
CHAPTER I

NECESSITY OF THE RESEARCH WORK

I.1. OBJECT, SCOPE AND APPLICATIONS

Research gathers information and investigates phenomena allowing us to test the theories and make prediction-solving problems. Research creates new knowledge and understanding. It reduces the chances of making poor or dangerous choices protecting us from false ideas and risky beliefs. Basic research leads to applied research in unusual ways. That why research is the force behind everything that we do, such as phones we use, medicine we take, power we consume. It is time for us all to embrace Research to find the evidence, to back up beliefs, to test out hunches and to discover better ways of doing things. 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 supramolecular chemistry discovered by Cramer, Pedersen, Cram, and Lehn, achieved the mountain high success and gained an enormous momentum in the last eras, with sometimes around 20,000 corresponding papers per year.¹⁻⁴ A vast applications regarding the host-guest supramolecular chemistry, e.g., sensing, separation, drug delivery, catalytic, and biomedical technologies strongly paved the first step of this field. It introduces the noncovalent interactions in an unprecedented way.⁵⁻⁸ Beside the calculation of binding energy, designing of the supramolecular systems and their applications are also of fundamental interest. These make able to understand biologically important association, techniques of complexation and designing a new host structure. Modern synthetic tool also helps to construct a suitable host structure, which can exert an extra potential for a particular guest compound. The interactions that results inclusion complexation can be identified and explained, based on the interactions their structures can also be elucidated. It has a great thermodynamics that can be determined quite accurately using binding constants of the inclusion complexes. 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) Short range repulsion (c) Electrostatic interactions (d) Hydrogen Bonding interaction (e) Interactions of Dipolar substances.

Macrocyclic host molecules are of immense importance in ICs, as their cyclized and constrained conformations offer the benefit of molecular selectivity.⁹ The cyclodextrins (CDs) are particularly interesting in this regard because of their amphiphilic nature.^{9,10} The interest in amphiphiles arises from their self-assembly in aqueous systems to form well-defined structures, such as micelles, nanotubes, nanorods, nanosheets, and vesicles, that can be applied in several fields ranging from nanodevices to drug delivery and cell imaging.¹¹⁻¹³ In recent times, cyclodextrin-modified nanoparticles have been the focus of great attention because they appreciably improve the characteristics of the resulting assemblies, such as the electronic, conductance, thermal, fluorescence, and catalytic properties, improving the potential applications of these assemblies as nanosensors and drug delivery vehicles.^{14,15} Various sophisticated probes have been designed for this purpose for applications in the manufacture of molecular switches, molecular machines, supramolecular polymers, chemosensors, transmembrane channels, molecule-based logic gates, and other interesting host-guest systems.¹⁶⁻¹⁸

Oligosaccharides, specially cyclodextrins (CDs) regarding host-guest inclusion complexation have very significant importance in food industries¹⁹⁻²¹ pharmaceuticals²² and consumer goods due to their unique conical-shaped cyclic structures. Cyclodextrins and their derivatives are commercially available and differ because of the presence of different glucopyranose residues. Cyclodextrins have their distinctive biphasic layers possessing hydrophilic outer and hydrophobic inner surfaces. The inner region allows incorporating hydrophobic surface of different guest or segment(s) of guest molecules into the cavity of a suitable and stable geometrical sized CDs through various kinds of non-covalent interactions.²³ Herein, α

and β -cyclodextrins 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.²⁴ The CDs have found widespread application in pharmaceuticals, food industries, cosmetics²⁵, 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, vitamins²⁶, and etc. to extend their light, air and thermal stability, enhancement of water solubility, bioavailability and shielding side effects.

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 drug molecules such as synephrine, phenylephrine hydrochloride, Vitamin B₁, alverine citrate, hexabromocyclododecane and with alpha and beta cyclodextrin have been studied to achieve the goal.

Structural characterization of Host-Guest inclusion complexes of α -CD and β -CD with two bio-active molecules, synephrine (SNP) and phenylephrine hydrochloride (PEH) were done over here in terms of geometry and structural preferences by means of a variety of physical and spectroscopic methods in solid state and solution phase. Phenylephrine hydrochloride is a selective α_1 -adrenergic receptor agonist of the phenethylamine class used primarily in cold and flu conditions as an antipyretic, analgesic drug to relief pain.²⁷ In the United States PEH is used as nasal decongestant. Phenylpropanolamine, pseudoephedrine and ephedrine are also used as nasal decongestant as the substitute of PEH.^{28,29} However, due to serious side effect (hemorrhagic stroke) phenylpropanolamine was withdrawn from market.³⁰ Alkaloid synephrine are used as bronchial muscle reluctant, increases blood pressure in the patients suffering from low blood pressure. Lipolytic stimulation by synephrine increases thermogenesis which leads to the increase in metabolic rate and fat oxidation.³¹⁻³⁴ In weight loss products as well as in the dietary supplement "ephedra free" synephrine is frequently used and starts to earn enormous attention after the banned product ephedrine.^{35,36} Most of the cases patients suffering from obesity are

often found to suffer from type-2 diabetes and hence synephrine in weight loss products frequently becomes beneficial to the diabetic patients.³⁷ Cyclodextrins, mostly α -cyclodextrin, are found to form complexes with the dietary fat which are stable enough to undergo enzymatic hydrolysis by lipase. This restrains accumulation of fat in human body.³⁸ Hence, inclusion complex of SNP and cyclodextrins can be of a great deal for the weight loss/weight management dietary food supplement for sportsman or obese person.³⁹

The inclusion of THC has been aimed within the cavity of α and β -CD separately in both solution and solid states to explore their formation of inclusion complex (IC) for enhancing the stability of THC. Regular release without any chemical alteration of THC in the presence of HSA from the cavity of α and β -CD develops the drug delivery system. The B vitamins and their derivatives are a class of water-soluble vitamins and naturally found in food substances. These have significant role in cell metabolism.⁴⁰ They have major importance for food processing and biological activities such as transferring the alkyl group, fitting carbon dioxide, decarboxylation and transamination of amino acids, lipids and sugars.^{41,42} Among all the B vitamins and their derivatives, one of the most common is thiamine (vitamin B1) chloro hydrochloride (THC, Figure 1), which is usually used as a component of single vitamin B complex and multivitamin preparations, food supplement, antioxidant, prooxidant, pharmaceutical industries and biological fluids. THC is used to treat in appetite and dermatophytosis. Moreover, it is also helpful to metabolize in human body.^{43,44} Due to the lack of thiamine hydrochloride, neurotransmission in human body can be affected. Deficiency of THC leads to the occurrence of various malfunctions inside the human body such as beriberi, confabulation and an irreversible dementia; even extreme deficiency may lead to heart failure and death.⁴⁵ Thus thiamine hydrochloride is extensively used in human body; moreover, it can be utilized as feed in agriculture and synthetic intermediates in industry. But THC is very sensitive to light and high temperature processing and also has tend to get oxidize easily in the presence of oxygen which limits their applications to a great extent in different fields. Chemical degradation is very common with vitamin B1 and the main route of the degradation is its reduction which is caused in the presence of food preservatives

such as sodium meta-bisulfite, with very low concentrations (~ 1 mmol/L). Moreover, it can degrade by some of cell surface enzymes and plant thiamine antagonists⁴⁶

1,2,5,6,9,10-hexabromocyclododecane(HBCDD) is mainly globally used as fire retardant additives for producing extruded or expanded polystyrene foam materials in bulk amount and has been widely manufactured from 1960. Moreover, it is used as an additive to manufacture various things such as upholstered furniture, automobile interior textiles, car cushions, electric and electronic equipment etc^{47,48}. In recent past decades it is one of the major environmental concern pollutant due to its persistent, toxic, bio-accumulative and biomagnifying or bio-transformative nature in environment.⁴⁹ For these reasons this cyclic aliphatic brominated compound is very harmful to aquatic life. Global market demand of HBCDD was 22000 tons per year in 2003. The major portion of HBCDD was used in Europe, which was estimated at 11,000 tons in 2006, of which about 96% were used in expanded and extruded polymer⁹. Recently, it is included in Annex A of the Stockholm Convention on Persistent Organic Pollutants (POP), 2009 and in 2013 for elimination with restricted uses.⁵⁰ Due to all of the adverse effects on environment it is restricted in Japan in 2014 and in Republic of Korea (South Korea) in 2015 permanently till the advanced substituted one upto 2020.⁵¹ In recent years, the bromine industry has taken important steps to reduce discharges from manufacture and use of HBCDD and other fire retardants, notably its production is closed in HBCDD manufacturing site Newton Aycliffenearly NE coast of England. Because of its dramatically negative environmental impact, the development of efficient HBCDD removal technologies has increasingly become a significant environmental concern. The bio-accumulative and biomagnifying nature of HBCDD is critically threaten to the sustainable development of our planet as influence of HBCDD on the environment is long-term and difficult to repair. Some traditional methods had applied to remove this HBCDD from water for example, debromination technique, adsorption technique etc. and also some micro biodegradation technique etc. had applied to degrade it.⁵²⁻⁵⁴ Here we approach a new novel way to remove this POP via making an encapsulation complex with β -cyclodextrin (β -CD) and hydroxypropyl- β -cyclodextrin (HP- β -CD) respectively. As HBCDD is a hydrophobic molecule, it may be inserted into the hydrophobic cavity of

cyclodextrin forming inclusion complex, its solubility in water increases enhancing bioavailability and faster biodegradability of HBCDD by microorganisms.

Irritable bowel syndrome (IBS), a gastrointestinal disorder is a most commonly diagnosed gastroenterological problem in medical sciences. Patients suffering from IBS are found to suffer frequently from various gastrointestinal disorder like, abdominal pain or dis-comfort, altered bowel habit, bloating⁵⁵ associated with the symptoms, incomplete bowel movement, urgency and tenesmus.⁵⁶ Thus, irritable bowel syndrome is a functional gastrointestinal disorder showing a lot of abnormalities. Hyper-reactive intestinal motility and visceral hypersensitivity are also found in patients suffering from IBS. Alverine citrate (ALVC) belonging from a class of antispasmodic drugs used to treat irritable bowel syndrome and diverticular disease. Alverine citrate acts as a muscle relaxant and relieves abdominal pain, constipation or diarrhoea caused by the abnormal activity of the gut muscle. It also relaxes the muscle in the womb which is caused by the muscle spasms in uterus. Voltage-gated calcium channels are the main transducers of membrane potential changes into intracellular Ca^{2+} transients such a way they intervene smooth muscle contraction and activate endocrine to release hormone.^{57,58} The supramolecular interaction between ALVC and cyclodextrins (CDs) to form inclusion complex was justified satisfactorily by the improvement of two novel aspects - (a) solubility of the ALVC into water for bioavailability, (b) drug delivery through HSA assisted controlled release from inclusion complexes.

A drug to show greater therapeutic effectiveness needs its bioavailability and solubility to a large scale. Pharmacological response to be shown by a drug a minimum concentration of it must be achieved, in this connection aqueous solubility of the drug to a desired level is significant. Solubility of a molecule may be defined qualitatively as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. It is found that ALVC-CDs inclusion complexes enhances the aqueous solubility of drug making it more bioavailable. Encapsulation of the hydrophobic part of the guest (ALVC) molecule into the hydrophobic cavity of suitable dimension of CDs makes it to increase aqueous solubility. The cavity dimension of the CDs should be moderate to reduce the contact between water and

the nonpolar regions of host and guest molecule. Among the CDs, α and β -cyclodextrins were used for the dimensional suitability of their cavity size.

As the organism – environment interaction is essential for its survival, on the molecular level small molecule like drug – protein/drug – gene product interaction is also essential that underlie the organism's ability to adapt to environmental changes and include those that bind, transport, and metabolize small molecules. Human serum albumin, the most abundant protein in blood plasma found to act as a carrier protein for vitamin, nutrients, hormone, steroid, drug like small molecule of low water solubility and binding ability of these molecules to HSA constitute a vibrant matter in pharmacokinetics.⁵⁹⁻⁶¹ Here, binding property of ALVC to the HSA had been studied spectrofluorimetrically. Thus, HSA assists the drug ALVC to release from the inclusion complexes and makes its transportation to the affective area where adsorption of the drug molecule to be needed. Our study to form inclusion complexes of ALVC thus become moralised by the solubility enhancement and HSA assisted transformation and controlled release of the drug in human body.

An analysis on the diverse molecular interactions of implausible food preservatives, Sodium benzoate (SBz), Sodium salicylate (Scyt) in the aqueous solutions of Benzyltriethylammonium chloride (BTEACl), Benzyltrimethylammonium chloride (BTMACl) having durable anti-bacterial effect have been explored thoroughly. According to our knowledge, it is well known to all, there is so much of food productions all over the world but because of the process of spoilage, a large proportion of these is lost. So, it requires a great attention to preserve foods, produced industrially and even at home. There are so many preservatives known, that utterly prevents spoilage of foods, but having some sort of side effects, usedness of these is an inherent threat to the world health. Now, use of these in minimal amount i.e. below the MIC (Minimal Inhibitory Concentration) in food would overcome the threat. The challenge was accepted and reveals that, two well-known food preservatives (FPs), sodium benzoate and sodium salicylate works properly against various fungus as well as gram-positive and gram-negative bacteria below their MIC in the presence of negligible amount of two ammonium based ionic liquids (ILs) benzyltriethylammonium chloride (BTEACl) and benzyltrimethylammonium

chloride (BTMACl), as a consequence reduces the unnecessary consumption of hazardous food preservative.

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 Molecule, Host Molecules, Ionic Liquids, Food Preservatives and Solvent molecules are listed below

Biologically Active Molecule:

- Synephrine
- Phenylephrine hydrochloride
- Thiamine hydrochloride
- Alverine citrate
- 1,2,5,6,9,10-hexabromocyclododecane

Host Molecules:

- α -Cyclodextrin
- β -Cyclodextrin
- 18-Crown-6 ether

Ionic Liquids:

- Benzyltriethylammonium chloride
- Benzyltrimethylammonium chloride

Food Preservatives:

- Sodium benzoate
- Sodium salicylate

Solvents:

- Water
- Dimethyl sulfoxide

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

Names 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
- ^1H NMR spectroscopy
- 2D ROESY
- Surface tension study
- Conductivity study
- Density study
- Viscosity study
- Refractive Index study
- Antimicrobial activity