CHAPTER I

1. INTRODUCTION:

This chapter gives a background on supramolecular chemistry and graphene based materials. The chapter mainly focuses on total overview of cyclodextrin based supramolecular assembly and graphene based nanocomposites and its wide range of applications especially drug delivery and different biological applications. The general features of graphene and cyclodextrin 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, research problem, scope and objectives and the expected outcomes of the proposed work are provided at the end of the chapter.

1.1 Supramolecular chemistry and graphene nanocomposites: a historical review:

The term supramolecular chemistry was first noted by Jean-Marie Lehn and won Nobel Prize for is extensive work in is 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; (1) host-guest chemistry (2) clathrates and (3) 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., CDs, 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.

Supramolecular species are somewhat different from individual molecules which are formed through covalent bond. Supramolecules are formed through non-covalent intermolecular interaction like hydrogen bonds, van der Waals interactions, π-π stacking, hydrophobic and electrostatic forces [6,7,8]. Supramolecular chemistry is a broad field which discussed about different aspects include molecular self-assembly, molecular recognition, host-guest chemistry, molecular machines and dynamic covalent chemistry.

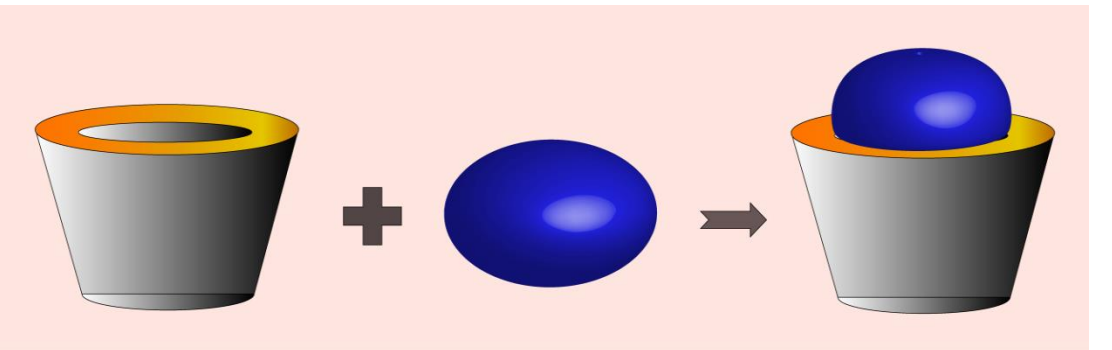

Figure 1: Schematic illustration of the association of a guest and host and thus making a supramolecular inclusion complex

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. [9,10]. 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 [11,12]. Our mother nature is also full of natural host-guest systems include antigen-antibody, DNA-ligand, enzyme-substrate, and protein-carbohydrate complexes.

The last two decades has seen an enormous improvement in the field of nanotechnology ranging from material science to molecular medicine [13,14]. The emergence of the field of nanotechnology has strongly influenced supramolecular chemistry. Recently, nanomedicine has open up a wide range of new strategies

useful in diagnosis, treatment and therapy and disease prevention [15,16]. Graphene and graphene based nanomaterials and nanocomposites have become an attraction in the field of material science due to its excellent properties [17,18]. Although, graphene is a two dimensional (2D) carbon allotrope having a honey comb lattice structure with carbon-carbon distance of 0.142 nm has been studied for more than 60 years theoretically [19]. Graphene was first isolated by the scientists Andre Geim and Konstantin Novoselov at the University of Manchester [20]. They used a simple but effective mechanical exfoliation method for extracting thin layers of graphene from a graphite crystal with Scotch tape. Subsequently, they got the Nobel Prize in Physics 2010 for successfully producing, isolating, identifying and characterizing graphene [21].

1.2 Macromolecular systems and nanomaterials:

1.2.1 Macrocyclic hosts:

In supramolecular chemistry, a macrocyclic host is usually a ring containing a number of binding sites that are arranged around the closed system. There are lots of different types of host molecules have been established. All these host molecules contain either an oxygen or nitrogen atom within the scaffold (Figure 3).

Cyclodextrins are a class of cyclic oligosaccharides consist of six and eight d-glucopyranoside units that are linked together by a 1,4-glycosidic link [22]. There are mainly three important different types of cyclodextrins, i.e. α-cyclodextrin, β-cyclodextrin (Figure 3a) and γ-cyclodextrin consisting of six, seven and eight glucopyranose units, respectively (Figure 2b) [23]. Cyclodextrins have a truncated cone shaped and consists of an upper (wide) and lower (narrow) rim. The secondary hydroxyl groups are attached in the upper rim and the primary hydroxyl groups are attached at the lower rim (Figure 2c) [24]. CDs have variety of industrial applications in food, cosmetics, pharmaceuticals and the environment [25]. When cyclodextrins are taking into consideration as a host, hydrophobic effects and hydrogen-bonding interactions among the different non-covalent forces are mainly responsible in the binding of organic guests. An illustration of the two as well as three dimensional molecular structure of αCD is shown below (Figure 2a).

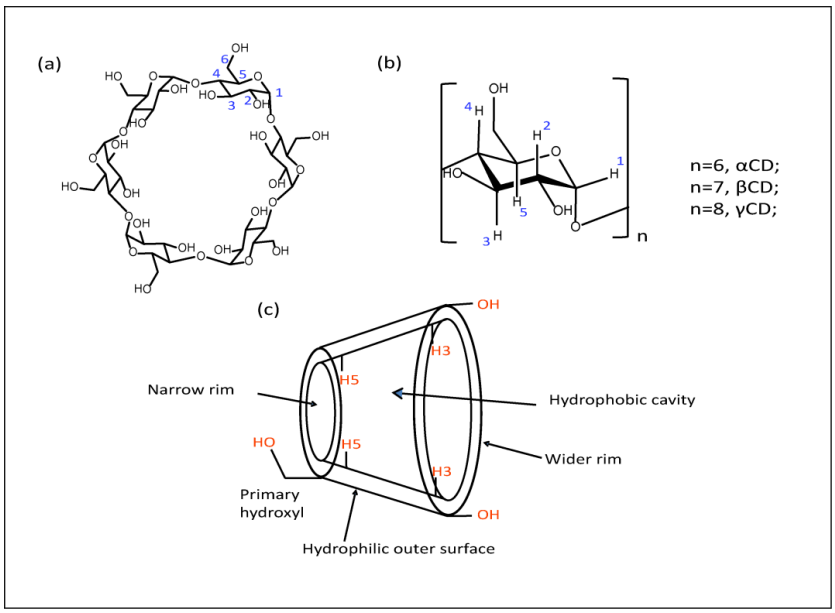

Figure 2: Schematic representation of (a) two dimensional structure of αCD (b) chair conformation of α-1,4-gluocopyranose unit (c) three dimensional truncated cone based repesentation of cyclodextrin

Unlike cyclodextrins, calixarenes are cyclic hosts having 'cone' shape synthesised by the condensation reaction between a p-substituted phenol and formaldehyde (Figure 3b) [26]. It can have several variations of calix[n]arenes (where n = the number of phenol-derived repeat units) for n=4-16, with the most common macrocycles being n = 4, 6 and 8 depending on bases, varying thermal conditions and altering the ratio of reactants [27]. Due to its cone shape, the t-butyl substituent are located at wider rim, whereas, the hydroxyl groups are located at the on the lower or narrow rim [28]. Mainly van der Waals interaction and crystal packing are responsible for calixarenes host molecules to encapsulate organic molecules.

Crown ethers also known as cyclic polyether are one of the most common class of cation binding host utilises more than one non-covalent interaction (Figure 3c). These cations binding host utilises more than one non-covalent interaction, in particular, a combination of electrostatic interactions and hydrogen bonding [29]. Larger crown ether hosts are capable of binding the cation complexes which play an essential role in many biological systems. There are different types of crown ethers are available depending on the number of total ring atom with number of oxygen donor atoms such as [12]crown-4, [15]crown-5, [18]crown-6, Benzo[18]crown-6

whereas, guest can be different metal cations such as, Na^+, K^+, Rb^+, Cs^+, Ca^{2+}, NH_4^+. Depending on the size of both guest and host, it has been found that [18]crown-6 is selective for K^+ as binding constant is highest among the metal cations [30,31].

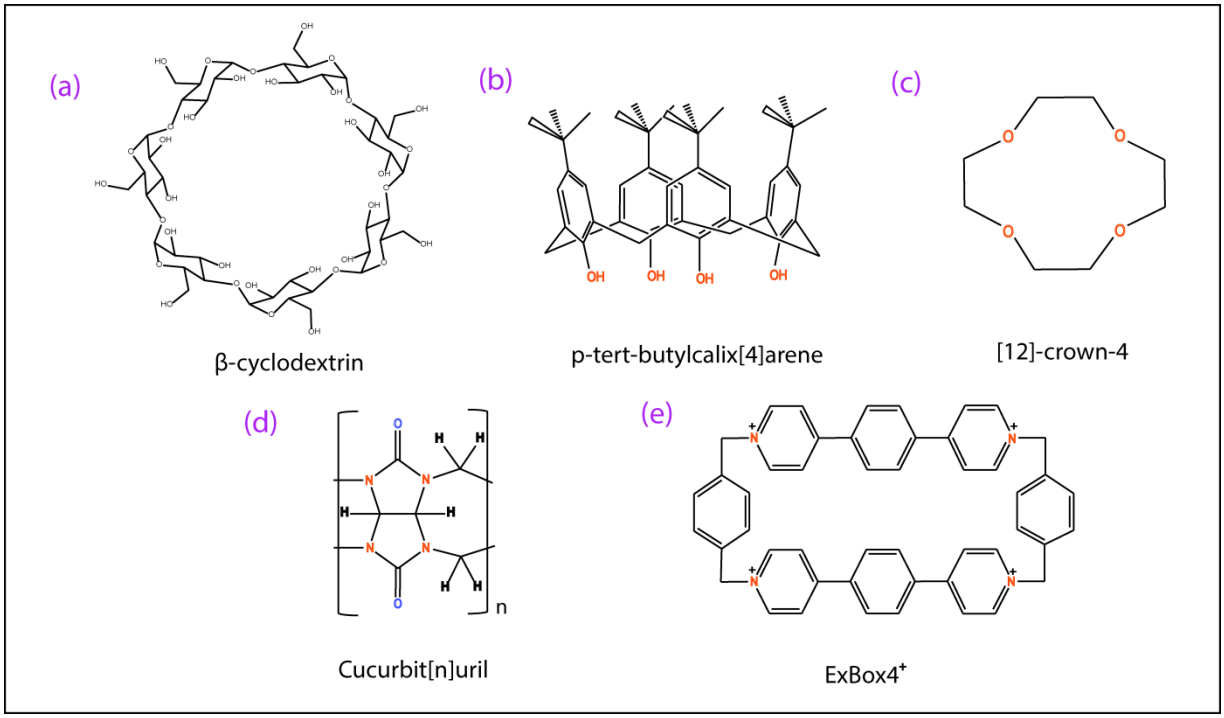

Figure 3: Examples of different types of natural and synthesized host molecules; (a) β-cyclodextrin, (b) p-tert-butylclix[4]arene, (c) [12]-crown-4, (d) ucurbit[n]uril, (e) ExBox4⁺

Cucurbit[n]urils (CBn with n = 5–8,10) are highly symmetric, rigid macrocyclic compound composed of n number of glycouril units linked by 2n methylene bridges and contain a hydrophobic inner cavity (Figure 3d) [32]. CBn has very high binding affinities towards neutral charged guests with high affinity, K_a up to 10^{10} M^{-1} of High binding affinity of CBn is associated with a combination of ion–dipole interactions with the portals and hydrophobic forces inside their inner cavity [33,34].

Stoddart et al., have synthesized a box like rigid structured cyclophane called 'ExBox' almost three decades ago (Figure 3e) [35]. It is tetracationic in nature and capable of acting as a host by encapsulating a guest like porphyrin, regulating its photodynamic anticancer activity [36]. It can also be used as the selective host to bind polycyclic aromatic hydrocarbons (PAHs) of different size and shape by means

of donor–acceptor interactions ranging in size from two to seven aromatic rings [37].

1.2.2 Biologically active guest molecules:

In order to obtain the better association strengths in inclusion complex, specific host as well as guest should be selected [38]. As the strength of CD complexes relies on the space available in the CD cavity, the size of the guest should be in such a way so that it can fit into the CD cavity [39]. As host-guest supramolecular field was initiated with the motivation to increase the solubility of a poorly water soluble drug [40]. In this Ph.D. thesis, various biologically active molecules for inclusion complexation have been studied for potential application in pharmaceuticals, industrials, dyes and agrochemicals. Ambroxol hydrochloride, a drug molecule has been used for inclusion complexation with αCD and βCD and is reported in greater detail in Chapter IV [41]. The binding properties of the Nile blue toward graphene nanocomposites have been studied in chapter V. Other drug molecules are trigonelline hydrochloride, rebamipide and umbelliferone and their complexation and characterizations are discussed in chapter VI, VII and VIII respectively.

Till now, various biologically organic molecules have been studied all over the world in supramolecular chemistry. Flavone derivatives being poorly water soluble drug have been extensively studied with different cyclodextrin derivatives in host guest chemistry [42]. Qiu et al., worked with water-insoluble anticancer agent barbigerone with water soluble hydroxypropyl-β-cyclodextrin to increase water solubility by forming inclusion complex [43]. Low water solubility and bioavailability of different biopesticides or herbal medicines could also be increased. After successful formation of crassicauline A CLA/β-CD complex will be potentially useful for its application as herbal medicine or healthcare products [44]. The most frequently utilized guest group for β-CD is adamantyl, which fits very efficiently into the β-CD cavity (logK = 5.04) [45]. Different inorganic complexes or different dye are also used as a guest for complexations [47].

1.2.3 Graphene and graphene based materials:

Based on the various chemical modifications, graphene nanomaterials can be categories in different components such as graphene, graphene oxide (GO) and reduced graphene oxide (rGO). Graphene is the 2D building material for carbon nanomaterials of all other dimensionalities as shown in Figure 4.

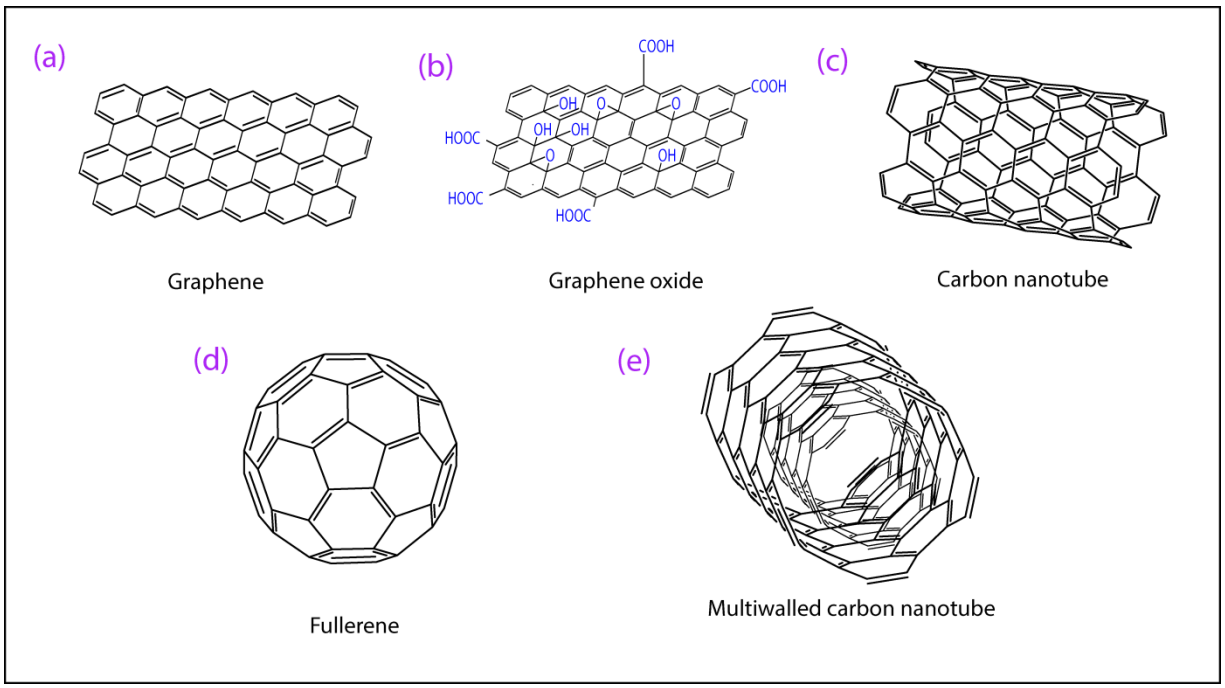

Figure 4: Typical molecular structures of different carbon nanomaterials; (a) Graphene, (b) Graphene oxide, (c) Carbon nanotube, (d) Fullerene (e) Multiwalled carbon nanotube.

1.2.3.1 Graphene:

Graphene is a two dimensional with hexagonal array of carbon atoms having single layered structured provides unique properties such as chemical inertness, high electrical conductivity, high flexibility, and large surface area (Figure 4a) [47,48]. It is composed of sp^2-hybridized carbon atoms arranged in monolayer formation with large surface (2630 m^2/g) [49]. Graphene has the ability to interact with different organic molecules and even enter into the cells through membranes by electrostatic interactions without damaging it. Over the last two decades, several researches have been carried out all over the world on the biological application of graphene materials ranges from drug and gene delivery systems, photothermal and photodynamic therapy (PTT & PDT), bio-imaging of different cancer cells and tissue engineering [50,51].

1.2.3.2 Graphene oxide (GO):

Graphene oxide (GO) has sparked an enormous interest to the scientific and technological community due to its intriguing electronic, mechanical, thermal and optical properties (Figure 4b) [52,53]. GO has the natural tendency to be highly dispersible both in water and in polar organic solvent due to the presence of oxygen containing functional group at the basal as well as at the edges of graphene [54,55].

1.2.3.3 Single wall carbon nanotube (SWCNT):

Single-walled carbon nanotubes (SWCNTs) are generally quasi-one dimensional substances constituted by one of the allotropic forms of carbon (Figure 4c) [56]. They can be recognised as perfectly rolled up hollow cylinders composed of graphene (a sheet of carbon atoms) having diameters in between the range of 0.4 and 5 nm [57,58]. They have appreciable electrical, thermal, optical and mechanical characteristics. The CNTS can be formed by the method of chirality or chiral vector, which is represented by (m,n) indices, can be described as the number of unit vectors along two directions of the hexagonal lattice [59]. Based on the concept organic chemistry, CNTs can be prepared theoretically by applying total synthesis. This method involves the formation of a particular type of CNT seed, in which some appropriate building blocks are added to elongate a CNT. On exposure of light, CNTs can be utilised for biological imaging, it can also be used as solar cell components. CNTs often be used as aqueous chemical sensor and also they have high thermal conductivity, for which they can be recognised as good heat sink objects [60].

1.2.3.4 Fullerene:

Fullerene is the third allotropic form of carbon having shapes like a hollow sphere, tube or ellipsoid and three dimensional analogues of benzene (Figure 4d) [61]. In fullerene, all the carbon atoms are interconnected in hexagonal and pentagonal rings containing 12 pentagones and 20 hexagones. In 1960s, Architect buck minster fuller had given the name to this special allotropic form as fullerene. The most available form of fullerenes is buckminster fullerene (C-60), a spherical structure with 60 atoms [62]. Fullerenes can be synthesised synthesized through arc heating of graphite and laser irradiation of Poly aromatic hydrocarbon (PAHs). Different types of fullerene can be known to us such as bucky ball clustures,

nanotubes, megatubes, nano onions etc [63]. Fullerenes undergo different type of reactions such as nucleophilic reactions, halogenations, hydrogenation, transition metal complex reactions, regioselective reactions etc. Fullerenes have wide applications such as it can be used as polymer additives, as catalyst, in portable power devices, water purification and bio–hazard protection catalysis [64].

1.2.3.5 Multi wall carbon nanotube:

Apart from single wall carbon nanotube (SWCNT), multi-walled carbon nanotubes (MWCNTs) composed of two or several cylinder, each of which formed by graphene sheets having diameter in between 1 to 3 nm (Figure 4e) [65]. The requirement of a catalytic agent is not mandatory for MWNT. The first discovery of MWCNTs was done by Iijima by arc discharge method [66]. Due to the sp^2 bonds between individual carbon atoms in MWCNTs, they possess greater tensile strength than steel. MWCNTs have several biomedical applications, biosensors; Fabricated MWCNT based sensor on lead pencil used for the simultaneous detection of hydroquinone and catechol [67]. Multi walled carbon nanotubes have been utilized in cancer diagnosis; they are extensively used in drug targeting as well as in controlled release of drug. MWCNTs are suitable carriers for peptides, proteins and genes as these macromolecules get easily degraded by enzymes present on cell surface or cell inside. MNCTs also exhibit anti microbial and anti fungal activity [68].

1.3 A brief review on different applications:

Supramolecular based formulation by cyclodextrins often leads to modification of molecular properties such as physicochemical and biological properties of the certain guest molecule [69]. Therefore, it has been used in a large number of applications which includes improvement of the solubility and enhancing stability and bioavailability of poorly soluble drugs (Figure 5) [70,71].

Apart from increasing water solubility, cyclodextrin inclusion complexes are also being used in increasing loading capacity, chiral separation, targeted drug delivery, organic synthesis, controlled release, enhanced thermal and photochemical stability, controlled bioadhesion etc [72]. Li et al., have synthesized cucurbit CB[8] based shape-controllable and fluorescent supramolecular organic frameworks exhibit stimuli-responsive turn-off/turn-on fluorescence, which can be applied in

cellular imaging [73]. Cyclodextrin can also be used as a catalyst in organic synthesis. Floresta et al., have designed a green and efficient method where catalytic amount of γ-cyclodextrin (γ-CD) has been used to synthesize substituted isoxazolidines [74].

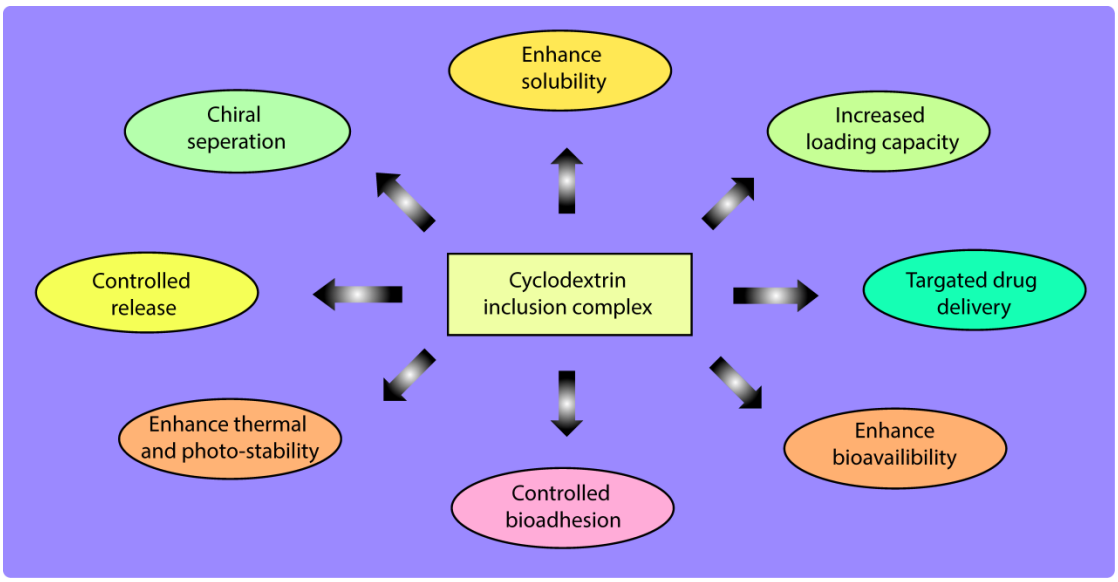

Figure 5: Applications of cyclodextrin based inclusion complexes in different fields

Farras et al., showed that molecular encapsulation by cyclodextrin enhanced the photostability of a ruthenium(II) polypyridyl complex in aqueous environment [75]. Photosensitizers are very reaction and upon light absorption, it can cause photooxidative damage to different cells. Stoddart et al., has developing new pharmaceutical formulations of photosensitive drugs through 1:1 host–guest complex by a newly developed host ($ExBox4^{+}$) in anticancer therapy through regulated PDT [76].

Cyclodextrin can also be used as a biodegradable capsules for the potential use in the controlled delivery of hydrophobic drugs to cancer cells. Jing et al., have showed that Cyclodextrin/Paclitaxel Complex in Biodegradable Capsules in a controlled way for Breast Cancer Treatment [77].

Cyclodextrins have been used for different industrial purposes such as reduce bitterness; improve taste and flavours of pastry and meat products so that they can be easily stored. Cholesterol may be removed from milk using β-

cyclodextrin by inclusion complexation to produce dairy products low in cholesterol [78].

Supramolecular nanotechnology through host-guest interaction has been recognised as a special area owing to its targeted delivery nature. A recent experimental fact leads to understand that host–guest nanoparticles and nanocomposites show enhanced efficacy in delivery especially to tumors and show effective cancer phototherapy as well as imaging (Figure 6). Nurunnabi et al., were studied on Graphene-based nanomaterials and they found that Their composites more than 70% kills MDAMB231 cancer cells (more than 70%) through both photodynamic and photothermal effects [79].

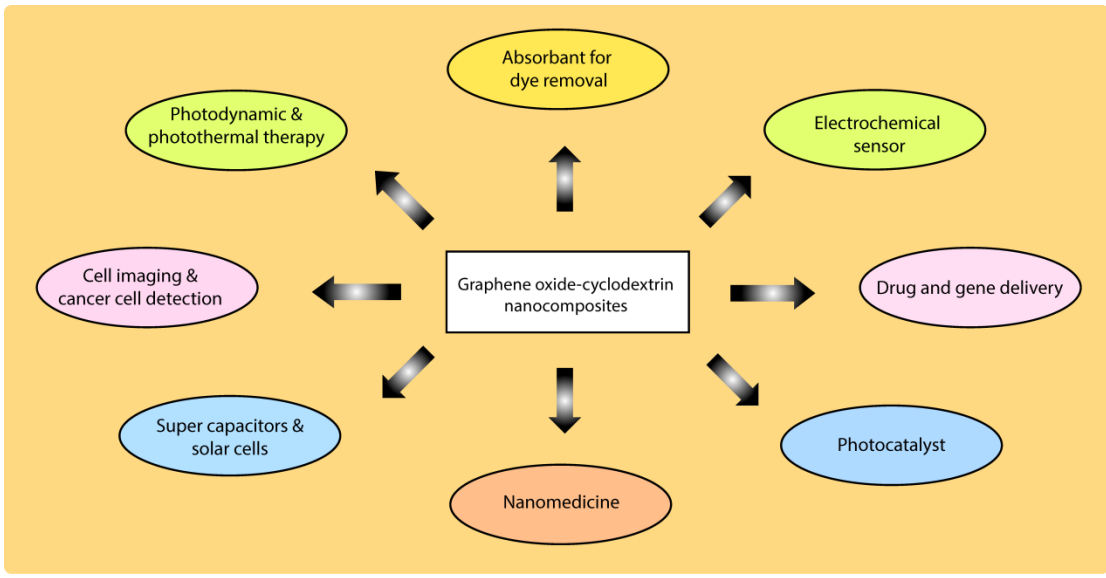

Figure 6: Applications of graphene oxide-cyclodextrin based nanocomposites in different fields

Graphene oxide-cyclodextrin based nanocomposites are being used in wastewater application. Nafie et al., have synthesized β-cyclodextrin grafted nanopyroxenes to offer an ecofriendly platform for the selective removal of organic compounds such as cyclopentanecarboxylic and trans4-pentylcyclohexane carboxylic acids [80].

They can also be used as promising materials for super capacitors and solar cells. Li et al., has showed that graphene-film based electric double layer capacitors (EDLCs) has the potentiality to be use as supercapacitors or ultracapacitors due to its excellent flexibility and as well as high performances [81].

GO hybrid cyclodextrin-based supramolecular hydrogels with NIR-light-, temperature-, and pH-responsive behaviors with improved thermal stability are able to show promising result in the field of delivery systems [82].

1.4 Scopes and objectives of the thesis:

The main focus of our research work is to improve the bioavailability of various biologically potent drugs via supramolecular assembly and also in presence of graphene oxide (GO) nanocomposites using as a nano carrier and also its structural characterization in depth with experimental augmented with theoretical and computational calculation. Encapsulation of drugs in nanocavity of supramolecular host enhances the solubility and pharmacokinetics of the drug. The structural factors that are responsible for the thermodynamics of the interaction between guest and various CD derivatives can be achieved. Cyclodextrin based supramolecular chemistry can be used for the competitive binding of guest molecules in a given CD-guest complex with a guest that has a higher binding affinity. The major aim of this thesis is to improve the solubility and bioavailability of different guest of low soluble drugs by conjugating or encapsulating it in different nanoformulations.

In order to achieve the goal, the following objectives have been set.

- Preparation and characterization of different supramolecular complexes with different cyclodextrin by experimentally as well as theoretically in depth.
- Investigation of the structural characterization and dynamic properties of the host-guest ICs.
- Development of supramolecular complex for the sustained release of the drug molecule from the encapsulated complex.
- Encapsulation of low soluble drug and its characterization through spectroscopic method for enhancing bioavailability and sustained dischargement.