# CHAPTER I

## NECESSITY OF THE RESEARCH WORK

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

Host –guest inclusion and release are extremely significant in pharmaceutical and bio medical fields for improving drug delivery science in recent years.[1] Host–guest interactions include a complementary stereoelectronic order of binding sites in host and guest.[2] Studies regarding the supramolecular host–guest complexes are a fast advancing research domain in chemistry, where encapsulation of a guest molecule inside the macrocyclic cavity of a host takes place. Various host molecules are used for the encapsulation purpose such as calixarenes, pillararenes, cucurbiturils, cyclodextrins. The host guest inclusion complexes have applications as supramolecular materials, in enzyme activation, photo sensing, temperature dependence, changes in pH/redox and competitive binding for regulatory release of guest molecules.[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.

Macrocyclic host molecules are of immense importance in Inclusion Complexes (ICs) as the cyclized and constrained conformation offer the benefit of molecular selectivity. The cyclodextrins (CDs) are exclusively interesting in this regard, due to their amphiphilic nature. CD got prior importance in the field of supramolecular chemistry due to construction of metal nano-particle modified CDs.[4] Conjugation of CD and various nano-particles enhances their characteristics such as electronic, thermal and catalytic properties of the guest and modify the macrocyclic host as nanosensors, drug delivery vehicles and

recycling extraction agents.[4] CDs have potential application in formulation of molecular switches, molecular machines, supramolecular polymers, etc. 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.

cyclodextrins family is considered as one of the most popular potential hosts for several reasons. It can be formed from starch by enzymatic bacterial degradation.[5] They are financially affordable for industrial purpose and also safe for the human consumption as they are non toxic in nature. Cyclodextrins have a shape like truncated cone. This cyclic oligosaccharides contains six ($\alpha$-CD), seven ($\beta$-CD) and eight ($\gamma$-CD) glucopyranose units, bound by $\alpha$-(1–4) linkages.[6] 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. It has exceptional ability to form inclusion complexes with a series of guest molecules by encapsulating the non-polar part of the guest into its hydrophobic cavity and stabilizing the polar part by the polar rims.[7] 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.

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. The experimental conditions, such as concentration, temperature, pH, etc. also play crucial roles exhibiting their potential applications in gene and drug delivery. 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.[8] 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. The chemical stability of guest molecule also increases due to encapsulation inside the cavity.

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 materials. Series of external stimuli, e.g., enzyme activation, light, temperature, changes in pH or redox and competitive binding may be employed to operate the release of guest molecules from the inclusion composites.[9] Recently cyclodextrin modified nanoparticles are of great interest as these supramolecular macrocycles significantly combines and enhances the characteristics of the entities, such as the electronic, conductance, thermal, fluorescence and catalytic properties expanding their potential applications as nanosensors, drug delivery vehicles and recycling extraction agents. Different sophisticated probes based on semiconductor nanocrystals and other nanoparticles have been designed for this purpose, because of their potential applications in the fabrication of molecular switches, molecular machines, supramolecular polymers, chemosensors, transmembrane channels, molecule-based logic gates and other interesting host  guest systems.

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.[10] 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.

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. If a drug is 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.[11]

In this thesis the four studied amino acids have biological activity in human bodies. **:** L-Leucine is used in the biosynthesis of proteins and is essential in humans, *i.e.*, our body cannot synthesize it and thus it must be incorporated from outside, which may be done using α and β-Cyclodextrins as carriers. It is a major component of the subunits in ferritin, astacin etc. proteins and L-Leucine is also activates mTOR; the later can be explained as the only nutritional amino acid that has the capability to immediately kindle muscle protein synthesis.[12] It is also present in the liver, adipose tissue and mussle tissue. Adipose and mussle tissue uses L-Leucine in the creation of sterols. This amino acid is quickly reached in the brain and the astrocytes present there translate it to alpha-ketoisocaproate through the path of transmission of alpha-ketoglutarate to glutamate.[13]

L-Isoleucine helps in biosynthesis of proteins in human and considered as essential in humans. L-Isoleucine is prepared using pyruvate utilizing leucine biosynthesis enzymes in various microorganisms such as bacteria.[14] Isoleucine is known to be a glucogenic and a ketogenic amino acid. The process of trans amination with alpha-ketoglutarate, the carbon skeleton has a tendency to convert into either succinyl CoA, and become a part of the TCA cycle for oxidation or can be transformed to oxaloacetate and used in gluconeogenesis. The same process can be done by transforming into acetyl CoA and enter into the TCA cycle by condensation with oxaloacetate forming citrate. For mammals Acetyl CoA is unable to transform again to carbohydrate but helps in the synthesis of ketone bodies or fatty acids, and that's why considered as ketogenic.

Biotin, commonly known as vitamin B7 or vitamin H, is a necessary obligation for the complete catabolism of isoleucine (also for leucine). Without

sufficient biotin, the humans are not able to fully break down isoleucine molecules.[15]

L-Asparagine helps in biosynthesis of proteins in humans. L-Asparagine is also necessary for the improvement of brain and has a vital role in the preparation of ammonia. Uaually the reaction between asparagine and some reducing carbohydrates or other compounds using carbonyls fabricates acrylamide in food after heating to optimum temperature. The products thus formed are present in baked goods such as French fries, potato chips, and toasted bread.

The asparagine amino acids form long chains by the hydrogen bond interactions with the peptide backbone, this amino acid residues are commonly found at the starting of alpha-helices as asx turns and asx motifs, and in similar turn motifs, or as amide rings, in beta sheets. Its main function is to cap the hydrogen bond communications that can be also fulfilled by the polypeptide backbone. The Glutamines having an extra methylene group, have higher entropy due to conformation and hence they are less capable for capping. Asparagines also helpful providing free sites for N-linked glycosylation, amendment of the protein chain with the accumulation of carbohydrate chains.[16] Naturally, a carbohydrate side chain can exclusively be summed up to an asparagine residue if it is edged on the C side by X-serine or X-threonine, here X is any amino acid with the exception of proline. L-Aspartic acid is the precursor of many essential amino acids and participates in gluconeogenesis process in mammals. In the human body, aspartate is most frequently synthesized through the transamination of oxaloacetate. The biosynthesis of aspartate is facilitated by an aminotransferase enzyme: the transfer of an amine group from another molecule such as alanine or glutamine yields aspartate and an alpha-keto acid. Aspartate is also a byproduct of the urea cycle.

In plants and microorganisms, aspartate is the precursor to several amino acids, including four that are essential for humans: methionine, threonine, isoleucine, and lysine. The conversion of

aspartate to these other amino acids begins with reduction of aspartate to its "semialdehyde," $O_2CCH(NH_2)CH_2CHO$. Aspartate has many other biochemical roles. It is a metabolite in the urea cycle and participates in gluconeogenesis.
Two drugs are used in two different experiments in forming inclusion complexes with α and β-CD. chloroquine diphosphate is mainly used to prevent malaria. Certain types of complicated cases of malaria typically require additional medication. Chloroquine diphosphate is mostly used to treat malaria. Many categories of complex cases of malaria characteristically need some special medication. This drug is frequently used for the treatment of amebiasis, rehumatoid artharitis and lupus erythematosus.[17] Women can safely take this during pregnancy time. It is useful to prevent the asexual type of malaria within the red blood cell. Chloroquine is widely used in along the field of medicines, this can be supplied to the materialization and extend of resistance. There is still recommendation to verify whether chloroquine is helpful in the province prior to using it. However in areas where conflict is present, additional antimalarials, as for example mefloquine or atovaquone, can be used. The Centers for Disease Control and Prevention suggest in the healing of malaria with chloroquine alone owing to higher efficient combinations. In order to cure amoebic liver abscess, this drug may be used with other efficient medications in case of failure of development with metronidazole or another nitroimidazole with a time limit of 5 days or bigotry to metronidazole or a nitroimidazole. This drug have the capability to mildly restrain the immune system, it has use in some autoimmune disorders, namely rheumatoid arthritis and lupus erythematosus.[18]

Probenecid is a vital drug which is particularly used to prevent gout and hyperurecemia. Besides this the drug is also helpful to restrain renal secretion of some other drugs, resulting an increase in the concentration of plasma and extending their functions. Probenecid is often employed to enhance the attentiveness of some antibiotics and also defend the kidneys by giving with cidofovir.[19] Particularly, a few evidences support the employ of intravenous cefazolin only one time inspite of using it three times a day in

combined with probenecid. The other function of probenecid is masking agent, particularly assisting athletes by performing as enhancing substances to shun recognition by drug tests.

Probenecid perhaps has various pharmacological functions, such as blocking pannexins. Probenecid is very efficient in the treatment of gout and the method of action is considerd to be centered on the kidney. Probenecid has the main action on kidneys' organic anion transporter (OAT), which regains uric acid from the urine and precedes it to the plasma. If this drug is present, the OAT is attached particularly to it (without binding to uric acid), it prevent the reaccumulation of the uric acid. Consequently, the urine contains more uric acid, decreasing uric acid concentration in the cell fluid.[20]

Three ionic liquids used for research experiments have vast applications as green solvents. 1-butyl-3-methylimidazolium chloride is liquid at room temperature. This ionic liquid has vast applications in chemical reactions, synthesis, cellulose processing, nuclear fuel reprocessing, waste recycling, metal air batteries etc. They are considered as green solvents as they do not produce any environmental hazards.[21] Because of its distinctive properties they are attracting increasing attention in many fields such as organic chemistry, electrochemistry, catalysis, physical chemistry and applied supramolecular chemistry.

1-butyl-1-methylpyrolidinium chloride is liquid at room temperature. The ionic liquid are good examples of neoteric solvents, new types of solvents, or older materials that are finding new applications as solvents, which is environmentally friendly (or eco-friendly) because they are less hazardous for human body as well as less toxic for living organisms, used as recyclable solvents for organic reactions and separation processes, lubricating fluids, heat transfer fluids for processing biomass and electrically conductive liquids as electrochemical device in the field of electrochemistry (batteries and solar cells).

Trihexyltetradecylphosphonium chloride is liquid at room temperature and appears as colourless. Ionic liquids are generally constituted with a large organic cataion and a small anion. They have vast applications in various chemical industries because of their green nature. They produce less hazardous compounds during their use.Phosphonium based ionic liquids are less toxic and more thermally stable than nitrogen based ionic liquids. This ionic liquid is highly used in separation of different dyes including methylene blue from aqueous media. This has also application as additives to improve the yield of essential oils in the hydrodistillation process.[23]

## I.2. Choice of Host and Guest Molecules:

**Host Molecules:**

(i) α-Cyclodextrin
(ii) β-Cyclodextrin

**Guest Molecules:**

**Amino Acids:**

(i) L-Leucine

(ii) L-Isoleucine

(iii) L-Asparagine

(iv) L-Aspertic Acid

**Ionic Liquids:**

(i) 1-butyl-3-methylimidazolium chloride

(ii) 1-butyl-1-methylpyrolidinium chloride

(iii) Trihexyltetradecylphosphonium chloride

**Drugs:**

(i) Probenecid

(ii) Chloroquine diphosphate

## I. 3. Methods of Investigation

Names of the investigation methods are listed below:

*$^{1}$H NMR spectroscopy*

*2D ROESY*

*FTIR spectroscopy*

*UV-visible spectroscopy*

*High resolution mass spectrometry*

*Surface tension study*

*Conductivity study*

*pH study*

*Density study*

*Viscosity study*

*Refractive index study*