CHAPTER I

NECESSITY OF THE RESEARCH WORK

I.1. OBJECT, SCOPE AND APPLICATION OF THE RESEARCH WORK

Research is a continuous effort of exploring new ideas, new definitions where all the new dimensions arise in a broader way. It can be done in any field. In the field of science research based ideas are of immense importance. Scientific research comprises of collecting data and establishing new facts with evidences. It's not only developing concepts, perceptions but also help in diminishing faulty beliefs. Research is a construction of new base. It is a practice to understand the human race and to find out how this understanding can be made beneficial for the whole world. For the betterment/growth of science researches are going on in every field of science especially in medicinal/pharmaceutical chemistry.

Nowadays supramolecular chemistry based researches are enforcing great impact in biomedical science unlike conventional chemistry. Supramolecular assemblies consist of discrete number of molecules by non-covalent interactions . There are enormous applications in the area of host-guest supramolecular chemistry, likewise drug delivery, catalysis, pharmaceutical and nano technologies. The exceptional noncovalent interactions between guest and the complementary host molecule result unbelievable outcomes ;that is why it is also termed as molecular recognition. Molecular self assembly can be constructed in order to get a pre-planned stoichiometry by taking a biologically potent or chemically reactive entity and a template which can be made according to requirements of the chemistry we are looking for. Modern approaches are progressing in the same technique. In making of larger structures e.g. vesicles, micelles, nano rods, membranes etc. this technique is somewhat difficult to maintain as they are not energetically favourable in such situations.

Inclusion complexation is an inevitable part of the host-guest chemistry. It is possible to explicate their structures by monitoring their type of interactions or attachments. The association constant of the above type of binding can be determined by

studying the thermodynamics of the complexation process. The main benefit of host-guest supramolecular chemistry is in the manufacture of functionalised biomimetic systems. It has great biomedical advantages. Targeted drug delivery and controlled release of the drug by using supramolecular systems reduce the side effects of the drug and make it more efficient in its therapeutic activity. Other important advantages in cell biology applying supramolecular designs are of utmost credit. The stability of the typical host-guest inclusion complex depends on a number of non-bonding interactions e.g. hydrogen bonding, van der Waals forces, hydrophobic forces and some electrostatic interactions.

Cyclic macromolecules introduce innovative thoughts in host-guest chemistry associated researches. Basically they don't have any end group. Porphyrins, cyclodextrins, crown ethers, calixarenes are the standard examples of macrocycles or macromolecules. They all have molecular hollow space. Among a lot of these macromolecules cyclodextrins are quite fascinating due to various reasons. Cyclodextrins have hydrophobic interior cavity with hydrophobic exterior which makes them soluble in water and by choosing specific guest molecules, they are competent enough to make inclusion structures.

Cyclodextrin-tailored nano particles are presenting great influence to improve the features of the consequential systems, such as conductance, electronic, thermal, and catalytic actions. A number of molecular architects have been planned to advance the efficacy of certain macromolecular systems. These are proved to be effectual in making different type of molecular machines, nanosensing, nanotubes etc. [1] [2] [3] [4] [5]

Host-guest inclusion complexation including cyclodextrins or cycloamyloses covered a huge region in advanced science. They have vast application starting from food, chemical industries, cosmetics ,drug delivery, pharmaceutical to environmental and agricultural sciences.[6] CDs can be produced by the enzymatic degradation of starch. There are three types of CDs in nature such as α ,β and $^{\gamma}$ CDs with 6,7,8 glucopyranose units. Due to the toroidal cone shaped CDs can completely or partially trap hydrophobic molecules into the cavity. The moderate cavity diameter of β- CD is suitable for encapsulating the guest or the entering molecule. Inclusion complexes of CDs are acceptable worldwide due to various reasons e.g. CDs have low production cost, water

soluble, low toxicity and appropriate cavity diameter. The host molecules that are taken here are α and β-cyclodextrins. The properties of the entering guest molecule have been changed greatly after complexation. These include solubility, permeability, stability, effectiveness, bioavailabiity, taste etc. Several bioactive molecules are being entrapped inside the CD moiety to improve their physical and chemical properties. [7, 8]

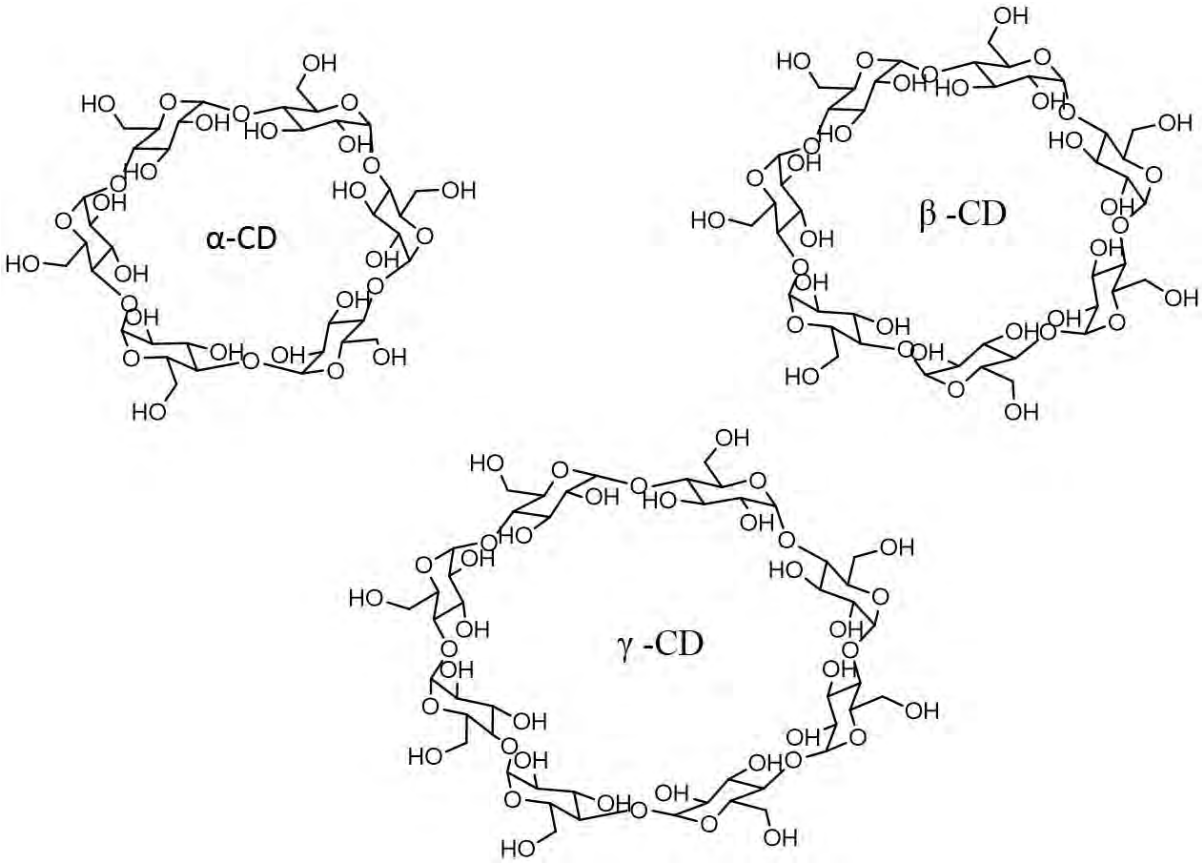

Fig.I.1. Structures of the Cyclodextrin molecules

To understand the chemistry of solution one need to understand the change in properties of an entity when it is dissolved in a new substance i.e. one needs to have a clear idea about the nature of solute and solvent molecules. What changes are taking place after they get into solution phase and also what kind of interactions are going on etc. should be taken into consideration . There will always be some anomaly when different solutes and solvents are mixed together. Studies of the aqueous solution of different molecules have given a variety of information regarding various thermodynamic properties. The contribution of a lot of intermolecular forces are there in

the process of solvation, these are dipole-dipole,ion-dipole,H-bonding, van der Waals forces. [9] The solute-solute interaction of uracil and gallic acid has been studied here.

In pharmacology, sustained release of the drug molecules is a very crucial thing. These types of bioactive molecules can be protected by encapsulating them inside the cavity of cyclodextrins to retain their therapeutic activity and to lessen their adverse effects. Moreover, it is also important to confirm that the inclusion complex has been formed between the host and the guest molecules. In order to achieve such goals inclusion complex of some biologically potent molecules with α and β cyclodextrins have been investigated. The concerned bioactive molecules are DL-Aminoglutethimide, Amiloride hydrochloride, Alibendol. On the other hand to minimize the harmful effects of certain dyes, complexation of the dye with cyclodextrin is considered to be a unique approach. Here complexation of Indigosulfonic Acid Dipotassium Salt with β-CD has been studied.

The synthesis of inclusion complex of the dye, Indigosulfonic acid dipotassium salt (ISD) and β- CD has been done along with characterization by different types of spectroscopic studies and calorimetric study. ISD is a derivative of indigo which is a natural dye. The application of dyes are vast including colouring food, textiles, medicinal products, cosmetics and they generally have complicated aromatic chemical structures. [10] Because of intramolecular H-bonds ISD seems to have high melting point. Moreover every synthetic dye must have some toxicity likewise ISD shows some toxicity when consumed. Whereas, β-CD has very low toxicity. So, formation of inclusion complex makes the overall moiety less toxic. The inclusion phenomenon of ISD and β-CD was studied to reduce the toxic effect of the dye. [11-14] [14-17]

The encapsulation of the drug DL-AGT and β-CD was investigated both in solid and liquid phase and it was also examined by the cell viability study that the inclusion complex is non-toxic. DL-Aminoglutethimide (DL-AGT) is an aromatase inhibitory drug. Formerly it was used as an anticonvulsant in United States but soon withdrawn from market due to some unanticipated side effects. It showed unusual side effects on various endocrine organs particularly adrenal. It restricts quite a lot of enzymes in the adrenal cortex. Its anomalous side effect turned out to be a blessing in the endocrine therapy. Hormone dependent human breast neoplasms are generally stimulated by estrogen

hormone can generate hormone dependent breast carcinoma. It can be possible to suppress the estrogen level by the use of some aromatase inhibitory drug and for that DL-AGT has been seemed to be effective. The antitumor efficacy of DL-AGT by suppressing estrogen found to be more productive in drug induced medical adrenalectomy than surgical adrenalectomy for the treatment of advanced breast cancer. Besides all these benefits DL-AGT has lack of specificity and side effects that are not linked to deprivation of estrogen. To make the drug more specific in its therapeutic activity and to decrease the side effects of it, an inclusion process has been done between the drug and a cyclic oligosaccharide β- CD. [18] [19] [20] [21]

There are already diverse pharmacological applications of the drug Amiloride hydrochloride (AMHCl) in literature. AMHCl is a guanidine diuretic, having an unlikeable taste ,synthesized first withinside the lab of Merck, Sharp and Dohme. It is 3,5 diamino-N-(aminoiminomethyl)-6-Chloropyrazine carboxamide. It has minor natriuretic, diuretic effects. It can expel sodium, holding potassium and used as a remedy in hypokalemia. AMHCl is used for the treatment of congestive heart collapse or high blood pressure. As the extra sodium is discarded in conjunction with excess water from the body, the blood pressure automatically reduced. Being a BCS drug of class III it possesses high solubility and less permeability.

The administration of any drug orally includes an array of limitations due to enzymatic degradation and first pass metabolism in the GI tract. Most of the drugs are metabolised in the gastrointestinal tract and thus the effective dose for the activity of the drug decreases i.e. its bioavailability decreases. Due to belonging to a BCS class III drug AMHCl has less efficient to pass through biological membrane. Now a days buccal routes are considered to be the smartest way for the drug to be orally administered. The drugs that can't be administered due to unpleasant odour can be taken as a form of mucoadhesive films as reported in the literature .

The inclusion complexation of the drug AMHCl with α and β-CDs not yet been discussed so far our knowledge is concerned. The approach may lead to various chemical advantages of the drug. The inclusion complex may diminish its side effects and increase its bioavailability or can be orally administered without any risk of hepatic first pass metabolism. [22, 23] [24]

2-hydroxy-*N*-(2-hydroxyethyl)-3-methoxy-5-(2-propenyl)benzamide or alibendol is of important pharmaceutical interest due to its activity in the field of medicinal chemistry. It has the formula of $C_{13}H_{17}NO_4$ and melting point of 95^0C and can be prepared by mixing ethyl ester of 2-hydroxy-3-methoxy-5-allyl-benzoic acid and ethanolamine under suitable experimental condition. The number of patents for the synthesis of alibendol is very little even the mentioning of alibendol is very less in literature.

The maximum choleretic, antispasmodic and cholekinetic drugs use alibendol in their composition as an active component. In biliary insufficiency it shows outstanding activity i.e. in dyspepsia, other uses are in alimentary intolerance and in hepatic origin constipation.

But as an active ingredient in antispasmodics it must have some sort of side effect such as vomiting, headache, dizziness, nervousness, irritation and in some cases allergic reactions. To consume such drugs may cause allergic reactions.

Alibendol included β –CD inclusion complex has not been studied earlier. We have studied the inclusion system with the help of some spectroscopic investigations. The pronounced inclusion system may decrease the limitations of the drug either by increasing bioavailability or by decreasing its side effects. [25] [26]

The pyrimidines and nitrogen heterocycles are mostly seen in a number of bio-active molecules and exhibit substantial therapeutic efficacy. Among these pyrimidine is found to be the most suitable architect in the chemistry of nucleic acid . The common natural derivative of pyrimidine is uracil. Uracil is the necleobase present in RNA but absent in DNA or it can be said that it is dimethylated in DNA, which is thymine. Uracil linked to the nucleobase adenine through two hydrogen bonds in RNA. It is reported earlier that uracil can be generated from pyrimidine by the action of UV light.

Uracil has a very little acidity. It can go through tautomeric shifts into amide-imidic acid due to nuclear unsteadiness which may occur owing to loss of aromaticity. The amide tautomer is the lactam structure and the imidic acid tautomer is the lactim structure. Generally uracil is more common in the lactum structure. Uracil has a variety of

application in drug discovery. Most of the synthesized drugs having antitumor, anticancer efficiency include uracil moiety in them.[27]

Gallic acid is a polyphenolic compound having tremendous antioxidant properties. There occurs some oxidative stresses due to normal metabolism, antioxidants of natural origin serve for the prevention of the cells from those oxidation. Oxidative stress is basically the formation of free radicals in our body. These reactive oxygen are not good for cells and gathering of these may lead to inflammatory disease, cardiovascular disease and chronic disease cancer. Gallic acid or 3,4,5-trihydroxy benzoic acid is a naturally occurring compound having low molecular weight. Its presence is widespread in the plants like oak barks, grapes, different types of berries, tea, mango, gallnuts and in vegetables.

We have investigated that there exists a strong interaction between uracil and gallic acid in the ternary system of uracil and aqueous gallic acid solution based on some physicochemical methodologies and some important parameters have also been derived. [28] [29] [30]

Therefore the objectives, scope and applications of the research work in brief are

- The most important objective of the research work is to study certain non-bonding interactions of some significant molecules especially in diverse system and also to collect the appropriate information regarding the phenomenon for future upgradation.
- The solvation consequences of certain bioactive molecules in ternary system provide us valuable information about changes in their physical properties.
- The thermodynamic results obtained from our research works are no doubt of extreme importance.
- Selection of biologically active molecule and by studying its biological activity after inclusion opens up new scopes for numerous future researches. This also has vast application for the betterment of bioactive compounds in their activity.

- By the course of this research work we understand different hydrophilic and hydrophobic interactions with the help of some physicochemical methodologies.
- The application of different host-guest inclusion systems here is to enhance the bioavailability and to reduce the side effects or toxic effects of certain bioactive and industrially important molecules.

I.2. SELECTION OF BIOACTIVE MOLECULES, DYE MOLECULE, HOST MOLECULES, SOLVENTS USED IN THE RESEARCH WORK

The names of the bioactive molecules, dye molecule, host molecules and solvents used in the research work are listed below.

Dye molecule:

- Indigosulfonic Acid Dipotassium Salt (ISD)

Bioactive Molecules :

- DL-Aminoglutethimide
- Amiloride hydrochloride
- Alibendol
- Uracil
- Gallic acid

Host Molecules:

- α – Cyclodextrin
- β -Cyclodextrin

Solvents:

- Water
- Dimethyl sulfoxide
- Acetonitrile
- Ethanol

I.3. METHODS OF INVESTIGATION

The following methods of investigation have been used in our research work.

- NMR spectroscopy (2D NOESY,ROESY and ^{1}H NMR)
- UV-Vis spectroscopy
- Powder X-Ray Diffraction
- Fluorescence spectroscopy
- FTIR spectroscopy
- Isothermal Titration Calorimetric Study
- Mass spectroscopy
- Scanning Electron Microscopy
- Density study
- Refractive index study
- Viscosity study
- Cell viability study
- Antimicrobial study

The partial molar volume, viscosity B co-efficient and other thermodynamic parameters give us important information to explain solute-solute, ion-ion and ion-solvent interactions. The nature and extent of ion-solvent interaction can also be predicted from the sign and magnitude of the partial molar volume.