CHAPTER I

NECESSITY OF THE RESEARCH WORK

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

Molecular encapsulation and release are exceptionally significant in pharmacology and drug delivery science in recent years.[1] For this purpose various host molecules, such as calixarenes, pillararenes, cucurbiturils, cyclodextrins, etc. have been widely used as excellent receptors for drug recognition.[2] The host-guest complexes could be applied to construct stimuli-responsive supramolecular materials, where series of external stimuli, such as, enzyme activation, photo sensing, temperature dependence, changes in pH/redox and competitive binding may be employed to operate the release of guest molecules from the inclusion complexes (ICs).[3] In the last decade attention has been focused on molecular sensing, anti-cancer drug release, gene transfection etc. with the help of mechanized nanoparticles capable of trapping and regulating the release of cargo molecules by a range of external stimuli.[4] Macrocyclic host molecules are of immense importance in ICs as the cyclized and constrained conformation offer the benefit of molecular selectivity.[5] The cyclodextrins (CDs) are exclusively interesting in this regard, due to their amphiphilic nature.[6] The interest in amphiphiles comes up from their self-assembly in aqueous systems to form well defined structures, such as micelles, nanotubes, nanorods, nanosheets and vesicles, which can be applied in several grounds ranging from nano-devices, drug delivery and cell imaging.[7] In recent times cyclodextrin modified nanoparticles are of great attention as they appreciably improve the characteristics of the assemblies, such as the electronic, conductance, thermal, fluorescence and catalytic properties improving their potential applications as nanosensors and drug delivery vehicles.[8] Various sophisticated probes have been designed for this purpose for their applications in the manufacture of molecular switches, molecular machines, supramolecular polymers, chemosensors, transmembrane channels, molecule-based logic gates and other interesting host–guest systems.[9]

Cyclodextrins (CDs) are the cyclic oligosaccharides containing six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, bound by α-(1–4) linkages forming a truncated conical structure.[10] Thus because of their unique structure, i.e., fairly rigid and well-defined hydrophobic cavities and hydrophilic rims having primary and secondary –OH groups they are of particular interest in the modern science.[11] CDs are used for controlled delivery of organic, inorganic, biological and pharmaceutical molecules due to their ability to form inclusion complexes with diverse guest molecules by encapsulating the non-polar part of the guest into its hydrophobic cavity and stabilizing the polar part by the polar rims.[12] The use of CDs already has a long history in pharmaceuticals, pesticides, foodstuffs etc. for the solubility, bioavailability, safety, stability and as a carrier of the guest molecules.[13]

The structures and the properties of the ICs formed by CDs are determined by their architectures, i.e., interplay between the hydrophilic–hydrophobic balance and geometric packing constraints.[14] The experimental conditions, such as concentration, temperature, pH, etc. also play crucial roles exhibiting their potential applications in gene and drug delivery.[15] Due to their above mentioned advantages, the ICs are being widely investigated in materials and biomedical sciences, especially, the applications in biologically and pharmaceutically relevant fields have produced tremendous interest of researchers in recent years.[16] The exterior of the CD cavity is highly polar due to the hydroxyl groups, while the interior is non-polar, making them suitable and fascinating hosts for supramolecular chemistry.[17] The chemical stability of guest molecule also increases due to encapsulation inside the cavity.[18]

CDs have been widely employed as not only excellent receptors for molecular recognition but also excellent building blocks to construct functional materials, where they could be applied to construct stimuli-responsive supramolecular devices.[19] Series of external stimuli, for example, enzyme activation, light, temperature, changes in pH or redox and competitive binding are employed to activate the release of guest molecules from the inclusion composites.[20]

Molecular recognition is of profound importance in biology and therapeutics, the physical chemistry of this phenomenon acknowledges that binding is often associated with loss in configurational entropy, but the overall thermodynamics is yet to be well understood.[21] Among various approaches CDs have contributed a lot to this aspect of drug delivery, because of having fairly rigid and well-defined hydrophobic cavities and hydrophilic outer surfaces, they can act as molecular receptors (hosts) for a wide variety of organic and inorganic, as well as biological and pharmaceutical guest molecules, forming host–guest complexes or supramolecular assemblies.[22]

The drugs, to be pharmacologically active, must possess some degree of aqueous solubility, as well as they should be lipophilic to permeate the biological membranes via passive diffusion.[23] If a drug is highly hydrophilic, the dissolved drug molecule will not penetrate from the aqueous exterior into a lipophilic bio-membrane. The use of cyclodextrins on drug solubility, bioavailability, safety, stability and as a carrier in drug formulation may be achieved by formation of inclusion complexes with drug molecules; in fact, the use of cyclodextrins already has a long history in pharmacy.[24]

Crown ethers (CEs) are cyclic polyether based macrocyclic molecules having utmost interest in the field of supramolecular chemistry.[25] CEs form diverse complexes with versatile species, such as, metal ions and cationic species by various non-covalent interactions.[26] These macrocyclic polyethers have the unique property of molecular recognition in solution phase, thus can act as phase transfer catalyst, photo sensor, delivery vehicle, etc.[27] In modern days researches are also interested in making supramolecular polymers, transmembrane channels, nanosensors, molecule-based logic gates and other interesting supramolecular systems improving the potential applications of CEs.[28] Thus, fundamental studies involving the interactions of CEs with cationic species are significant for their advanced applications.[29] The assemblies of CEs with cationic species are already known, but mechanistic study involving similarly substituted various ionic liquids (ILs) with a CE provides multidimensional information in this field of research.[30]

Crown ethers (CEs) are used as important hosts in supramolecular chemistry, where the host–guest interaction mimics natural systems as well as constructs various

materials.[31] CEs are macromolecular heterocyclic compounds with essential repeating unit -CH_2CH_2O-.[32] A number of researchers are working on fabrication of crown-ether-based stimuli-responsive materials that have unique characters of ion recognize ability.[33] A variety of current supramolecular materials, for instance rotaxanes are made on these unique recognition properties of CEs.[34] Binding of CEs with cations with high selectivity and affinity has found remarkable importance in chemistry.[35] Formation of molecular assemblies has vast implication for the building of molecular machines having plausible use as analogous to sophisticated machines of natural systems.[36] Hence, fundamental investigations of the interactions between CEs and cationic species are important for their advanced applications.[37]

In this thesis the studied two vitamins, namely, nicotinic acid and ascorbic acid are the essential human nutrients with many important functions in biological systems. Nicotinic acid is used to treat hypercholesterolemia and pellagra while its deficiency causes nausea, skin and mouth lesions, anemia, headaches, and tiredness.[38] On the other hand scurvy, fatigue, depression, and connective tissue defects are the common syndromes caused by deficiency of ascorbic acid.[39] Thus to protect these important bio-molecules from external effects (i.e., oxidation, structural modification etc.) and for their regulatory release, it is crucial to investigate whether these molecules can be encapsulated into the CD molecule and to explore the thermodynamic aspect of the process. Guorong *et al.,* Okazaki *et al.* and Delicado *et al.* showed different interactions of ascorbic acid with CD, while Manzanares *et al.,* Silva *et al.,* Pardave *et al. and* Hu *et al.* indicated the formation of inclusion complexes between ascorbic acid with *β*-CD by different electro and physicochemical methods. On the other hand Terekhova *et al.* demonstrated nicotinic acid-CD interactions by volumetric and heat capacity studies.

Dopamine is an important neurotransmitter (NT) in the mammalian central nervous system and is a member of catecholamines.[40] It is involved in neuropsychiatric disorders such as Perkinson's disease, which is the second most common central nervous system disorder. Tyramine is also a NT and acts as a catecholamine releasing agent, having nonpsychoactive peripheral sympathomimetic effects.[41]

Epinephrine is a hormone and a NT, serves as chemical mediators for conveying the nerve impulses to effectors organs. Epinephrine remains a useful medicine for several emergency indications and is used as a drug to treat cardiac arrest and other cardiac dysrhythmias.[42]

The stabilization and regulatory release of the sulfa-drugs are of great concern in pharmacology.[43] Thus to protect these drugs from external effects and for their regulatory release, it is crucial to investigate whether they can be encapsulated into the CD molecule. Sulfonamides are bacteriostatic material and their range of activity is analogous for all. Sulfonamides restrain bacterial synthesis of dihydrofolic acid by inhibiting the condensation of the pteridine with aminobenzoic acid by competitive inhibition of the enzyme dihydropteroate synthetase.[44] Topically applied sulfonamides act against vulnerable strains of various bacterial eye pathogens, for example, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus, Haemophilus influenzae, Klebsiella species, Enterobacter species, etc. Sulfacetamide sodium (SS) 10% topical lotion is approved for the treatment of acne, seborrheic dermatitis, conjunctivitis and various external visual infections due to susceptible for microorganisms. SS has been considered in the treatment of pityriasis versicolor and rosacea.[45] It also has anti-inflammatory property while used to treat conjunctivitis. It is found that SS may be used in the treatment of mild forms of hidradenitis suppurativa. There are a number of topical products containing SS, e.g., foams, shampoos, cream, etc. Sulfacetamide is a competitive inhibitor of bacterial para-aminobenzoic acid, which is necessary for bacterial synthesis of folic acid, a vital constituent for bacterial growth.[46] The multiplication of bacteria is thus inhibited by the action of sulfacetamide. SS can also be used orally to treat urinary tract infections and the oral absorption of SS is found to be 100%. Sulfacetamide causes slight irritation in presence of UV-A light, as it gets sensitized and degraded leading to toxicity when used continuously. Thus, stabilization from external hazards, i.e., oxidation, sensitization, photolytic cleavage etc.; for the regulatory delivery of required amount of SS at the targeted site for a period of time professionally and accurately and to prevent overdose, encapsulation of the drug is very important.

RNA nucleosides are very important biomolecules having enormous applications in the field of modern biological sciences, *e.g.*, RNA-based information technologies, RNA cloning, recombinant RNA technology and other genetic engineering processes. Xiang *et. al*. demonstrated the formation of inclusion complexes of purine nucleosides with *β*-CD, as well as their stability and carrying capacity by solubility, circular dichroism, ultraviolet spectrophotometry and NMR techniques.[47] Formoso illustrated the binding of nucleic acid monomer units as well as dinucleoside phosphates with *β*-CD by circular dichroism studies and calculated the binding constants and thermodynamic parameters, which show significant interactions between *β*-CD and the nucleotide moieties.[48] In this thesis the inclusions of all the four RNA nucleosides inside into aqueous *α* and *β*-CD have been explored, especially towards their formation, stabilization, carrying and controlled release without chemical modification by different reliable methods focusing mainly on the encapsulation of the RNA nucleosides into the cavity of *α* and *β*-CD.

In this thesis five naturally occurring amino acids (namely, L-Lysine, L-Phenylalanine, L-Glutamic acid, L-Arginine and L-Histidine) have been studied with *α* and *β*-cyclodextrins to observe whether they form host-guest inclusion complexes by various physicochemical, transport and spectroscopic studies. Nature of the inclusion complexes are established by density, viscosity and refractive index measurements by calculating the contributions towards the limiting apparent molar volume and viscosity-B coefficient of different groups of the guest molecules, solvation number and limiting molar refraction by taking different mass fractions of *α* and *β*-cyclodextrins in aqueous medium. ^{1}H NMR study confirms the encapsulation of the amino acids into the hydrophobic cavity of cyclodextrins.

In this thesis 1-butyl-3-methylimidazolium chloride [BMIm]Cl, 1-butyl-4-methylpyridinium chloride [BMPy]Cl and 1-butyl-1-methylpyrrolidinium chloride [BMP]Cl have been investigated with 18-crown-6 (18-C-6) in CH_3CN solution to elucidate their complexation process in molecular level with specific atomic interactions. Imidazolium, pyridinium and pyrrolidinium based ILs are biologically highly significant as they play important roles in enzymatic reactions.[49] They are also important in organometallic, organic and material chemistry for their exceptional physical, chemical and electrical

properties.[50] Here, the complexation processes for the three ILs are different and require special considerations. Various non-covalent type interactions are present in solution, but H-bond type attraction predominates over others in the complexations of [BMIm]Cl and [BMPy]Cl with 18-C-6, while ion-dipolar attraction prevails in complexation of [BMP]Cl with the CE in CH_3CN medium. These studies furnish specific information about the complexation processes for potential applications in supramolecular host-guest chemistry.

In this thesis cetylpyridinium chloride (CPCl) has been investigated as the cationic species, which is structurally significant because of having long lipophilic chain and pyridinium cationic head and also has medicinal applications, while three CEs, namely, dibenzo-18-crown-6 (DB-18-C-6), 18-crown-6 (18-C-6) and dicyclohexano-18-crown-6 (DCH-18-C-6) have been selected with similar cavity dimension, but having different and tailored abilities to construct supramolecular complexes. The complexation processes have been explored in CH_3CN solution with definite host-guest type interactions in molecular level. Pyridinium based ionic liquids (ILs) are biologically extremely significant and also have role in material chemistry for their extraordinary properties.[51] Here, the structure of CPCl is very important to make supramolecular materials and also has biological and medicinal functions. In this study the three complexation processes require special attention to explore the various interactions taking place in molecular level. Conductivity measurement and programmed mathematical treatment of the data offer quantitative idea about association constant and thermodynamic parameters, whereas FT-IR and ^1H NMR spectroscopic studies deliver specific information about the complexation processes for the potential applications in supramolecular host-guest chemistry.[52]

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

Names of the cyclodextrins, crown ethers, biologically active molecules, ionic liquids and solvents are listed below:

Cyclodextrins:

- α-Cyclodextrin
- β-Cyclodextrin

Crown ethers:

- 18-Crown-6
- Dibenzo-18-crown-6
- Dicyclohexano-18-crown-6

Biologically active molecules:

- Nicotinic acid
- Ascorbic acid
- Dopamine hydrochloride
- Tyramine hydrochloride
- (±)-Epinephrine hydrochloride
- Sulfacetamide sodium monohydrate
- L-Lysine
- L-Phenylalanine
- L-Glutamic acid
- L-Arginine
- L-Histidine
- Adenosine
- Guanosine
- Uridine
- Cytidine

Ionic liquids:

- 1-Butyl-3-methylimidazolium chloride
- 1-Butyl-4-methylpyridinium chloride
- 1-Butyl-1-methylpyrrolidinium chloride
- Cetylpyridinium chloride

Solvents:

- Water
- Acetonitrile

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

Names of the investigation methods are listed below:

- ^{1}H NMR spectroscopy
- 2D ROESY
- FTIR spectroscopy
- UV-visible spectroscopy
- High resolution mass spectrometry
- Isothermal titration calorimetry
- Surface tension study
- Conductivity study
- pH study
- Density study
- Viscosity study
- Refractive index study
- Ultrasonic speed study