CHAPTER I

NECESSITY OF THE RESEARCH WORK

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

We live in a quickly growing and transient world with a numerous possibility. Gradual growth in technology, science makes greater impact in our society. Growth in scientific research led to many changes to traditional belief, systems, structures and mode of working thus exciting advanced opportunities are arising. These developments are leading to the change in the world of chemistry as a discipline and the role which chemists play. The environment where we work also changing. Scientific research helps to accumulate information and investigates different phenomena which in turn supports us to develop various theories and finally to understand nature. The gradual development in scientific research makes prediction-solving problems. Continuous involvement in research may create surprising outcomes. They may have direct impact in the immediate environment, such as related to chemical processes, or their developments in much broader context. Research creates new knowledge and understanding, thus helping us to plan the future to transform to the world of promising prospects. Understanding how existing trends in research have developed can help us to progress towards new paradigm of many unexplored fields in the future. Hence, scientific research is the key to realize everything that we have been doing, where we live, this earth and most importantly the whole universe. We human beings are doing research from centuries and science has reached to this stage through gradual contribution made by numerous scientist and researchers across the globe. This world is made up of molecules and interaction among variety of molecules such as living or non-living is the reason for our existence. As a result, a great deal of chemical research is dedicated to exploring matter – living organism interactions. These interactions can be of several types but the most important that we observe in living world is either covalent or non-covalent.

Non covalent interaction covers the broader area of supramolecular chemistry and solution chemistry.

The area of supramolecular chemistry was pioneered by Jean-Marie Lehn, Charles J. Pedersen and Donald J. Cram in 1987 when they were honoured with the Nobel Prize for their outstanding work in this field [1]. In this field of science, we deal with the chemistry of non-covalent bond. The introduction of lock and key principle by Emil Fischer in the year 1894 laid the philosophical roots of supramolecular chemistry [2]. The word supramolecule was coined in the 1930 after intermolecular interactions led to the invention of molecular aggregation. During 1950s Cramer worked extensively on cyclodextrins. Likewise in 1960s, Pedersen's work on host-guest complexes with crown-ether, Cram's research on spherands, cavitands, and very recently Stoddart's excellent work on box container like molecules forwarded the supramolecular chemistry research to manyfold [3,4].

Supramolecular chemistry is generally divided into three broad types: (i) clathrates, (ii) self-assembly and (iii) host-guest chemistry. In clathrates, the guest molecules with relevant size and shape are encapsulated within the pothole lattice of host molecules [5]. In the field of host-guest chemistry, variety of guest molecules are accommodated inside the nanocage of various host molecules (e.g., calixarenes, cucurbiturils, cyclodextrins) [6]. Likewise self-assembly are the class of molecules that form automatically assembled aggregates [7].

Supramolecular compounds generally involve non-covalent intermolecular interactions like π-π interactions, hydrogen bonding, electrostatic forces, hydrophobic and van der Waals forces which led to their formation [8-10]. Supramolecular chemistry covers the very significant concepts like molecular recognition, molecular self-assembly, host-guest chemistry, molecular machineries and dynamic covalent chemistry etc [11].

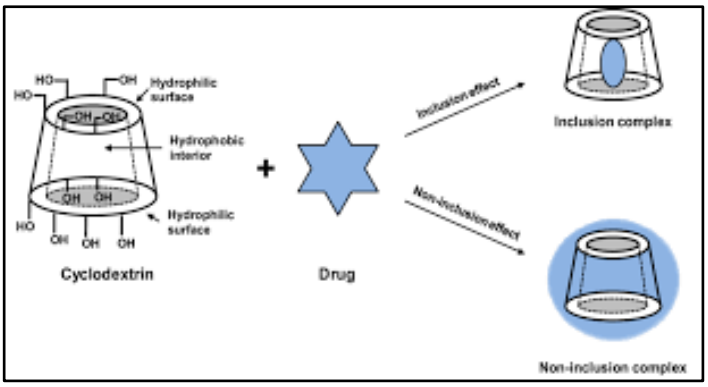

Figure 1. Schematic illustration of the encapsulation of a host and a guest forming supramolecular inclusion complex.

A schematic representation of inclusion complexation with non-covalent interaction between a guest and a host has been shown in Figure 1. The host molecule is generally a large macrocyclic compound such as cyclodextrins, crown ethers and calixarenes or sometimes enzyme, containing suitable cavity [12,13]. The guest that have been used may include inorganic anions, ion pair, inorganic or organic cation, or a complex organic molecule such as anticancer drug [14,15]. Naturally existing host-guest systems includes antigen-antibody, DNA-ligand, enzyme-substrate, antigen-antibody and protein-carbohydrate complexes.

The research in macrocycle-based host–guest compounds greatly led to the advancement of supramolecular chemistry [16]. Compounds like calixarenes, cyclodextrins, pillararenes, cucurbiturils, crown ethers, and other macrocycles serves as host molecules [17-22]. These compounds have blessed cyclic conformations that promotes the selectivity for different molecules with versatile prospects for encapsulating different guest molecules [16,23]. The cyclodextrins (CDs), due their amphiphilic properties, are considered mostly for this purpose [23,24]. They have capacity to self-assemble in aqueous environment to generate explicit geometries like nanotubes, nanorods, vesicles, nanosheets, and micelles etc. They find application in various fields such as cell nanodevices, imaging and drug delivery [25-27]. Recently reported cyclodextrin-modified nanoparticles have come up as a potential agents as they improve electronic, fluorescence, catalytic, conductance, and thermal properties thereby enhancing their significant applications in the form of nano sensors and drug delivery vehicles [28,29]. Subsequently, a great deal of research have been dedicated to develop the state of art methods to manufacture compounds that can be used as

transmembrane channels, chemosensors, supramolecular polymers, molecular switches, molecular machines, molecule-based logic gates, and other interesting host–guest systems [30-32].

Inclusion complexes of cyclodextrin find their application pharmaceuticals, goods [33], and even in food industries [33-36]. CDs have distinctive two-phase layers with inside hydrophobic and outside hydrophilic surfaces. The hydrophobic cavity encapsulates the of non-polar portion of the guest compounds through the variety of non-covalent interactions [37]. Here in this thesis, α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) containing 6 and 7 glucopyranose units, have been selected as host molecules because easy availability and suitable cavity dimensions, great encapsulation capacity with subtle toxicity [38,39]. The CDs are extensively used in cosmetic industries [40], food industries, tissue engineering, pharmaceutical materials and devices used in bio-medical and pharmaceutical fields. Inclusion complexes are used to protect the large range of compounds includes drugs, bioactive molecules, volatile organic compounds, flavonoids, essential oils, taxols, enzymes, vitamins [41], etc. to expand their stability towards heat, light and air along with their biocompatibility, solubility in water and to lessen toxic effects.

To enhance the stability of biologically potent molecules and their controlled release are presently the major challenge in pharmacology. Apart from these the enhancement in the utility of drugs is a priority among researchers in the present scenario. Hence, it is of great importance to minimize the consequences due to the side effect of the drug. One way to achieve this to entrap guest molecules into cyclodextrins and other hosts. Therefore, to attain this objective, the inclusion complexes of different three biologically potent molecules such as Rhodanine (RH), Nitrofurantoin (NTF) with both α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) and of Gemcitabine (GEM) with β- cyclodextrin (β-CD) have been investigated.

In this thesis inclusion of RH into both α-CD and β-CD have been studied. Rhodanines are used in solar cells and pigmentation as it has the capacity to adjust the spectroscopic and electrochemical properties [45]. Rhodanine, a heterocyclic molecule has a wide spectrum of biological applicability like antibacterial [49–56], antioxidant [46], HIV integrase inhibitor [43, 57], anti-inflammatory [47, 48], antifungal [59,60], and the very vital one is anticancer [58–60]. Rhodanines are

among few compounds that can inhibit the penicillin-binding proteins (PBPs), serine (SBL)-β-lactamase and metallo-β-lactamase (MBL)s [62,63]. RH is extensively used in silver estimation, its derivatives can be applied in the medicament of diabetes, obesity, Alzheimer's disease, cystic fibrosis, thrombocytopenia, cancer, sleep, mood, and central nervous system disorders as well as chronic inflammation [63, 65, 66]. Hence it is considered as a privileged structure with wider spectrum of applicability. They are also act as thyromimetic and anti-ischaemic agents [43, 44, 64]. Thus, to enhance the biocompatibility of RH molecule in medical application as well to increase its resistance towards temperature changes, light and acidic environment for its controlled and safe delivery at the selected site, it is very specific to investigate the encapsulation process of RH molecule into the cavity of different CD molecule along with thermodynamic angle of such inclusion process.

Enhancement in the utility of drugs is an utmost priority among researchers in the present world. One way to achieve it, is by minimizing the consequences due to the side effect of the drug. Certainly, it is very important to develop methods or to modify existing procedures to increase the bioavailability of drug molecule as more than half of the population around the globe suffers from the various diseases caused by bacterial, fungal, microbial infections. But with the success of available vaccines and antibiotics the mortality rate due to such diseases has been brought down drastically. Along with this, the current improvement in primary and secondary precaution for cardio-vascular diseases have made this clear now that cancer is actually the first or second cause of premature death in more than 180 countries in recent time [67]. In the year 2008 over 12 million people were diagnosed globally by cancer and the number is increasing rapidly till date [68]. During last few decades many chemotherapeutic drugs have shown significant activity against different types of human carcinoma. Among various chemotherapy agents gemcitabine (2'-deoxy 2' 2'-difluorocytidine) is found to be one of the most promising and effective agents against different types of cancers [69-71]. Gemcitabine (GEM) is a chemotherapeutic drug with huge potential for treatment of different type of cancers [72-74]. It is an anticancer drug with promising clinical application but has limited effectiveness because of its toxicity and inactiveness in serum. It is a leading drug for pancreatic cancer treatment but has limited efficiency due to fast deamination of N-4 amine thus making it inactive in blood plasma [75-77]. A great variety of gemcitabine prodrugs

have been investigated and developed to protect GEM from deamination; still, the clinical application of such prodrugs has been restricted due to their toxicity [78-80]. When gemcitabine is combined with the FOLFIRINOX, the regimen has found to increase its activity even in case of metastatic pancreatic cancer but also sufficiently increases the toxic effect to manifold, thus leading to the patient in an adverse condition [81-83]. Therefore, a great deal of research was increasingly being carried out in past few decades to improve the drug delivery system of GEM [84-86]. A phase III clinical trial of gemcitabine in combination with erlotinib reported a slight improvement in overall survival (6.24 vs 5.91 months) [81,82,87-95]. Likewise, the phase II trial of ACOSOG Z5041 showed positive results with when gemcitabine is given along with erlotinib [77] However, the scattered or arbitrary medical trial LAP07 reported that the combination of drug GEM failed to enhance survival despite patient consent [78]. The multicenter scattered phase III trial CONKO-005 disclosed almost no change in the universal survival of pancreatic cancer. In fact, most of the medical trials have highlighted the adverse effect due to the use of combination chemotherapeutic drug [74–80]. Encapsulating cancer drugs within nanoparticles (NPs) can lessen their side effects through selected delivery and guided release. The U.S. Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) has approved Polylactic-co-glycolic acid (PLGA) NPs as drug delivery systems. PLGA has various supreme properties such as surface tunability, biocompatibility, biodegradability, and controlled release [81–83]. Jaidev et al. evaluated the biophysical and photophysical properties of gemcitabine-loaded PLGA nanocavities in vitro [84], and Aggarwal et al. prepared PLGA–polyethylene glycol (PEG) NPs layered with the anti-EGFR monoclonal antibody for sustained delivery to malignant cells [85]. However, one limitation these NPs suffer is that they are acknowledged by the reticuloendothelial system as foreign particles and are subsequently partly removed by the immune cells [86]. Recently camouflaged NPs have been developed and investigated for the sustained release of drug. Although a great variety of work has been reported yet this technique has not been standardized for large population. Again, cost effectiveness is also a serious concern as half of the world suffering badly from cancer comes from under developed nations [67, 68]. Furthermore, the treatment of cancer with cancer drugs may cause additional difficulties of eroding the immune system and in some cases may even lead to

immunodeficiency. Therefore, cancer patients undergoing chemotherapy treatment are more susceptible to acquire different kind of microbial infections. According to various reports, microbial infections is among the major reason of mortality among malignant patients [70]. Thus, there is an absolute need for a drug which has anti-cancer properties along with antibacterial properties. Gemcitabine fits very well in this criterion [87-92]. But, the rapid decrease in gemcitabine concentration in blood plasma requires the excess doses of the drug in chemotherapy treatment. Also, as mentioned above there is an urgent requirement to develop a delivery system which enhances its sustained release with decreased rate of deamination along with retaining/enhancing its antimicrobial property as. The drug with such potential application could lead to decrease the secondary effect and increase the probability of rapid recovery and ultimately the survival of cancer patients. Hence, considering all above conditions we have explored the inclusion complex of GEM with β-cyclodextrin (β-CD).

Nitrofurantoin is a compound with significant antibacterial activity as well as photophysical properties. Nitrofurantoin is used for the prohibition and treatment of urinary tract infections. Nitrofurantoin also shows cytotoxic activity against various cancer cells such as colon, prostate, cervix cancer cell lines and human leukaemia by inhibiting the proliferation [93]. Nitrofurantoin is a potential pharmacophore molecule with pro-oxidant activities and is involved in the mitochondrial reactive oxygen species (ROS) pathway, producing oxidative stress, which is responsible for its anticancer activity [94,95]. Nitrofurantoin is a (E)-1-[(5-nitro-2-furyl) methylideneamino] imidazolidine-2,4-dione [96,97] and it's used in clinical application because it can suitably tune their electrochemical and spectral features [98]. However, due to their negligible solubility in water, they find limited application in fields like drug delivery and bioavailability. Not only this, but there are two serious NTF- associated disadvantageous reactions are pulmonary and hepatic [99,100]. Hence, to address all these issues i.e., to increase biocompatibility and decrease its adverse effects on human health we have prepared two inclusion complexes (ICs) of NTF. A major advantage of this approach is remodeling of an already well-known /approved drug in terms of improving physicochemical and pharmacokinetic properties.

In order to investigate the stability of proteins, ionic liquids (ILs) are generally employed as a novel medium [101]. Amino acids are considered ideal system for investigating the characteristics of proteins [102-104]. Further denaturation, solvation and dissociation of enzyme are highly affected by the neighbouring environment [105]. In this thesis, a study featuring the variety of physicochemical characteristics of amino acid in solution of ionic liquid in water have been explored [106]. This work helps in interpretating the behavior of these compounds in complex structures of proteins [107-110]. Here we have selected an Ionic liquid as an additive (electrolyte) as they are blessed with various advantages as a function of concentration, temperature, and ambient pressure [111]. Thermodynamic, viscometry, volumetric, refractometric, surface tension measurements have been carried out as these properties are susceptible towards the solute-solute and solute-solvent interaction [112]. Investigation of these properties greatly support to understand the structure and characteristics of solutes in aqueous medium and gives a reliable explanation for the complicated nature of molecular interactions in various biochemical processes occurring in the human body [113]. In this work we have carried out the systematic measurements of density, viscosity, molar refractive index, surface tension, and conductance of L-Leucine in two aqueous solutions of ILs namely Benzyltriethylammonium chloride (BTEACl) and Benzyltributylammonium chloride (BTBACl) aiming to understand solute-solvent interactions. In this regard, different thermodynamic parameters have also been obtained and explain in order to understand better such interactions among them. These data are used to compute apparent molar volume (ϕ_V), limiting apparent molar volume (ϕ_V^0), Falkenhagen coefficient (A), and viscosity B- coefficients.

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

Names of the Host Molecules, Biologically Active Guest Molecules and Solvents are listed below:

Host Molecules:

- β-Cyclodextrin
- α-cyclodextrin

Biologically Active Guest Molecules:

- Rhodanine (2-thioxo-1,3-thiazolidin-4-ones)
- Gemcitabine (2'-deoxy 2' 2'-difluorocytidine)
- Nitrofurantoin{(E)-1-[(5-nitro-2-furyl)methylideneamino] imidazolidine-2,4-dione}

Amino Acids:

- Leucine

Ionic Liquids:

- Benzyltributylammoniun chloride
- Benzyltriethylammonium chloride

Solvents:

- Water
- Dimethyl sulfoxide
- Ethanol

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

Name of the Investigation Methods are listed below:

- UV-Visible spectroscopy
- Powder X-Ray Diffraction
- Differential Scanning Calorimetry
- Scanning Electron Microscopy
- ^{1}H NMR spectroscopy
- FT-IR spectroscopy
- Surface tension study

- Conductivity study
- Density Study
- Viscosity Study
- Refractive Index Study
- Mass spectrometry
- Molecular Docking Study
- Antibacterial activity study
- Cell viability assay
- Photostability Study
- CT-DNA Study