CHAPTER I

NECESSITY OF THE RESEARCH WORK

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

The field of supramolecular chemistry was awarded in 1987 when Jean-Marie Lehn, Charles J. Pedersen and Donald J. Cram acquired the Nobel Prize for their significant work in this area.[1] It is a rapidly growing field in chemistry, which deals with the chemistry of non-covalent bond. In 1894, the lock and key principle suggested by Emil Fischer introduces the philosophical roots of supramolecular chemistry.[2] The discovery of the molecular aggregation through intermolecular interactions in 1930s led to the invention of the word supramolecule. Extensive works by Cramer on cyclodextrins during 1950s, Pedersen on crown ether based host-guest complexes in 1960s, Cram on spherands, cavitands, and recently Stoddart on box like container molecules advances the research in the area of supramolecular chemistry.[3,4]

In recent years, supramolecular chemistry is categorized into three broad types : (i) clathrates, (ii) self-assembly and (iii) host-guest chemistry. Clathrates are complexes where guest molecules with appropriate size are encapsulated into the cavities of a lattice of host molecules.[5] In host-guest chemistry, guest molecules are accommodated into the permanent intramolecular cavities of host molecules (e.g., cucurbiturils, calixarenes and cyclodextrins).[6] Another supramolecular entity includes self-assembly, in which molecules form spontaneously organized aggregates.[7]

Supramolecular species differ from individual molecules which are produced via covalent bonds. Non-covalent intermolecular interactions such as π-π interactions, electrostatic forces, hydrogen bonding, hydrophobic and van der Waals forces gives rise to supramolecules.[8-10] Supramolecular chemistry is a vast discipline in which important concepts including molecular recognition, host-guest chemistry, molecular self-assembly, dynamic covalent chemistry, and molecular machineries, are explored.[11]

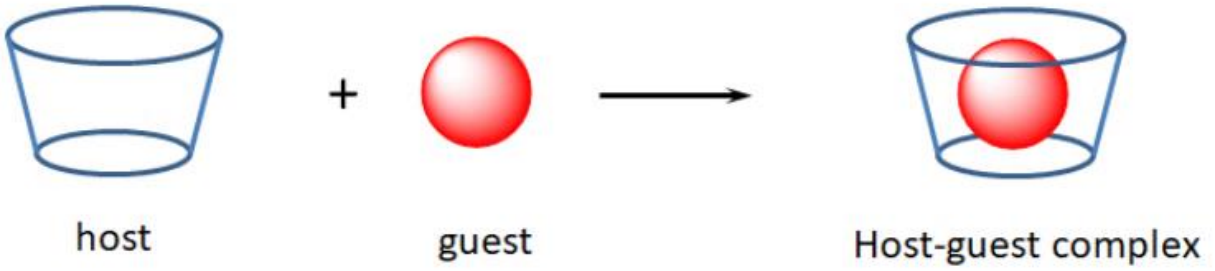

Figure 1. Schematic illustration of the association of a host and a guest forming supramolecular inclusion complex.

Supramolecular host-guest chemistry is concerned with non-covalent complexation or binding between a guest and a host as shown in Figure 1. The host is commonly defined as a large molecule such as macrocyclic compound (e.g., crown ethers, cyclodextrins, calixarenes and so on) or an enzyme, which possess a pre-organized sizeable cavity.[12,13] The guest may be a simple inorganic anion, an inorganic or organic cation, an ion pair, or a complex organic molecule like anticancer drug.[14,15] There are also natural host-guest systems abound in our mother nature which includes enzyme-substrate, DNA-ligand, antigen-antibody and protein-carbohydrate complexes.

The advancement of supramolecular chemistry has been aided by macrocycle-based host–guest chemistry.[16] The macrocyclic hosts include calixarenes, pillararenes, crown ethers, cyclodextrins, cucurbiturils, and other macrocycles.[17-22] These host molecules are very much significant as their constrained cyclized conformations offer a molecular selectivity benefit with versatile potentiality for binding different guest molecules.[16,23] The cyclodextrins (CDs), owing to their amphiphilic character, are especially interesting in this aspect.[23,24] The interest with amphiphiles stems from their ability in aqueous environment to self-assemble into well-defined structures, such as nanorods, nanosheets, nanotubes, vesicles and micelles, that can be applied in various fields including cell imaging, drug delivery and nanodevices.[25-27] In recent years, cyclodextrin-modified nanoparticles have received a lot of attention as they significantly improve the properties of the assemblies, such as fluorescence, conductance, electronic, catalytic and thermal properties, increasing

their potential applications as drug delivery vehicles and nanosensors.[28,29] Therefore, a variety of sophisticated probes have been developed for applications in the manufacturing of chemosensors, molecule-based logic gates, transmembrane channels, molecular switches, supramolecular polymers, molecular machines, and other interesting host–guest systems.[30-32]

Cyclodextrin-based host-guest inclusion complexation have very important significance in consumer goods, pharmaceuticals[33] and food industries[33-36] due to the peculiar cyclic cone-shaped structures of CDs. CDs possess unique biphasic layers with hydrophobic inner and hydrophilic outer surfaces. Their hydrophobic inner region or cavity allows encapsulation of non-polar part of the various guest molecules through different types of non-covalent interactions.[37] Here in, β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) bearing 7 glucopyranose units have been chosen as host molecules because of low price, fitting cavity dimensions, high inclusion efficiency and negligible toxicity.[38,39] The CDs have found extensive application in cosmetics,[40] tissue engineering, foodstuffs, pharmaceuticals and bio-medical devices. Inclusion complexation into the non-polar toroidal cavity of CDs is used for safeguarding the various drugs, volatile organic compounds, enzymes, bioactive molecules, taxols, flavonoids,[41] essential oils, vitamins, etc. to extend their thermal, light and air stability, bioavailability, water solubility and shielding side effects.

Calixarenes, in supramolecular chemistry, are one of the most important classes of macrocyclic organic compounds.[42-44] They have received importance because of their flexible hydrophobic pockets and susceptibility to undergo facile rim modification to encapsulate specific targets.[45,46] This flexible behavior allows for efficient permeation of cell membrane without cell structure deformation.[47] In the calixarene family, sulphonated calixarenes are water-soluble, highly stable and less toxic supramolecules having widespread applications in the host-guest chemistry field.[48-51] Sulphonated calixarenes possess hydrophobic aromatic cavity and hydrophilic lower and upper rims.[52,53] They have achieved increasing attention over the last three decades owing to their inclusion properties with a wide range of guests.[54] Having high selectivity and affinity for various

kinds of guests in an aqueous environment[55-58] and due to nontoxic character,[59,60] sulphonated calixarenes have been widely employed in the fields of smart materials,[61,62] chemical sensors,[63-65] supramolecular amphiphiles,[66] drug delivery,[67,68] protein surface recognition,[69] including molecular capsules synthesis in biocompatible environments.[70-72] Hence, the fundamental investigations involving the interactions of sulphonated calixarenes with different types of guests are important for their advanced applications.[73]

The stabilisation of bioactive guest molecules and their controlled delivery are currently of major concern in pharmacology. To protect these guest molecules from harsh external environment and to reduce their side effects by making them effectual at lower doses for controlled release, it is essential to study their entrapment into cyclodextrins and calixarenes. Therefore, to accomplish such objective, the formation of inclusion complexes of different guest molecules such as Indole-3-methanol (IM), Ticlopidine hydrochloride (TCP) with β-cyclodextrin (β-CD) and/or hydroxypropyl-β-cyclodextrin (HP-β-CD), and Sulisobenzone (SBZ), L-Valine (Val), L-Aspartic acid (Asp) with *p*-sulfonatothiacalix[4]arene (TSC4X) have been studied.

In this thesis encapsulation of IM into β-CD and HP-β-CD have been investigated. IM is a phytochemical compound which can be found in a relatively high level in the vegetables of Brassica genus.[74] The significant role of IM has been examined in cancer management.[74-76] The study on the effect of IM on human melanoma cells showed that IM leads to inhibition of proliferation and induction of apoptosis.[77] IM has also been found to have an anti-inflammatory effect and its role as a chemoprotective agent in prostrate and breast cancer.[78-80] Thus to protect and stabilize this important bioactive IM molecule from external effects (i.e., temperature changes, light and acidic environment) and for its regulatory delivery at the targeted site, it is significant to explore whether this molecule can be included into the cavity of CD molecule and to study the thermodynamic aspect of such inclusion process.

Thienopyridine compounds inhibit thrombus formation thereby acting as antiplatelet agents. They suppress platelet secretion reaction, inhibit functions of platelet such as adhesion, aggregation, and lower circulating platelet aggregates, in addition to blood clotting in peripheral vascular disease, cerebrovascular disease and coronary artery

disease.[81-83] Apart from anti-aggregating effects, other pharmacological effects of thienopyridines include stimulation of NO production, and lowering erythrocyte filterability as well as circulating fibrinogen.[84-86] Thienopyridine derivatives have also shown anti-cancer and anti-proliferative activity against hepatocellular carcinoma as well as pro-apoptotic effect towards cancer cells.[87,88] Ticlopidine hydrochloride (TCP), an FDA-approved drug, belongs to the thienopyridine family and has its potential applications as antiplatelet agent. TCP prevents aggregation of platelet and formation of clot inside blood vessels suppressing thrombus development.[89,90] It also has anticancer and antibacterial activities.[91,92] However, TCP is sensitive towards temperature, light, air, acidic and alkaline environment,[93] which may result in the deterioration of its bioactivity. Therefore, to extend the stability and improve bioactivity of TCP, inclusion complexation within the β-CD cavity is employed.

The benzophenones are a group of aromatic ketones that are used as ultraviolet curing agents, flavor ingredients, fragrance enhancers and photoinitiators ; they are also used in manufacturing pharmaceuticals, agricultural chemicals, insecticides, and as an additive for adhesives, coatings and plastics.[94,95] They also found their applications in plastic surface coatings and toiletries to extend shelf life or delay photodegradation.[95] Sulisobenzone (SBZ) falls under the drug category of benzophenones. It is an FDA approved sunscreen agent which acts as a UV filter by filtering out both UVA and UVB ultraviolet light protecting the skin from damage.[96] However, sunscreen ingredient such as sulisobenzone undergo degradation when exposed to UV-radiation. Hence, to protect and improve the stability of SBZ in order to retain its efficacy, encapsulation of SBZ into the cavity of TSC4X have been explored.

In this thesis, host-guest inclusion complex formation of two naturally occuring amino acids (namely, L-Valine and L-Aspartic acid) with *p*-sulfonatothiacalix[4]arene (TSC4X) have been studied by molecular docking and various physicochemical techniques. Encapsulation with TSC4X results in an enhancement in the thermal stability of these amino acids. ^{1}H NMR spectral titration offer quantitative idea with regard to binding affinity and spontaneity of inclusion process, while FT-IR and ^{1}H NMR spectroscopic

studies along with molecular docking provide specific information on the binding mode confirming the inclusion of amino acids within the TSC4X hydrophobic cavity.

I.2. Choice of Host Molecules, Biologically Active Guest Molecules and Solvents Used in the Research Work

Names of the Host Molecules, Biologically Active Guest Molecules and Solvents are listed below :

Host Molecules :

- β-Cyclodextrin
- Hydroxypropyl-β-cyclodextrin
- *p*-Sulfonatothiacalix[4]arene

Biologically Active Guest Molecules :

- Indole-3-methanol
- Ticlopidine hydrochloride
- Sulisobenzone
- L-Valine
- L-Aspartic acid

Solvents :

- Water
- Dimethyl sulfoxide
- Acetonitrile

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

Name of the Investigation Methods are listed below :

- Fluorescence spectroscopy
- UV-Visible spectroscopy
- Powder X-Ray Diffraction
- Differential Scanning Calorimetry
- Scanning Electron Microscopy

- ^{1}H NMR spectroscopy
- FT-IR spectroscopy
- Surface tension study
- Mass spectrometry
- Thermogravimetric analysis
- Molecular Docking Study
- Antibacterial activity study
- Cell viability assay
- Reactive Oxygen Species (ROS) generation study