# CHAPTER I

## INTRODUCTION

*This chapter gives a background on supramolecular and solution chemistry. The chapter mainly focuses on total overview of cyclodextrin and calix[4]arene 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 and calix[4]arene based inclusion complexes, their characterization and the importance of their hybrids are discussed along with 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 conducted for the purpose of contributing towards science by the systematic collection, interpretation and evaluation of data. Research creates new knowledge and understanding. We human beings are doing research as the development of the world made. The world made up of matters and living organisms, so most of the research work confined into the group exploring matter – living organism interactions.

The term supramolecular chemistry was first introduced by Jean-Marie Lehn and 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 bond. The fundamental of supramolecular chemistry was first described in 1894 when the *lock and key principle* 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 1950s, extensive works on cyclodextrins by Cramer, Pedersen in 1960s on the host-guest complexes of crown ether compounds 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 is divided into three broad categories; (a) host-guest chemistry (b) clathrates and (c) self-assembly depending in 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 complex when two or more host molecules cause gap in between thus generate an extramolecular cavity. Self-assembly is another supramolecular entity where two molecules, which neither belongs to the typical descriptions of host nor guest.

A particular advantage of supramolecular complexes is that they allow elucidation of the existence and the limitation of additivity of binding energies, which is inherently assumed in most applications, e.g., for rational drug design. Another advantage is that, in typical supramolecular complexes, several interactions contribute, and the loss of entropy of translation for any intermolecular association is already paid by a single association step. Several non-covalent forces stabilize the inclusion complexes, which are (a) Van der waals forces of interactions (b) Hydrogen Bonding interaction (c) π-π stacking (d) Electrostatic interactions (e) hydrophobic interactions [6]. Supramolecular chemistry is a wide field which discussed about different aspects include molecular self-assembly, molecular recognition, host-guest chemistry, molecular machines and dynamic covalent chemistry.

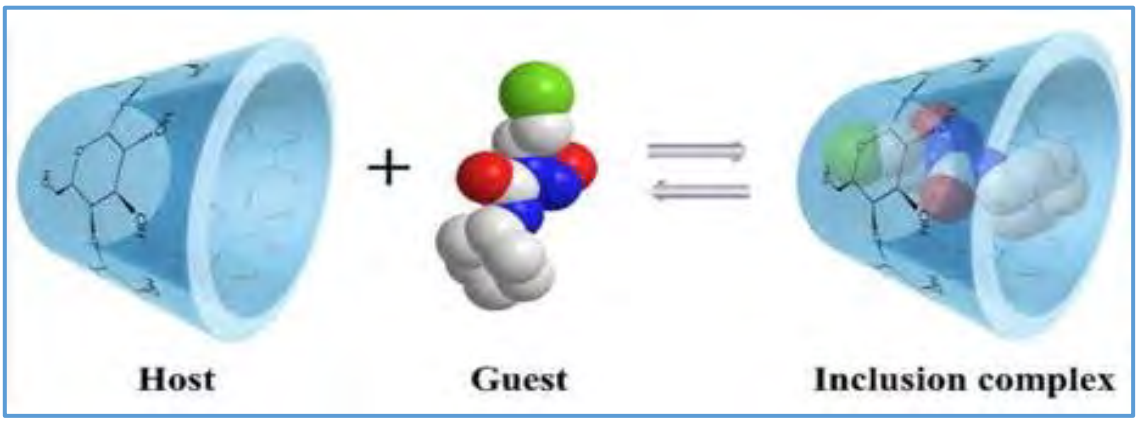


**Figure 1: Schematic illustration of host-guest supramolecular inclusion complex**

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 possess 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 anticancer

drug [9,10]. Our mother nature is also full of natural host-guest systems include 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]. The cyclodextrins (CDs) are particularly interesting in this regard because of their amphiphilic character [14]. The interest in amphiphiles arises from their self-assembly in aqueous systems to form well-defined structures, such as nanotubes, nanorods, nanosheets, micelles and vesicles, that can be applied in various fields including drug delivery, nanodevices and cell imaging [15,16]. In recent years, cyclodextrin-modified nanoparticles have received a lot of attention as they significantly improve the properties of the assemblies, such as conductance, electronic, fluorescence, catalytic and thermal properties, improving the potential applications of these assemblies as nanosensors and drug delivery vehicles [17,18]. Consequently, a variety of sophisticated probes have been designed for applications in the manufacturing of molecular machines, molecular switches, supramolecular polymers, chemosensors, transmembrane channels, molecule based logic gates, and other interesting 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 and also they are nontoxic nanocarrier 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 guest including small molecules and different drug molecules CDs can be act as host [22]. Due to the binding properties of CDs, it has the stability to form stable inclusion complexes with several biologically active compounds, for health products ranging from drugs to food compounds [23,24]. Herein, α-cyclodextrin and β-cyclodextrin bearing 6 and 7 glucopyranose units, respectively, have taken as host molecules due to high inclusion efficiency, fitting cavity dimensions, low price, and negligible toxicity [25]. The CDs have found widespread application in pharmaceuticals, food industries, cosmetics [26], tissue engineering, bio-medical devices. Inclusion complexation within the non-polar cavity of CDs is employed for protecting the hydrophobic part of different bioactive molecules, enzymes, drugs, volatile organic compounds, flavors, essential oils, taxols, flavonoids, vitamins [27], and etc. to extend

their light, air and thermal stability, enhancement of water solubility, bioavailability and shielding side effects.

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 class 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 have ability to make rim modification to incorporate specific guest molecules such as drug, vitamin & metal ions [31,32]. The *p*-sulfonatothiacalix[4]arene (TSC4X) which is derivative of calixarene, composed of phenolic groups linked by 'S' atom at the 2 & 6 positions. The sulfonated calixarenes from calixarene family are less toxic, sufficiently soluble in water & possesses high stability [33-35]. It has been explored that, TSC4X compound finds application in the field of smart materials, drug delivery, chemical sensors, molecular recognition owing to their nontoxic behaviour & high selectivity and affinity for various kind of guests in aqueous medium [36,37]. π-π stacking, electrostatic & hydrophobic interacts 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.

The stabilisation and the controlled release of the drugs now days, are of great concern in pharmacology. To guard drug molecules from environmental effects and to reduce the side effects for their controlled release it is vital to investigate whether they can be encapsulated into the cyclodextrin molecule. Thus to complete such aim, the inclusion complex formation of bioactive guest molecules such as Mephenesin (MEP), 6-Propyl-2-thiauracil (PTU), Riboflavin (RIBO), Nile blue (NB) with host molecules viz. α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and *p*-sulfonatothiacalix[4]arene (TSC4X) have been studied to achieve the goal.

In this thesis the encapsulation of MEP into β-CD cavity has been investigated. One of the most important drug from the family of glycerol ether is MEP known as (3-(2-methylphenoxy)propane-1,2-diol), also regarded as the blockbuster drugs known as centrally acting skeletal muscle relaxants [39]. It is a colourless, odourless, crystalline solid soluble in ethyl alcohol and propylene glycol [40]. The specific action of MEP on

spinal interneurons owing 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 also observed [44-46]. Thus, to protect and stabilize this important bioactive MEP molecule from external effects (i.e., temperature changes, light and acidic environment) and to increase the aqueous solubility of Mephenesin for minimizing the doses in human body, with this aim MEP is complexed with β-CD. The interaction of MEP and MEP + βCD inclusion complex with Calf thymus DNA and BSA have also been studied separately.

Hyperthyroidism is one of the major disease found in human beings. When the thyroid gland produces excess of triiodothyronine (T3) and thyroxine (T4) thyroid hormones causing hyperthyroidism [47,48]. There are some inhibitors known as antithyroid agents may be effective to control these processes [49,50]. Antithyroid agents are basically thiourea-based (thionamides) compounds with a thione moiety containing a heterocyclic structure. Some important and useful antithyroid agents are 6-propyl-2-thiouracil (PTU), methimazole (MMI), 6-methyl-2-thiouracil (MTU), carbimazole (CBZ) etc. [51,52]. The patients who medicate with irradiation i.e those who are suffering from Hodgkin's disease having high risk of thyroid cancer, Grave's disease and hypothyroidism as well after the treatment for them radioprotective substances are very useful [53,54]. It has been noticed that among the radioprotective substances, PTU and methylthiouracil can protect the thyroid gland from radiation injury in both man and rat [55]. Like many radioprotective substances, this thioureylene drug (PTU and thiourea) having simple chemical structure holding sulphur atom [56]. Thus, the presence of such properties lead them to be used as radioprotectors. Although PTU is an effective drug but it has some side effects too like severe idiosyncratic toxicity, hepatitis, skin reactions, aplastic anaemia and cholestasis [57]. In USA, PTU is considered to be the third most frequent source of drug induced liver transplant. Also PTU is light, air sensitive and has less solubility in water. Therefore, in order to overcome such problems related to PTU and increase its efficacy, stability as a drug molecule the encapsulation of PTU with α-cyclodextrin (α-CD) is employed.

Early detection of disease having several advantages induce considerable interest in biomedical field specifically in case of breast cancer, specific tumour diagnosis [58]. Initially, an exogenous contrasting agent is being used as optically imaging biomarkers that are unique to specific cell types at the onset of a disease [59]. Biomarkers are basically small molecule dyes which are used to determine an abnormal growth of cells present in the body. Fluoroprobes are generally fluorescent dye that are being developed in order to improve the selective imaging sensitivity in the near-infrared (NIR, 700-900 nm) spectrum, a wavelength region of low absorptivity by tissue chromophores as well as deeper tissue penetration [60,61]. This helps to reduce intrinsic background interference with detectable fluorescence signal intensities through several centimetres of tissue [62,63]. Nile blue (NB) is a class of benzophenoxazine derivative dye which has the ability to show sharp and strong absorption in the near-infrared (NIR) region [64,65]. It is a cationic dye and readily water soluble and show intense fluorescence. Because of its intense fluorescence and good photostability, it has been anticipated for wide applications such as metal ion sensors and in long-wavelength fluorescent biological labelling probe and as photosensitizers in photodynamic therapy (PDT) [66,67]. The photostability of a fluorescent dye molecule depends on how it can protect itself from being biodegradation. Such protection is often accomplished by the employing of a drug carrier system, which not only enhances the molecule's water solubility but also its stability under physiological conditions [68,69]. In this context, unlike cyclodextrins, calixarene as well as thiacalixarene have emerged as carrier systems having ability to bind guest molecules such as metal ions, drug molecules in their cavities [70,71]. Thus, to improve NB chemical/photostability, enhance its bioactivity and fluorescence imaging capability, the encapsulation of host molecule TSC4X with guest molecule Nile blue has been carried out and the most probable inclusion mechanism amplified by nuclear magnetic resonance ($^1$H-NMR), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA) methods and DFT approach. The binding characteristics of NB-TSC4X with BSA has been investigated employing spectroscopic techniques to understand the interaction and also to determine the binding affinity of NB-TSC4X towards BSA.

Riboflavin (RIBO) which is 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine belongs to the Flavin family [72], commonly known as vitamin B2 . RIBO is very essential compound

in order to initiate major cellular process, especially it is associated with various redox process & biological $e^{-1}$ transport. RIBO is extensively utilized in the field of medicine and food chemistry [73]. RIBO subsists as orange-yellow crystal which is stable towards heat, acid or oxidation but it appears to be highly sensitive towards light especially UV radiation of sunlight. Vitamin B2 can easily be absorbed in body and mixed with blood, which supply it to the tissues. But if anyone consume excess RIBO, it will be excreted from body through urine in the form of other metabolites such as 7-hydroxymethyl riboflavin & lumiflavin, as they are water soluble substances [74,75]. RIBO is a nontoxic compound approved by FDA, stored in the liver & kidney of humans with very small quantity. RIBO also shows its medicinal effect to those who are suffering from visionary related issue (like eye burning, excess tearing), growth of the cells, skin, hair etc. Riboflavin-5-phosphate can be assimilated readily instead of RIBO itself for the people suffering from allergies & chemical sensitivities [76]. The alcohol related problems, digestive difficulties, ulcers, leg pain can also be eradicated with the help of RIBO. During cancer treatment doctors often prescribe RIBO supplements to the patients [77]. Recent observation reveals that humans do not consume sufficient RIBO despite having accessible RIBO rich foods. The deficiency in RIBO intake may causes several health related problems such as sore tongue, cheilosis, scaly rashes on scrotum or vulva [78,79]. Its deficiency also causes night blindness, lake of growth, mild anaemia & fatigue. The scarcity of RIBO may also increases the risk of cancer & cardiovascular like major diseases [80]**.** Generally, RIBO has poor aqueous solubility and high photosensitivity, as a result its medicinal & biological activity get reduced. Therefore, in order to enhance the aqueous solubility, photo stability & biological activity of RIBO, it is encapsulated with *p*-sulfonatothiacalix[4]arene (TSC4X), a special type of host molecule belongs to Calixarene class compounds [81]**.**

Ionic liquids (ILs) are the special class of substances that have substantial ability to provide advances in various biomedical fields, as well as the formulation of biologics proposed for delivery into the human body [82]. Ionic liquids are generally synthesised from imidazolium-based cations and highly fluorinated anions, irrespective of that they also obtained from salts, sugars, amino acids, and biomolecules that exist in nature- many of them used as pharmaceutical additives [83]. In many inorganic and biocatalytic reactions they are used as designer solvents [84] and “green” alternative for volatile

organic solvents. They can be used as heat transfer fluids in processing biomass, as conductive liquids in batteries and solar cells and also in analytical equipment. They make up electrolytes in lithium-ion batteries, super capacitors and metal plating baths [85,86]. Benzyltributylammonium chloride (BTBACl) is 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 etc. are the important parameters to measure the physicochemical properties of ionic liquids [87]. 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 [88]. L-Proline is found as major amino acid and it is non-essential for humans, as it can automatically be synthesized in our body from the non-essential amino acid L-glutamate. It is found to be a major amino acid in cartilage and manifest significant role in repairing of muscle, skin damage and connective tissue as well as essential for the immune system. Proline is inimitable in that it is the only amino acid in which a five-membered ring containing nitrogen atom is formed by connecting the side chain to the protein backbone twice [89,90]. L-Valine is an α-amino acid soluble in water, essential for humans which means our body cannot synthesize it and must be received from dietary sources such as meats, dairy products, soy products, beans and legumes. L-Valine stimulates muscle growth and regeneration and is involved in energy production. L-Valine is used as food additive and an essential amino acid for vertebrates [91,92].

The solute –solvent interaction between BTBACl and L-Valine/L-Proline in aqueous media plays a significant role for the optimization of a number of important biotechnological processes. L-Valine and L-Proline (two solute molecules) interact with an ionic liquid (Benzyltributylammoniumchloride) in 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 from density, viscosity, refractive index, conductance measurements have been used to explain the molecular level interactions which was supported by $^{1}$H-NMR and UV-vis studies. Using 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 Jones-Doles equation explained the solute-solute and solute-solvent interactions in the solution. Lorentz-Lorenz equation has used to calculate the molar refraction. Further the findings have been supported by NMR study of the solutions and also considerable amount of theoretical analysis has been done which was in good agreement with the experimental result. The behaviour of many other bio-molecules can be explained by considering amino acids as model and the mechanism has been extended to elucidate the behaviour of other (biological) systems.

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

Names of the Biologically Active Molecules, Host Molecules, Ionic Liquids, Amino Acids and Solvent molecules are listed below-

**Biologically Active Molecule:**

- Mephenesin
- 6-Propyl-2-thiouracil
- Riboflavin
- Nile blue

**Host Molecules:**

- α-cyclodextrin
- β-cyclodextrin
- *p*-sulfonatothiacalix[4]arene

**Ionic Liquids:**

- Benzyltributylammoniun chloride

**Amino Acids:**

- L-Valine
- L-Proline

**Solvents:**

- Water
- Ethanol
- Dimethyl sulfoxide

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

Name of the Investigation Methods are listed below:

- UV-vis Spectroscopy
- Fluorescence 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
- Antioxidant Activity