

ABSTRACT

As the title of the thesis, I would like to show my deep insight into the field of Supramolecular Host-Guest Inclusion Complexation and the Food Preservative-Ionic Liquid interaction in solution phase resulting molecular synergism to show better microbial activity. In the supramolecular chemistry, various biologically potent molecules like, drugs, proteins and vitamins that are merely water-soluble and the delivery system of whose are still not so developed, were chosen for the formation of inclusion complexes with cyclodextrins in order to enhance its solubility as well as bioavailability. Different physicochemical as well as spectroscopic methodologies usually used to study the inclusion complexation exploring mechanism of their formation, stability, thermodynamic feasibility, binding ability to albumin protein and sustained oozing of the drug molecules from inclusion complexes to the dispersive media. In the field of solution chemistry, Food Preservative-Ionic liquid interaction showing molecular synergism to antimicrobial activity would become a great aspect in the area of food chemistry, as we know, 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 genesis of diverse interactions is usually exposed by measurement of the apparent molar volume (ϕ_v), limiting apparent molar volume (ϕ_v^0), molar refraction (R_M), limiting molar refraction (R_M^0) viscosity B coefficients obtained from different physicochemical methodologies. Food Preservative-Ionic liquid combinations were found to show better antimicrobial activity as obtained from the antimicrobial study on some gram positive and gram negative bacteria.

Importance of host-guest chemistry of cyclodextrins:

My research work on the supramolecular chemistry associated with and justified satisfactorily by the improvement of the following novel aspects - (a) solubility of the

drug into water for bioavailability, (b) drug delivery through HSA assisted controlled release from inclusion complexes, (c) Enhancement of fire resistivity of fire retardant, (d) Promotion of biodegradability of a water pollutant.

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 was found, that drug-CDs inclusion complexes enhances the aqueous solubility of drug making it more bioavailable. Encapsulation of the hydrophobic part of the guest 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. Here, binding property of drug to the HSA had been studied spectrofluorimetrically. Thus, HSA assists the drug 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 drug thus become moralised by the solubility enhancement and HSA assisted transformation and controlled release of the drug in human body.

The higher melting point of the inclusion complexes compare to its parent compound, namely, HBCDD are because of the fact that extra amount of heat is required for that compound to come out from the corresponding cyclodextrins (β -CD and HP- β -

CD respectively) cavity and also these higher values of melting point i.e. 209.5°C and 210.5°C clearly indicate the formation of two new inclusion complexes i.e. IC-1 and IC-2 respectively.

The aqueous solubility of HBCDD is very low in a wide range of temperature. But, the inclusion complexes were found to be fairly soluble in water at room temperature. This is the indication of a molecule to be bio-available and also bio-degradable by the microorganisms. Thus, the pollutant HBCDD which, was a non-bio-degradable in nature, now has been converted into a bio-degradable material through the newly formed inclusion complexes with β and HP- β -CD respectively.

Importance of drugs and vitamins as guest molecule: Phenylephrine hydrochloride (PEH) 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. 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. However, due to serious side effect (haemorrhagic stroke) phenylpropanolamine was withdrawn from market. Now, it is imperative to find out the suitability of PEH as the same done by the Phenylpropanolamine, pseudoephedrine and ephedrine for the treatment of nasal or sinus congestion and to find out the way of delivery with biocompatibility.

Alkaloid synephrine (SNP) was first extracted as a natural product from the leaves of various citrus trees are used as bronchial muscle reluctant, increases blood pressure in the patients suffering from low blood pressure. Its presence and positive retort as a bio-marker makes the orange juice like soft drinks authentic. Lipolytic stimulation by synephrine increases thermogenesis which leads to the increase in metabolic rate and fat oxidation. 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. 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.

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. The visceral pronociceptive effect of 5-HT can be reduced as ALVC binds with 5-HT_{1A} acting as an antagonist. ALVC when combined with simethicone, found to act more effectively in the treatment of abdominal pain in IBS. It is marketed commercially by the name Spasmonal® Forte in the form of hard capsule (Alverine citrate 60/120 mg) and soft capsule (60 mg Alverine citrate/300 mg simethicone). ALVC ultimately metabolised to two secondary metabolites through the conversion of its primary active metabolite, para hydroxy alverine (PHA).

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. 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. Fruit juices are one of the main sources of vitamins and the quality of fruit juices in industry is maintained by different techniques including pasteurization, which led to the degradation of vitamins along with other valuable food nutrients.

Importance of water pollutant: 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. 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. For these reasons this cyclic aliphatic brominated compound is very harmful to aquatic life.

Importance of food preservatives: Sodium benzoate (SBz) having inhibitory effect on the microbial growth is strongly recommended as a food preservative and

commonly used in foods like soda, fruit juice and a variety of products, such as cosmetics and pharmaceuticals. In acidic medium SBz works better on yeasts, molds, bacterial growth and prevent spoilage. In patients with acute hyper-ammonaemia, who were born with urea cycle disorders SBz acts as a therapeutic agent and also treats dental carries, blocks D-dopa in the hemi-parkinsonian rat.

Sodium salicylate (Scyt) is a significant antibacterial agent, also enhances the activities of certain antibiotics. The bacterial strains, Salmonella, Shigella including Escherichia coli that causes diarrhoea used to inhibited by bismuth subsalicylate efficiently. Being an active anti-inflammatory compound Scyt have been used as anti-inflammatory, analgesic and anti-pyretic agent still it has gastric bleeding like serious disadvantage. Scyt acts synergistically with vancomycin and enhances anti-staphylococcal activity significantly. The presence of salicylate in 5 mM concentration, vancomycin prevents biofilm formation and kill bacteria effectively below its MIC.

Importance of ionic liquids: Quaternary ammonium based ionic liquids also execute some sort of anti-microbial effects for instance positive charge on the Nitrogen atom of these ionic liquids attracts naturally the negatively charged species, such as bacterial proteins and consequently disorganization in the protein chain makes it denature. Ionic liquids, benzyltriethylammonium chloride, benzyltrimethylammonium chloride were used herewith in all four possible combinations to the illustrious food preservative SBz and Scyt. Anti-microbial activities of the set of four combinations were studied warily at concentrations below their MIC and found synergistic to each other.

Summary of work done:

Chapter I: This chapter contains in details about the object of the research work, their scope and applications in the contemporary science. It also includes the reason of choosing the biomolecules, drugs, vitamins, water pollutant, food preservatives, ionic liquids, cyclodextrins and the solvent systems.

Chapter II: This chapter includes the review of the earlier works in this field of research done by various scientist and researchers across the world. This chapter also provide a detail theory of investigation, where the interacting forces between the molecules have been described. Here, the theory of ^1H NMR, 2D ROESY, FTIR spectroscopy, UV-Visible spectroscopy, Fluorescence spectroscopy, Differential Scanning Calorimetry, Scanning Electron microscopy, Powder X-Ray Diffraction, High Resolution Mass Spectroscopy, Surface tension, Conductivity, Density, Viscosity, Refractive index have been discussed thoroughly and the significance of their use in the research work described in this thesis have been shown.

Chapter III: This chapter contains the experimental section. It covers the name, structure, physical properties, method of purification and applications of biologically active molecules, drugs, vitamins, water pollutant, cyclodextrins, food preservatives, ionic liquids and solvents used in the research work. It also includes the details about the experimental methods, the descriptions and use of the instruments involved in the research work.

Chapter IV: Host-guest interaction of two significant drugs, phenylephrine hydrochloride and synephrine with α and β -cyclodextrins were studied systematically. Initially two simple but reliable physicochemical techniques namely conductance and surface tension were employed to find out saturation concentration for the inclusion and its stoichiometry. The obtained 1:1 stoichiometry was further confirmed by two spectrometric methods, UV-Vis study and spectrofluorimetry. Significant shifts in IR stretching frequency also support the inclusion process. Relative stabilities of the inclusion complexes were established by the association constants obtained from UV-Vis spectroscopic measurements, program based mathematical calculation of conductivity data. Calculations of the thermodynamic parameters dictates thermodynamic feasibility of the inclusion process. Spectrofluorometric measurement scaffolds the UV-Vis spectroscopic measurement validating stability of the ICs once again. Mass spectroscopic measurement gives the molecular ion peaks corresponding to the inclusion complex of 1:1 molar ratio of host and guest molecules. The mechanism of inclusion was drawn by ^1H -NMR and 2D ROESY spectroscopic analysis. Surface texture of the inclusion complexes was studied by SEM. Finally, the cytotoxic activities of the inclusion complexes were analyzed and found, Cell viability also balances for

non-toxic behavior of the ICs. Moreover, all the studies reveal the formation of inclusion complexes of two ephedra free, alternatively emerging drugs (after their banned product having ephedra) SNP, PEH with α and β -CD, which enriches the drug delivery system with their regulatory release without any chemical modification.

Chapter V: The host-guest inclusion of thiamine hydrochloride(guest) within the hydrophobic cavity of α and β -cyclodextrin molecules (hosts) have been studied scientifically in the solid and solution phases respectively. Various modern spectroscopic techniques had been used to establish the outcome of this work. The UV-Vis study supported the 1:1 stoichiometry of the inclusion complexes and also used to evaluate the association constants along with thermodynamic parameters with high accuracy for the determination of the feasibility of this inclusion process. From the mass spectrometric study, 1:1 stoichiometry of the inclusion complexes had been confirmed in their solid state. Differential scanning calorimetric and infrared studies also supported this fact. $^1\text{H-NMR}$ and 2D ROESY spectroscopic analysis had given the mechanism of inclusion process, and the SEM study exposed their surface structures. Finally, the sustained oozing of the guest molecule from the hydrophobic cavity of the respective cyclodextrin molecules separately had been studied in the presence of human serum albumin in their aqueous buffer solutions with the help of fluorescence spectroscopic technique. This study has a truly intense effect to the stabilization of the respective guest molecule from the external hazardous, such as photolytic degradation, oxidation-reduction, thermal cleavage etc., and also predicts the releasing behavior of thiamine hydrochloride in the presence of human serum albumin without any chemical modification.

Chapter VI: Inclusion complexation of a non-biodegradable pollutant in hydrophobic cavity of β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin were synthesized and characterized to retain its fire resistance property and converted it into bio-degradable molecule. $^1\text{H NMR}$, 2D ROESY, HRMS, SEM, etc. studies have been executed to establish this fact. The stoichiometry of the two complexes has been obtained as 1:1. The inclusion has been established by $^1\text{H-NMR}$ and 2D ROESY spectroscopic analysis. Substantial shifts in IR stretching frequency support the inclusion process. HRMS measurement gives the molecular ion peaks corresponding to the inclusion complex of 1:1 molar ratio of host and guest molecules. Surface texture properties of the inclusion

complexes were studied by SEM and the presence of bromine were proved by EDXS. Thermal stabilities of the inclusion complexes were illustrated by melting point analysis. The aqueous solubility of the inclusion complexes demonstrate that these are more bio-available to the microorganism making them biodegradable in nature. The biodegradability study confirms the conversion of non-biodegradable HBCDD into biodegradable material by encapsulating in the two cyclodextrins.

Chapter VII: Solubility development of supramolecular host-guest interaction between Alverine citrate with α and β -cyclodextrins were studied throughout the article. 2:1 host to guest stoichiometry of the inclusion complexation in the solution phase were confirmed by the Job's plot and further confirmation about the stoichiometry was also obtained from the mass spectra of the inclusion complexes. IR, DSC, SEM and PXRD data turn out to be supportive about the phenomenon, inclusion complexation. Association constants and thermodynamic parameters of the inclusion complexes were obtained using UV-vis and spectrofluorometric measurement. The mechanism of inclusion complexation was explored by ^1H and 2D ROESY NMR spectroscopy. Binding ability of the drug molecule, Alverine citrate with the HSA and the controlled release of the drug molecule from inclusion complexes were studied at PH-7.4 by spectrofluorimetrically. Studied phenomenon thus develops the solubility of merely soluble drug into water, consequently makes bioavailable and enriches the drug delivery system.

Chapter VIII: 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 by various physicochemical methodologies such as Density, Refractive index, Viscosity, Electrical conductivity, at five different temperatures ranging from 298.15 K to 318.15 K. Anti-bacterial as well as the anti-fungal effects of the ternary mixtures, (BTEACl+SBz+H₂O), (BTEACl+Scyt+H₂O), (BTMACl+SBz+H₂O) and (BTMACl+Scyt+H₂O) were further analysed for better results and found to act synergistically below the MIC of both the food preservative, thus minimises the hazardous threat, caused by unnecessarily excessive consumption of food preservatives. Association constants governed by diverse intermolecular interactions

in the solution phase were studied by UV-vis spectroscopy. Construction of thermodynamic background caused by innumerable interactions taking place in the ternary mixtures were explored by calculating the free energies of various molecular associations. The genesis of diverse interactions was exposed by measurement of the apparent molar volume, limiting apparent molar volume, molar refraction, limiting molar refraction, viscosity B coefficients and reveals as strong solute-solvent interaction, over the solute-solute and solvent-solvent interactions. Optimum energies with the optimised geometries of molecular assembly for (BTEACl+SBz), (BTEACl+Scyt), (BTMACl+SBz) and (BTMACl+Scyt) systems were calculated from Ab-initio quantum chemical calculations using Gaussian 09W quantum chemical package which found supportive to the practical outcomes.

Chapter IX: This chapter includes the concluding remarks about the research works done in this thesis.