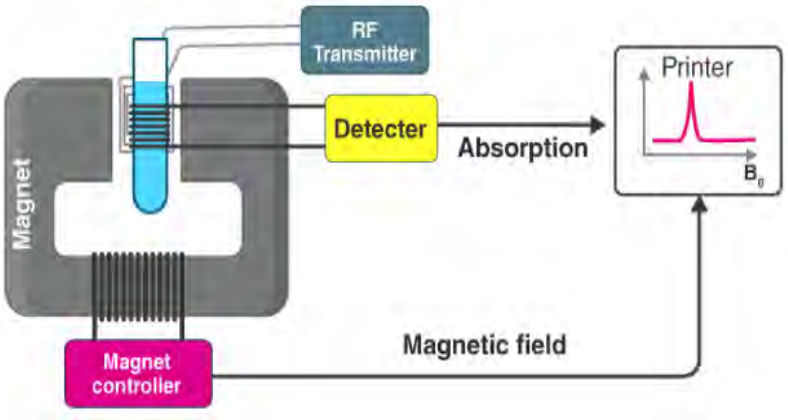

Figure 7: Schematic representation of an NMR instrument

NMR's extended timeframe makes it unable to see swift events, which results in an average spectrum. Large amounts of contaminants cannot be detected on an NMR spectra. Higher sensitivity can therefore be achieved by applying a stronger external magnetic field.

Chemical Equivalence

Some protons within a molecule present in chemically identical environments are said to be chemically equivalent. These chemically equivalent protons usually show the same chemical shift. A molecule possessing a set of protons that are chemically distinct from each other may give different absorption peaks, and such protons are chemically non-equivalent. 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

We can examine peak intensity and the quantity of various protons with the use of software. In the most basic NMR investigations, the number of protons is merely proportional to the strength, or integral, of the NMR signal. This is because we can readily estimate the signal intensity and, consequently, the number of hydrogen atoms present in that location by integrating the peak area. The integral of the signals in the case of carbon-13 NMR spectra is also dependent on the nucleus's relaxation rate as well as the scalar and dipolar coupling constants. However, insufficient knowledge about that means that complex system components continue to provide challenges.

Chemical Shift

Protons with various magnetic surroundings register their signals at different places in the 1H-NMR spectrum, depending on the nature of those environments. Though the signals' positions in the spectrum may change depending on how strong the external magnetic field is. An NMR signal is typically reported using a reference signal, which is typically the TMS (tetramethylsilane) signal. In addition to creating a local magnetic field and an opposition to the external magnetic field, the electron density surrounding a nucleus also acts as a shield against the external magnetic field. The 1H-NMR spectra then show an upfield shift. Currently, a proton that is close to an electronegative atom loses

electron density and experiences an external magnetic field, which causes the proton to shift downfield in the 1H NMR spectrum. Excited states, among other considerations, exert a substantial influence on the chemical changes for heavier nuclei.

In this study, the interacting protons from the host and guest molecules move as inclusion complexes develop, indicating that the host and guest protons are shielded paramagnetically or diamagnetically.

II.2.9. FTIR Spectroscopy

Since infrared (IR) spectroscopy measures bond stretching and bending rather than any atomic attribute, it offers a direct method of viewing these functional groups. Because IR spectroscopy is so effective at identifying the stretching of unsymmetrical bonds, such those present in functional groups like OH, C=O, NH_2, NO_2, etc., it is a wonderful addition to NMR as a structural investigation technique.

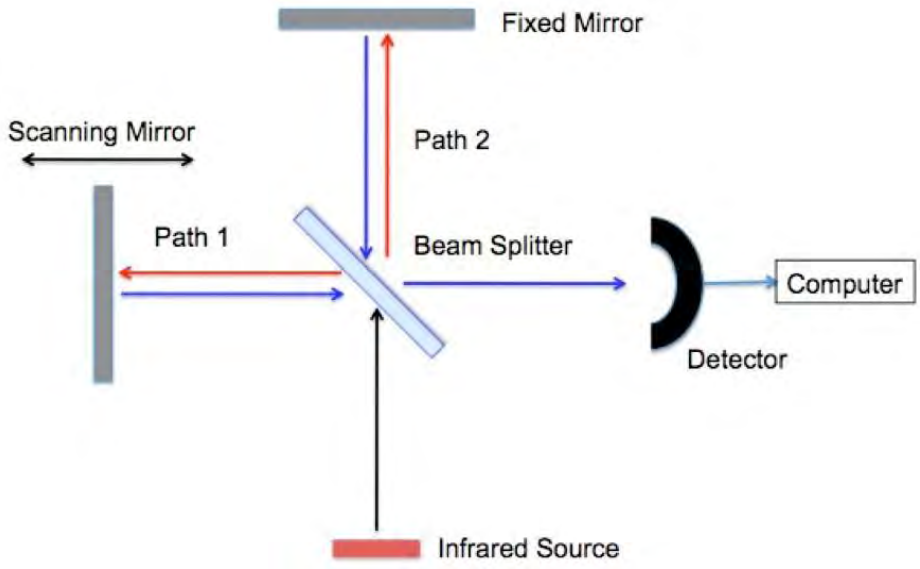

Figure 8: A schematic diagram of a Fourier Transform Infrared (FTIR) Spectrophotometer

The two atoms' bonds are thought of as a spring that vibrates similarly to a basic harmonic oscillator. The mass of the atoms, m_1, m_2, that the bond is holding together as well as the bond's strength (force constant K) determine the vibration frequency. The Hook's law states that the vibration frequency can be stated as follows:

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}} \qquad \text{(II.1)}$$

Where, μ is the reduced mass of the molecular system and can be expressed as follows-

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \quad \text{(II.2)}$$

The force constant K signifies the bond strength.

The Infrared Spectrophotometer

A tool used to ascertain a compound's absorption spectrum is an infrared spectrophotometer. Compound spectra across the frequency range of 4000-400 cm^{-1} are provided by this device.

Preparation of Samples

We prepared a palette by combining a small amount of sample with a big amount of dried KBr, and then we recorded all of the FTIR spectra in the solid state. The conditions of host-guest interaction are supported by the interpretation of the infrared spectroscopic data of the ICs as well as the pure host and guest molecules, which validates the validity of the process by which the ICs are generated.

II.2.10. UV-visible Spectroscopy

Molecules with different electronic energy levels can move from a lower energy state to a higher energy state that exhibits a large peak in the UV-visible spectrum. UV or visible light can be absorbed by electrons at different energy levels, both bonding and non-bonding, to excite them to higher anti-bonding molecular orbitals. The molecule will be excited at the longer wavelength of light more readily if the energy difference between the HOMO and LUMO is smaller. π–π*, n–π*, and σ–σ* are the four types of transitions that could occur, depending on the energy gap order. σ–σ* > n–σ* > π–π* > n–π*

According to the Lambert-Beers' law, UV-visible spectrometer shows the absorbance of a sample and records the spectrum.

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

A = Absorbance

I_0 = Intensity of incident light

I = Intensity of the light leaving sample

ε = Molar absorptivity

c = Molar concentration of solution containing sample

l = Length of cuvette containing sample

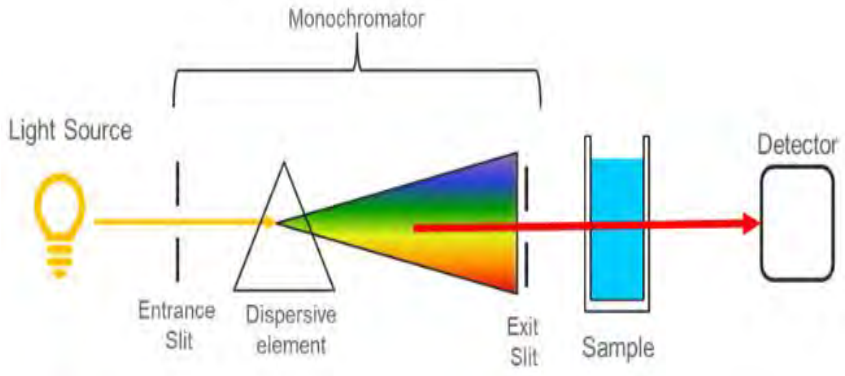

Figure 9: Schematic diagram of UV-visible spectrophotometer

In this thesis, the binding constants of the inclusion complexes generated by the cyclodextrin with different guest molecules were calculated using the Benesi-Hildebrand equation and the UV-visible spectroscopy data. With the use of the information obtained from the UV-visible spectroscopy, the stoichiometry of the inclusion complexes was also established.

II.2.11. Differential Scanning Calorimetry (DSC)

One important tool that helps us examine how a sample's heat changes as a result of temperature changes is the DSC. By examining the shift in melting point that registers a peak other than the melting temperature in the DSC thermograms, we can occasionally use this information to detect the presence of impurities or changes in crystal structure. About the thermal stability of the sample being studied, many thermodynamic parameters can also be acquired.

Heat Flux Type and Power Compensation Type are the two varieties of DSC. The sample and reference holder, heat sink, heating resistor, and heater are all part of the Heat Flux DSC system. Through the use of a heat resistor and heat sink, the heater provides heat to both the sample and the reference. Heat flow and the heat differential between the heat sink and holders are proportionately related. The sample's heat capacity is less than that of the heat sink. Heat sinks adjust for the sample's endothermic or exothermic processes. Maintaining a steady temperature differential between the

reference and sample is beneficial. The temperature differential between the two holders is directly correlated with the variation in heat supply to the sample and the reference. Quantitative measurement of the unknown sample is made possible through standard material calibration.

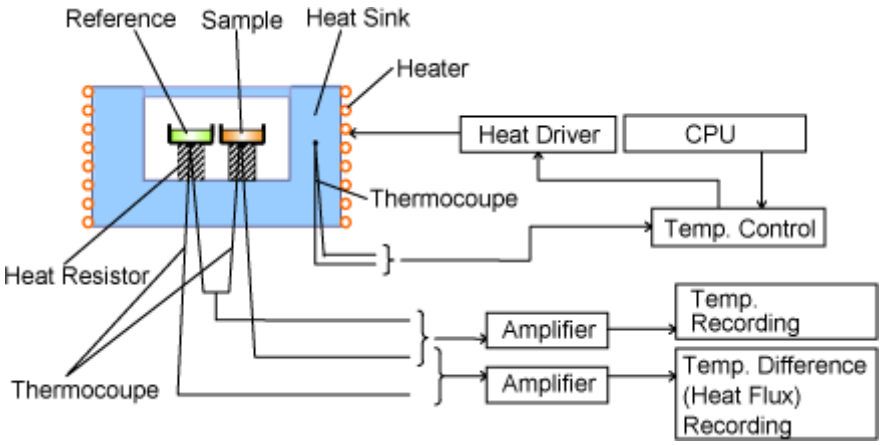

Figure 10: Diagrammatic representation of the working principle of DSC

The physicochemical condition of the guest upon encapsulation within the cyclodextrin cavity has been examined in this research. DSC thermograms typically show that the presence or absence of an endothermic peak for the pure guest molecule in the inclusion complexes, or the shifting of an endothermic peak to a different temperature, signifies a change in the melting point, crystal lattice, or sublimation point brought on by inclusion complexation.

II.2.12. Thermogravimetric Analysis

One kind of thermal analysis technique that monitors sample mass as temperature changes is thermogravimetric analysis (TGA). Many materials undergo deterioration and then lose volatile components, exhibiting mass change as a function of temperature. This occurrence may provide additional insights on the materials being tested.

Using a precise temperature program, a sample is subjected to TGA in a selected environment. A thermogravimetric curve is produced when a sample's mass is measured at regular intervals throughout the temperature profile. This allows for the plotting of mass against temperature. This characteristic curve shows how a sample decomposes thermally.

The thermal stability of the inclusion complex and the free guest molecules have been investigated and compared in this research effort using TGA.

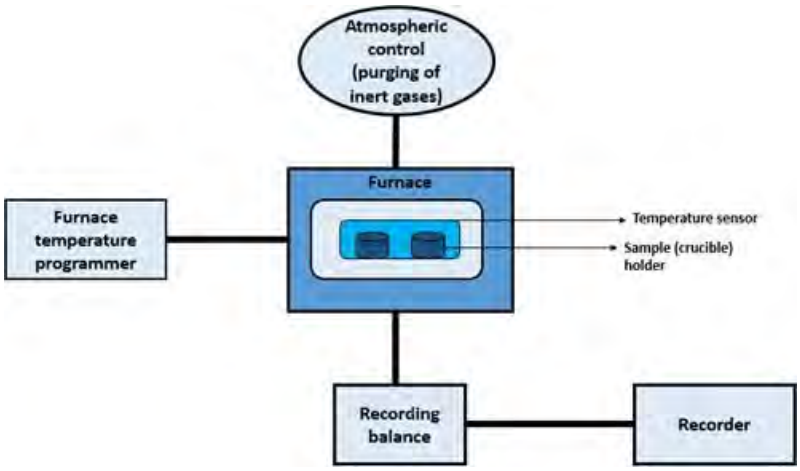

Figure 11: Schematic diagram of TGA instrument

II.2.13. Powder X-Ray Diffraction (PXRD)

PXRD is a rapid analytical technique that can yield information on unit cell dimensions and is mostly used to identify the phase of crystalline materials. The material under analysis is homogenized, finely powdered, and its average bulk composition is ascertained.

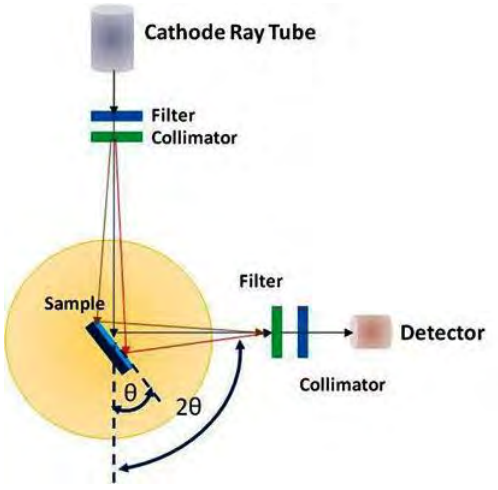

Figure 12: Diagrammatic representation of the working principle of PXRD

It was discovered that the plane spacing in a crystal lattice is similar to the wavelength of an X-ray, and that crystalline materials work as a three-dimensional diffraction grating for X-rays. Nowadays, XRD is widely used to determine crystal structures and atomic spacing. When light is scattered by a periodic array with long-range order, diffractions

occur, resulting in constructive interference at a particular angle. The crystalline nature of a substance can be explained by keeping an eye on the relationship between the wavelengths of X-rays and the distance between two atoms.

While meeting the requirements of Bragg's Law, the interactions between the incident rays and the sample result in constructive interference-

$$n\lambda = 2d\sin\theta \qquad \text{(II.3)}$$

Where d is the inter-planar spacing, θ is the Glancing angle, and λ is the wavelength of the X-ray that was employed.

After the sample has been exposed to X-rays produced by the X-ray tube, the diffracted X-rays are identified, handled, and tallied. Data were collected at various 2θ angles.

II.2.14. Scanning Electron Microscopy (SEM)

A concentrated beam of high-energy electrons is utilized in the Scanning Electron Microscope (SEM) to create an image of the material with a high energy resolution. Primary electrons accelerate and strike the material, producing secondary electrons (SE).

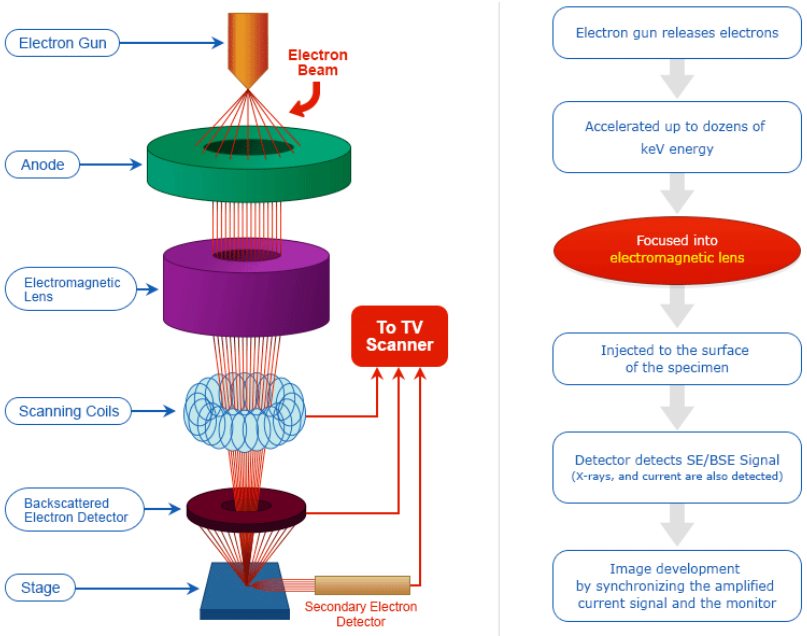

Figure 13: Diagrammatic representation of working principle of SEM

These secondary electrons are made up of a positively charged electron detector, which produces a three-dimensional image of the material. The signals resulting from the interactions between the electrons and the sample provide information about the

exterior morphology, crystalline structure, chemical composition, and orientations of the constituent materials of the sample. A region's breadth can be imaged via SEM scanning, from around 1 cm to 5 microns. Additionally, selected points and places on the sample can be analyzed using the SEM.

II.2.15. Molecular Docking Study

The process of forecasting a ligand's preferred orientation to a target's binding site when the two are joined together to create a stable adduct is known as molecular docking. Based on their preferred orientation, ligands and targets can be predicted to have a given binding affinity or degree of interaction. Different potential adduct conformations from molecular docking are ranked and organized using the scoring method. The lowest energy and most favorable docked conformer is selected based on the docking score.

The most popular technique in structure-based drug design is molecular docking, which may be used to anticipate how a ligand would attach to the right binding site on a target. Determining the binding behavior is important for both rational medication design and the understanding of basic biological processes.

Molecular docking studies have been carried out in this thesis to estimate potential binding modes and the free energy of interaction between various guests and cyclodextrin.

II.2.16. 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 period. Numerous pathogenic microorganisms are used as indicator strains 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 1-butyl-2,3-dimethylimidazolium tetrafluoroborate [Bdmim]BF_4, and its inclusion complex has been investigated considering some commonly known pathogens, which includes Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli*, *Salmonella* sp. and *Pseudomonas* sp. by agar well diffusion technique.

II.2.17. 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 colour. The purple coloured 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 coloured product, whose quantification is done by measuring its absorbance at a range of 500-600 nm wavelength using a spectrophotometer.

Thus, cytotoxicity assays find 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 Mephenesin (MEP), and its inclusion complex towards human kidney cancer cell line ACHN has been assessed by MTT assay.

II.2.18. Density Measurements

"Density" as a function of weight, volume mole fraction, and surplus volumes of mixing are contained in the volumetric information. It's a well-accepted technique for analyzing molecular interactions in liquids. The solute molecules' molar volume varies based on the type of solvent. Thus, we may monitor the type of interaction occurring between the solute and solvent in solution by determining the apparent molar volume of a solution system. In this case, volumetric information is quite important.

Apparent Molar Volumes

The geometric volume of the two solute molecules combined during solvation via solute-solvent interaction with the co-solvent is the apparent molar volume of a material in solution. One can compute the molar volume of a pure material using density data. It is challenging to calculate the volume that an ion added in one mole of a solvent, though.

This is because, upon entering the solvent, the ions cause the solvent's volume to change as a result of electrostriction—the compression of the solvent caused by the ions' electric field—and the disruption of the solvent structure close to the ions. When there

are electric fields of the order of 109–1010 V m^{-1}, electrostriction occurs, and there is probably going to be a large compression of ions and molecules. A directly obtained quantity-apparent molar volume (ϕ_v) can be used to calculate the partial molar volume, or effective volume, of an ion in solution. The following relation can be used to compute the solutes' apparent molar volumes, (ϕ_v):

$$\phi_v = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0} \qquad \text{(II.4)}$$

Where, M represents the molar mass of the solute, m is the molality of the solution, and ρ and ρ_0 represent the densities of the solution and solvent respectively.

Limiting Apparent Molar Volumes

Limiting apparent molar volume (ϕ_v^0) is the apparent molar volume at infinite dilution. Least squares fitting of linear plots of (ϕ_v) against the square root of molar concentrations ($m^{1/2}$) using the Masson equation gives the values of limiting molar apparent volume (ϕ_v^0) and experimental slopes (S_v^*).

$$\phi_v = \phi_v^0 + S_v^* . \sqrt{m} \qquad \text{(II.5)}$$

The solute-solvent interaction occurring in the solution is often indicated by the values of the limiting molar apparent volume (ϕ_v^0), which are always positive in all situations. Conversely, experimental slopes (S_v^*) indicate that there is a solute-solute interaction in the solution.

It was discovered throughout this study project that the experimental slope (S_v^*) which indicates the degree of ion-ion contact in the solution, has negative values, suggesting that there is less ion-ion interaction in the medium. The comparison shows, that the greater magnitude of ϕ_v^0 than S_v^*, it recommends that the ion-solvent interactions are more predominant over ion-ion interactions.

Structure Making/Structure Breaking Interaction

There are two types of solute-solvent interactions that have been researched so far: synergistic structure-making interactions and structure-breaking interactions. Hepler's

approach is useful for analyzing the type of solute-solvent interaction that occurs during the solution phase.

In this regard, the data were fitted using the following polynomial equation to get the limiting apparent molar volumes of solutions:

$$\phi_v^0 = a_o + a_1 T + a_2 T^2 \qquad \text{(II.6)}$$

where, a_0, a_1 and a_2 are the empirical coefficients depending on the environment of solute, and mass fraction (W) of co-solvent. T represents temperature in Kelvin scale.

First derivative of equation (II.6) gives the values of limiting apparent molar expansibilities (ϕ_E^0) which have been calculated for various temperatures.

$$\phi_E^0 = (\delta\phi_v^0 / \delta T)_P = a_1 + 2a_2 T \qquad \text{(II.7)}$$

Positive values of limiting apparent molar expansibilities (ϕ_E^0) suggests the absence of caging or packing effect in the solutions.

According to Hepler, values of $(\delta\phi_E^0/\delta T)_P$ in the expression given below, determines whether, it is structure breaker or structure maker interaction:

$$(\delta\phi_E^0/\delta T)_P = (\delta^2\phi_v^0/\delta T^2)_P = 2a_2 \qquad \text{(II.8)}$$

Based on the above expression, it has been stated that $(\delta^2\Phi_v^0 / \delta T^2)_P$ value is positive for structure-making solutes and negative for structure-breaking solutes. In this research work, the negative values of L-Tryptophan (**Table 10 b, Chapter V**) show that it acts as a structure-breaker solute and the positive values of L-Phenylalanine (**Table 10 a, Chapter V**) show that it acts as a structure-maker solute at 0.005m aqueous IL, and ascorbic acid solutions in Benzyl triethylammonium chloride (BTEAC) act as a better structure breaker than in Benzyl tributylammonium chloride (BTBAC) (**Table 6a, 6b, Chapter VII**)

II.2.19. Refractive Index Measurements

Refractive index or optical data, of electrolyte mixes offer useful details about the structure and molecular interactions of the solutions in addition to supplementary

information on useful processes like estimating other properties or measuring concentration.

The speed of light in a vacuum divided by the speed of light in another medium is known as the index of refraction (n_D) for a certain substance.

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

Light changes its speed when it crosses a boundary from one medium into another. The relationship between speed of light 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-

$$\frac{V_A}{V_B} = \frac{sin\theta_A}{sin\theta_B} = \frac{n_B}{n_A} \tag{II.9}$$

It is possible to calculate a sample's refractive index without having to measure its light speed. 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 precisely determine the refractive index of the sample as a stand-in.

The refractive index of mixing can be correlated by the application of a composition-dependent polynomial equation. Molar refractivity, was obtained from the Lorentz-Lorenz relation by using n_D, experimental data according to the following expression-

$$R_M = \frac{(n_D^2 - 1)}{(n_D^2 + 2)}\left(\frac{M}{\rho}\right) \tag{II.10}$$

where, R_M, n_D , M and ρ represents molar refraction, refractive index, molar mass and density of solution, respectively.

The limiting molar refraction can be calculated using the following equation-

$$R_M = R_M^0 + R_S\sqrt{m} \tag{II.11}$$

where, 'm' is the molality of solution and R_M^0 is the limiting molar refraction that indicates solute–solvent interaction. Therefore, this measurement operates as an exclusive tool for studying the molecular interaction in solution.

II.2.20. Viscosity Measurement

There are several different types of viscosity correlations in electrolytic solutions. Due to the fact that the solution involves ion-ion and ion-solvent interactions, it is challenging to separate the associated forces. However, thorough examination allows for the drawing of strong and reliable conclusions on the composition and kind of solvation of the specific system. Since viscosity is a measurement of the friction between parallel planes in a liquid that are near to each other and move relatively quickly, any change in the interaction between the planes will alter the friction, which in turn will alter the viscosity. Thus, while keeping an eye on the viscosities of the solution, we also address the different interactions that occur in the solution between the solute and the solvents.

Viscosity A- and B- Coefficients

The liquid's planes will close together when a huge spherical is submerged in it, raising the viscosity. Comparably, an increase in the average degree of hydrogen bonding between the planes will cause the planes to rub against one another more, which will increase viscosity. For an ion that promotes structure, a sizable rigid co-sphere will act as a rigid sphere submerged in liquid, increasing inter-planar friction.

In a similar vein, an ion raising the degree of correlation or hydrogen bonding between neighboring solvent molecules will raise the viscosity. On the other hand, the viscosity would drop if ions destroyed the correlation. An empirical equation quantitatively connecting the molar concentrations (c) and the relative viscosities of the electrolytes was proposed by Jones and Dole in 1929 is-

$$\frac{\eta}{\eta_o} = \eta_r = 1 + A\sqrt{c} + Bc \qquad \text{(II.12)}$$

The above equation can be rearranged as-

$$\frac{\eta_r - 1}{\sqrt{c}} = A + B\sqrt{c} \qquad \text{(II.13)}$$

where the constants A and B stand for the interactions between ions and solvents, respectively. The equation has been widely utilized and is equally relevant to aqueous and non-aqueous solvent systems when there is no ionic connection. The term A√c, originally recognized as Grüneisen effect, arose from the long-range columbic forces between the ions. The significance of the term had since then been realized due to the development of Debye-Hückel theory of inter-ionic attractions in 1923. The Falkenhagen Vernon equation, which derives the A-coefficient from interionic attraction theory, can be used to determine the ion-ion interactions.

$$A_{Theo} = \frac{0.2577\Lambda_o}{\eta_o(\varepsilon T)^{0.5}\lambda_+^o\lambda_-^o}\left[1 - 0.6863\left(\frac{\lambda_+^o\lambda_-^o}{\Lambda_o}\right)^2\right] \qquad \text{(II.14)}$$

where, the symbols have their usual significance.

The plots of $(\eta/\eta_o - 1)/\sqrt{c}$ against $\sqrt{c}$ for the electrolytes should give the value of A- and B-coefficient. But, sometimes, the values come out to be negative or considerably scatter with deviation from linearity. Thus, the Falkenhagen-Vernon equation is typically used to determine the A-coefficient rather than obtaining it from the plots or using the least squares method. Non-electrolytes should have an A-coefficient of zero. Jones and Dole suggest that the stiffening effect of the electric forces between the ions, which tend to retain a space-lattice structure, is likely represented by the A-coefficient in the solution.

The ion-solvent interaction parameter is represented by the viscosity B-coefficient, which can be either positive or negative. Using the least squares method, the B-coefficients are found as the slopes of the straight lines with intercepts equal to the A-values.

The Factors Affecting Viscosity B-Coefficients

(1) η or B-value increases as a result of ionic solvation and the ion field's ability to produce long-range order in solvent molecules.

(2) The breakdown of solvent molecules' three-dimensional structure, also known as the structure breaking effect, results in a drop in η values.

(3) High B-values for comparable solvents are produced by a low dielectric constant and a high molal volume.

(4) When one of the ions in a binary electrolyte cannot be precisely solvated, or when the primary solution of ions is sterically inhibited in large molal volume solvents, reduced B-values are obtained.

Temperature Dependence of Viscosity B-Coefficient

In both aqueous and non-aqueous solvents, regularity in the behavior of B and dB/dT has been noted, and Kaminsky has provided helpful generalizations. He noted that: (i) the B-ion values within a set of periodic tables drop as the crystal ionic radii grow; and (ii) the temperature co-efficient of B_{Ion} values within a group of periodic systems increases with the ionic radius. The following is a summary of the findings:

(i) A and dA /dT >0

(ii) $B_{Ion} < 0$ and $dB_{Ion} / dT > 0$, characteristic of the structure breaking ions.

(iii) $B_{Ion} > 0$ and $dB_{Ion} / dT < 0$, characteristic of the structure making ions.

An improvement of the viscosity B-coefficient in forecasting the kind of solute-solvent interaction as a structure-maker or structure-breaker is the first derivative of viscosity B-coefficient over temperature. The activation energy needed for the viscous flow in solution is measured by the value of dB/dT. For this reason, rather than the sign or size of the B-coefficient, the measure of dB/dT is indicative of the ability to create or break structures. Structure-maker (kosmotropic) is indicated by a negative or tiny positive value of dB/dT, while structure-breaker (chaotropic) is indicated by a greater positive value.

In our research work, it has been seen that the small positive value of dB/dT signifies L-Phenylalanine as structure-maker (kosmotropic), whereas the larger positive value of dB/dT identifies L-Tryptophan acts as a structure-breaker (chaotropic) in aqueous Benzyltributylammonium chloride solution.

II.2.21. Surface Tension Measurement

A liquid takes on the shape with the least surface area due to the attraction force that molecules below exert on its surface molecules, tending to drag the surface molecules into the liquid's bulk.

Surface tension values are decreasing with increase in concentration of the amino acids. The sign and extent of the limiting slopes ($\partial\sigma/\partial m$) of surface tension with reference to concentration are associated with the hydrophobic or hydrophilic character of the solute because it reflects the sort of interaction predominant on the surface. The amino acids have negative ($\partial\sigma/\partial m$) values, which are not the characteristics of electrolytes and very polar hydrophilic compounds.

II.2.22. Conductivity Measurement

An electrolyte solution's conductivity, often known as its specific conductance, is a measurement of its electrical conductivity. Conductivity is measured in Siemens per meter, or S/m, in SI units. In numerous industrial and environmental applications, conductivity measurements are a quick, affordable, and accurate method of determining the ionic content of a solution. For instance, one common method for tracking and continually trending the operation of water purification systems is the measurement of product conductivity.