

CHAPTER I

INTRODUCTION

An overview of solution and supramolecular chemistry is provided in this chapter. The chapter primarily covers a broad overview of supramolecular assembly based on cyclodextrin, as well as its numerous applications in drug delivery, biology, and a variety of molecular interactions involving ionic liquid, medicinal drugs, saccharides, and amino acids in aqueous medium. The primary goals of the thesis are covered, along with the general characteristics of cyclodextrin-based inclusion complexes, their characterization, and the significance of their hybrids. The chapter concludes with a brief discussion of the purpose, scope, goals, and applications of our research.

NECESSITY OF THE RESEARCH WORK

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

Research is done to advance knowledge through the systematic gathering, analysis, and assessment of data. New understanding and information are produced via research. Since the creation of the planet, humans have been conducting research. Since matter and living things make up the world, the majority of research efforts are focused on examining the interplay between matter and living things.

Jean-Marie Lehn coined the phrase "supramolecular chemistry" and, along with Pedersen and Cram, was awarded the Nobel Prize in 1987 for his substantial contributions to this field [1]. This is a new area of study in material science chemistry that includes both the chemistry of non-covalent bonds and the chemistry of molecular assemblies. The lock and key idea was identified by Fischer in 1894, marking the first description of supramolecular chemistry [2]. The term "supramole" was first used in the 1930s when it was discovered that molecules can aggregate through interactions between molecules. Research in the field of supramolecular chemistry has accelerated since the 1950s thanks to substantial works on cyclodextrins by Cramer, Pedersen in the 1960s on the host-guest complexes of crown ether compounds, and Cram on spherands, cavitands, and most recently, box-like container molecules found by Stoddart [3, 4].

According to size and shape, supramolecular chemistry is now classified into three main categories: (a) host-guest chemistry; (b) clathrates; and (c) self-assembly. Host molecules, sometimes referred to as cavitands in host-guest chemistry, are molecules that have permanent intramolecular cavities to enclose guest molecules (e.g., cyclodextrins, calixarenes, and cucurbiturils) [5]. When two or more host molecules create a gap between one another, an extramolecular cavity is created, forming a crystalline complex known as a clathrate. Another supramolecular entity that defies standard host and guest classification is self-assembly, which involves two molecules.

One unique benefit of supramolecular complexes is their ability to clarify the presence and bounds of binding energy additivity, which is a given in most applications, such as sensible medication design. An additional benefit is that several interactions typically contribute to supramolecular complexes, and a single association step already pays the loss of translation entropy for each intermolecular relationship. The Van der Waals forces of contacts, hydrogen bonding interactions, π - π stacking, electrostatic interactions, and hydrophobic interactions are among the non-covalent forces that stabilize the inclusion complexes [6]. The vast area of supramolecular chemistry covers a variety of topics, such as dynamic covalent chemistry, host-guest chemistry, molecular machinery, molecular self-assembly, and molecular recognition.

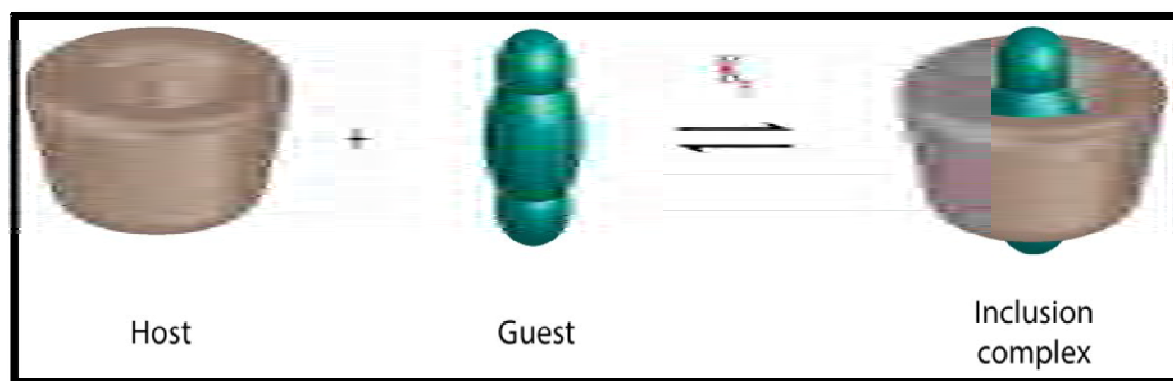


Figure 1: Schematic illustration of host-guest supramolecular inclusion complex.

The study of non-covalent complexation or binding between a host and a guest is the focus of supramolecular host-guest chemistry, as seen in Figure 1. A large molecule or aggregate, such as an enzyme or synthetic cyclic compound, with a sizable, pre-organized center hole or cavity, like CDs, calix[n]arenes, crown ethers, etc., is referred to as the host [7, 8]. A simple inorganic anion, an ion pair, an organic or inorganic cation,

or a more complex organic molecule, like an anticancer medication, could be the guest [9, 10]. The natural host-guest systems found in our mother nature also include protein-carbohydrate complexes, DNA-ligand pairs, antigen-antibody pairs, and enzyme-substrate pairs.

The host-guest chemistry based on macrocycles has contributed to the progress of supramolecular chemistry [11]. Cyclodextrins, calixarenes, crown ethers, cucurbiturils, and other macrocycles are some of the macrocyclic hosts [12, 13]. Because of their amphiphilic nature, cyclodextrins (CDs) are especially intriguing in this context [14]. Amphiphiles are of interest because they can self-assemble in aqueous systems to create well-defined structures including micelles, vesicles, nanorods, nanotubes, and sheets. These structures can be used in a variety of applications, such as drug administration, nanodevices, and cell imaging [15, 16]. The properties of the assemblies, including conductance, electronic, fluorescence, catalytic, and thermal properties, have been greatly enhanced by cyclodextrin-modified nanoparticles in recent years, which has attracted a lot of attention and improved the assemblies' potential applications as drug delivery vehicles and nanosensors [17, 18]. As a result, a wide range of complex probes have been developed for use in the production of molecular switches, supramolecular polymers, chemosensors, transmembrane channels, logic gates based on molecules, and other intriguing host-guest systems [19, 20].

Cyclodextrins (CDs) are harmless nanocarriers called cyclic oligosaccharides that are produced from starch and contain six (α -CD), seven (β -CD), eight (γ -CD), or more (α -1, 4)-linked α -D-glucopyranose units [21]. While the inside cavities of cyclodextrins are largely hydrophobic, the outside surface of CDs is hydrophilic. CDs can operate as hosts for both polar and nonpolar guests, such as tiny molecules and various medicinal compounds [22]. Owing to its binding characteristics, CDs can form stable inclusion complexes with a variety of physiologically active substances, including dietary and pharmaceutical components [23, 24]. Here, the host molecules are α -cyclodextrin and β -cyclodextrin, which have 6 and 7 glucopyranose units, respectively. These are chosen because of their high inclusion efficiency, small size, affordability, and minimal toxicity [25]. The pharmaceutical, food, and cosmetics sectors, as well as tissue engineering and biomedical devices, have all discovered extensive uses for CDs [26]. To extend the light,

air, and thermal stability of various bioactive molecules, enzymes, medications, volatile organic compounds, flavors, essential oils, taxols, flavonoids, vitamins, and so on, as well as to improve their water solubility, bioavailability, and side effect shielding, inclusion complexation is used within the non-polar cavity of CDs.

Pharmacology is now very concerned with the stabilization and sustained release of pharmaceuticals. It is essential to research if drug and dye molecules may be encapsulated inside the cyclodextrin molecule to protect them from environmental influences and minimize adverse effects for their controlled release. To accomplish this goal, research has been done on the inclusion of complex formation of bioactive guest molecules, such as Valacyclovir (VC) and Brilliant Blue FCF (BB), with host molecules, beta-cyclodextrin (β -CD) and gamma-cyclodextrin (γ -CD).

In this thesis, our study aimed to synthesize the molecular inclusion of BB with β -CD to find the applications of color additive BB to minimize the uses of synthetic dye in the human body by the application of the co-evaporation method. Brilliant Blue FCF (BB) is a synthetic organic compound categorized as a triarylmethane dye having IUPAC name ethyl-[4-[[4-[ethyl-[(3-sulfophenyl)methyl]amino]phenyl]-(2-sulfophenyl)methylidene]-1-cyclohexa-2, 5-dienylidene]-[(3-sulfophenyl)methyl]azanum. BB has various uses in cotton candy, ice creams, dairy products, and drinks to color in addition it, is utilized in soaps, shampoos, mouthwash, and cosmetic products, employed as a water tracing agent, and applied in hemostatic medical devices having non-toxic properties [27]. Through the use of UV-Vis and fluorescence spectroscopy techniques, it was determined that BB formed an inclusion complex in a water medium at a 1:1 stoichiometric ratio. Numerous spectroscopic methods, such as ^1H -NMR and FTIR, were utilized to forecast the solid-state interaction between BB and β -CD. To find out how this study influences the creation of ICs, the conductance and surface tension investigation was conducted. The creation of the BBIC was identified by PXRD studies and SEM imaging examinations. In the presence of Calf Thymus DNA (CT-DNA), the controlled release of BB from the β -CD cavity to the aqueous medium was investigated. After binding BB with β -CD, the photostability was enhanced in the existence of UV light so it can act as a photostable medicine color for its longevity. In the biological activity study of the IC, in vitro cytotoxicity studies were observed on the HEK-293 human kidney cell line and A-549 cancer cell line to determine the benefits of encapsulation.

Therefore BBIC destroyed the cancer cell line more compared to BB whereas both BB and BBIC showed no significant toxicity in normal human cell lines. DFT study suggested solid-state dye and β -CD interactions to correlate with the experimental results.

Herpes Simplex, commonly known as herpes simplex virus, can cause painful blisters or sores around the mouth or genitals. HSV-1 can spread by kissing or oral sex affecting lips or gums and by touching skin surfaces of the infection from one person to another, while HSV-2 is mainly contracted by vaginal, anal, or oral sex with the infected person and is also known as genital herpes. When the painful blister form of infection spreads, to relieve the pain and discomfort and to stop the reoccurrence of the disease frequently, anti-herpes drugs like Acyclovir and Valacyclovir (VC) are treated initially for the disease. Valacyclovir (VC) possesses 55-70% oral bioavailability among all the anti-herpes drugs and shows more effective performance than Acyclovir (15-20% oral bioavailability) [28, 29]. The objective of the current research was to synthesize and characterize the inclusion complex of VC with Gamma-Cyclodextrin (γ -CD) utilizing various spectroscopic and physicochemical techniques like UV-Vis, ^1H -NMR, FTIR, conductance, surface tension, SEM analysis. The studies confirmed that the 1:1 stoichiometry of the formed inclusion complex (ICVC) which was also well supported by molecular docking study. After complexation with γ -CD, the photostability was enhanced. The present study aimed to prepare VC-loaded and ICVC-loaded topical gel and to evaluate its potential for improved permeation of VC through the topical route by improving its pharmacokinetic profile. *In-vitro* drug release study and Ex-vivo skin permeation analysis were performed to show the improved efficacy of VC as the topical delivery of ICVC containing gel for the treatment of Herpes in comparison to VC containing gel. Ex-vivo skin permeation study of ICVC-loaded gel showed poor permeability and negligible flux value in comparison to VC-loaded gel that infer the retentability of the drug to dermal site as depot formulation from which drug will release and act in sustained manner. Based on the findings, it was confirmed that efficacy of VC can be much improved as the topical delivery of ICVC-loaded gel for the treatment of herpes with minimum dose requirements, less toxicity, rapid action, and improved bioavailability of the VC.

Ionic liquid (IL) defined mainly as organic salt, and they exist in liquid state even at room temperature having a low melting point less than 100°C [30]. It has gain considerable interest in academic as well as industry due to its wide applicability in many areas (lubricants, additives, heat storage) [31, 32]. Ionic liquids are also known as green solvent because it has ease of reuse, non-volatility, thermal stability and varieties of organic and organometallic compounds can easily dissolve in it [33]. Varieties of ILs can be easily prepared by choosing different combinations of cationic and anionic part which enhances their intrinsic characteristics, such as conductivity, density, viscosity, melting point, polarity, solvation power, hydrophilicity/ hydrophobicity, and acid/base character [34]. The thermophysical and thermochemical properties between ionic liquids and small organic molecules are of high importance in the development of industrial processes and biotechnologies. Benzyltriethylammonium chloride can catalyse poly condensation reactions to prepare polymers having high molecular weight under bi-phasic conditions and also using as liophilic phase-transfer catalyst [35, 36]. L-Alanine and L-Isoleucine which falls under non-essential and essential amino acids category are being used in the present study containing carboxylic acid side chain and generally known as acidic-polar amino acids [37]. The amino acids, the basic building units of proteins, have gain significant interests in multidisciplinary studies. L-Alanine is an example of α - amino acid which is used in biosynthesis of proteins, low blood sugar, in many metabolic processes, provides energy for muscles, brain and central nervous system. L-Isoleucine is essential to human for different biological functions such as formation of haemoglobin, muscle growth, immunity boosting, metabolism of protein and glucose transportation [38].

In this work, the systematic measurements of density, viscosity, refractive index, surface tension and conductance of L-Alanine & L-Isoleucine in aqueous Benzyltriethylammonium chloride have been investigated in different concentrations of solutions 0.001 m, 0.003 m and 0.005 m at different temperatures 298.15 K, 303.15 K, 308.15 K, 313.15 K. From the above data, we have determined different thermodynamic parameters like apparent molar volume (ϕ_v), limiting apparent molar volume (ϕ_v^0), Falkenhagen coefficient (A), viscosity B- coefficients (B), temperature derivative of B, dB/dT , $\Delta\mu_1^\circ$, $\Delta\mu_2^\circ$, ΔH° and ΔS° aiming to understand the solute-solvent interaction with deep attention. The significant thermodynamic parameters have been used to

explore such better interaction & the experimental data are well supported by UV-Vis & proton NMR study. Density functional theory is applied to calculate optimized geometries, molecular electrostatic potential maps and reduced density gradient. Our theoretical parameters also support the experimental observations.

The Macromolecule-Drug interaction in water medium takes part in a vital role in the biochemical process and provides noteworthy ideas like the attaching of drugs with the structure of proteins, transportation of a drug, and therapeutic action of a drug to the desired target [39]. This type of interaction affects the influence of the drug in the body of a living organism due to the receptor nature of drug molecules toward proteins as it has a difficult three-dimensional structure exhibiting different chemical properties [40]. The recent perception of biological macromolecules topic is unacceptable without being vigilant of the solvent atmosphere because hydration by water molecules explains a key function in the gathering of the three-dimensional construction of dynamic protein. The significant output in forming the stable native structures of biopolymers depends on the hydration of the hydrophobic part, charged functional groups, and an ionic portion which are the components of every biological system [41]. The experimentation and computational investigations of the alpha-AAs, viz., L-Valine (L-Val), L-Phenylalanine (L-Phe) in aqueous solution of Mephenesin have been done to detect probable interactions predominant in these type of ternary mixtures which can sustain the life metabolism with technical applications of drugs in aqueous phase by providing beneficial data [42-47].

The drug belongs to the glycerol ether family, significantly most useful is Mephenesin (MEPN), a chemically known as 3-(2-methylphenoxy) propane-1, 2-diol which is used as a centrally operating relaxant used for skeletal muscle anticonvulsant, local anesthesia, and muscle paralyzer [48]. The colourless, odourless, crystalline solids of MEPN have a specific action on spinal interneurons with contractions of polysynaptic reflexes and unaltered reflexes of monosynaptic knee-jerk [49]. L-Val is in the category of essential AA, i.e., a person can require its need from foods by consuming and gaining from dietary resources that help in the growth and regeneration of muscle, produce energy, increase endurance, and recover muscle tissue. L-Phe also belongs to the essential AA family and is a component of vital protein structures and enzymes, it

transforms into Tyrosine by the body, which can be used to synthesize Dopamine and Norepinephrine neurotransmitters.

In our current work, we have reported the methodical determinations of density, ρ , viscosity, η , refractive index, n_D , conductance, Λ , and surface tension, σ of L-Val and L-Phe in an aqueous solution of MEPN at altered concentrations (molality), temperatures and at atmospheric fixed pressure targeting to explain solute-solvent interactions prevailing in the investigated medium and to obtain the effect of variation on the phenomena of physicochemical manners of the aqueous drug solution in presence of both the AAs. With the help of these observations, we have calculated limiting molar apparent volume (ϕ_V^0), Jones-Dole coefficients (B), Falkenhagen coefficient (A), Hepler's constant (dB/dT), free energy of activation of viscous flow per mole of solvent ($\Delta\mu_1^\circ\#$) and solute ($\Delta\mu_2^\circ\#$), enthalpies ($\Delta H^\circ\#$) and entropies ($\Delta S^\circ\#$) of activation of viscous flow to explain the solute-solvent interaction among them in a better way and the experimental values are well supported by spectroscopic methods of UV-Vis and proton NMR study. L-Phe-MEPN interactions are observed to be greater than L-Val-MEPN interactions by experimental as well as theoretical data. DFT study is applied to find out the geometries of optimization, maps of molecular electrostatic potential (ESP), and reduced density gradient (RDG) determination. Our theoretical data and experimental observations correlate with each other.

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

Names of the Biologically Active Guest Molecules, Host Molecules, Ionic Liquids, Amino Acids, and Solvent molecules are listed below-

Biologically Active Guest Molecule:

- Brilliant Blue FCF
- Valacyclovir

Host Molecules:

- β -cyclodextrin
- γ -cyclodextrin

Ionic Liquids:

- Benzyltriethylammonium chloride

Drug:

- Mephenesin

Amino Acids:

- L-Alanine
- L-Isoleucine
- L-Valine
- L-Phenylalanine

Solvents:

- Water

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

Name of the Investigation Methods are listed below:

- UV-vis Spectroscopy
- Fluorescence Spectroscopy
- Powder X-Ray Diffraction (PXRD)
- Scanning Electron Microscopy (SEM)
- FTIR Spectroscopy
- ^1H -NMR Spectroscopy
- Surface Tension Study
- Conductivity Study
- Density Study
- Viscosity Study
- Refractive Index Study
- DNA binding Study

- MTT Assay
- *In-vitro* drug release study
- Ex-vivo skin permeation studies
- Stability study
- Molecular docking evaluation
- zeta potential
- DFT study