CHAPTER VIII

Concluding Remarks

In this thesis I investigated the formation of host-guest inclusion complexes of various bioactive molecules with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and *p*-sulfonatothiacalix[4]arene exploring particularly towards their formation, stabilization, solubility, bioavailability and biological activity without chemical modification by different dependable methods like ^{1}H NMR spectroscopy, FT-IR spectroscopy, UV-Visible spectroscopy, Mass spectrometry, Fluorescence spectroscopy, Thermogravimetric analysis, Differential Scanning Calorimetry, Powder X-ray Diffraction, Scanning Electron Microscopy, Surface tension study, Molecular docking study, Antibacterial activity study, Cytotoxicity study, Reactive Oxygen Species generation study, which primarily focus on the encapsulation of the bioactive molecules into the cavity of cyclodextrins and calix[4]arene. The stoichiometry, association constants and thermodynamic parameters for the inclusion complexes (ICs) have been determined to communicate a quantitative data regarding the encapsulation of the bioactive molecules inside into β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and *p*-sulfonatothiacalix[4]arene (TSC4X).

The findings are discussed chapter wise as follows :

Chapter IV : The supramolecular interactions of macrocyclic host (β-CD and HP-β-CD) with important phytochemical compound IM and formation of their ICs have been studied, which can be used in near future for an efficient regulatory delivery of IM retaining its bioactivity. The surface tension study and Job's method suggested the formation of mono-molecular encapsulated complex, which was further confirmed by mass spectral analysis. The stability constants and thermodynamic parameters determined from the reliable spectroscopic methods accounts for the stability of ICs formed and the thermodynamic feasibility of inclusion process respectively. The increase in ΔS^0 and a drop in ΔG^0 indicates the inclusion process to be thermodynamically favourable. The greater value of association constant, and hence more stability of IM-HP-β-CD IC compared to IM-β-CD IC is attributed to the larger

cavity diameter of HP-β-CD than β-CD. FT-IR study and SEM image analysis confirmed the formation of IC from structural and morphological details. ^{1}H NMR spectroscopic analysis provided a deep insight towards the mode of complexation in which aromatic ring of IM was encapsulated from the wider rim side of the cavity of CDs. DSC study revealed that the inclusion into CDs can enhance the thermal stability of IM. The molecular docking simulation revealed the possible interaction of IM with β- and HP-β-CD giving stable 3-D structures of the ICs. The antimicrobial activity of IM was considerably improved after its encapsulation in CDs, however, IM-HP-β-CD IC provided better activity than IM-β-CD IC. Further, both IM-β-CD and IM-HP-β-CD ICs expressed low cytotoxic effect than free IM on the normal liver cell line WRL-68. Therefore, the encapsulation of IM into CDs can be a potential approach to improve stability and biological activity of IM for applications in the pharmaceutical industries and biomedical sciences.

Chapter V : The TCP-β-CD complex was successfully prepared and the effect of inclusion on the *in vitro* bioactivity of TCP have been examined. The formulation of complex was confirmed by ^{1}H NMR, ESI-MS, FT-IR, TGA, SEM, PXRD, surface tension and UV-vis spectroscopic studies. The Job's method, surface tension study and ESI-MS experiment confirmed 1:1 stoichiometry of the TCP-β-CD complex. Higher binding constant value (K_a = 25.16 x 10^3 M^{-1}) accounts for the higher binding affinity of TCP with β-CD and the thermodynamic spontaneity of binding process was validated by the negative Gibb's free energy change (ΔG). ^{1}H NMR, FT-IR and molecular docking studies suggested a possible stable molecular conformation for TCP-β-CD complex in which the chlorophenyl and piperidine moieties are almost completely included into the cavity of β-CD, and the thiophene ring lie nearly outside the wider rim. PXRD and SEM analysis showed that the prepared complex has amorphous nature which is unlike from its pure components. From TG analysis we observed that encapsulation has enhanced the thermal stability of TCP. *In vitro* antibacterial test showed that the better activity was displayed by TCP after its complexation with β-CD. TCP-β-CD complex (IC_{50} = 24 μM) expressed significant cytotoxic effect than pure TCP (IC_{50} = 44 μM) towards human kidney cancer cell line (ACHN). Furthermore, the complex was found to induce the intracellular ROS generation more prominently than TCP, suggesting the enhancement in the apoptotic activity of TCP after complexation. Thus, TCP-β-CD inclusion complex

can be a promising approach for designing a novel formulation of TCP in drug delivery, thereby, extending the potential clinical purpose of TCP in biomedical sciences and pharmaceutical industries.

Chapter VI : The molecular recognition of SBZ by TSC4X has been investigated. The Job's plot indicated that SBZ forms 1:1 inclusion complex with TSC4X, which was further supported by ESI-MS study. SBZ was found to possess higher binding affinity for TSC4X (K_a = 6.01×10^3 M^{-1}), and the negative free energy of binding (ΔG = -5.15 kcal mol^{-1}) suggested the binding process to be thermodynamically feasible. FT-IR and ^{1}H NMR studies demonstrated a preferential occupancy of the hydrophobic cavity of TSC4X by unsubstituted aromatic moiety of SBZ, which is in accordance with the docking analysis. DSC study showed that the complexation with TSC4X improved the thermal stability of SBZ. Finally, encapsulation of SBZ into the cavity of TSC4X also improved its photo-stability. Thus, the overall study concluded that SBZ-TSC4X supramolecular hybrid would enable the investigation of its applications as highly photo-stable sunscreen agent for cosmetic industry.

Chapter VII : The inclusion complex formation of two naturally occurring amino acids, L-Valine (Val) and L-Aspartic acid (Asp), with TSC4X was confirmed by ^{1}H NMR study. ESI-MS study suggested the efficient 1:1 complexation of Val and Asp with TSC4X. ^{1}H NMR and molecular docking studies provided most stable binding orientation of Val and Asp within the cavity of TSC4X, such that $CH(CH_3)_2$, CH groups of Val and CH, CH_2 groups of Asp are included into the hydrophobic cavity of TSC4X. The greater binding affinity (K_a) value of Val-TSC4X IC (≃ 719 M^{-1}) compared to that of Asp-TSC4X IC (≃ 312 M^{-1}), obtained from ^{1}H NMR titration, revealed that the Val binds to TSC4X strongly than the Asp. ^{1}H NMR titration also accounts for the thermodynamic feasibility of the inclusion process, which was validated by the negative value of ΔG (≃ -3.9 kcal/mol for Val-TSC4X and ≃ -3.4 kcal/mol for Asp-TSC4X). The formation of Val-TSC4X and Asp-TSC4X complexes was further confirmed by PXRD study and SEM image analysis. Finally, the complexation with TSC4X improved the thermal stability of Val and Asp. Thus, the present work may add a dimension to the modern field of science of controlled delivery of these two amino acids by using TSC4X.

My research work may be justified by the following novel outcomes –

- All the studies supports successfully the formation of inclusion complexes.
- Hydrophobic interactions provides the stability to the inclusion complexes. Sometimes H-bonding interaction gives the extra stability to the inclusion complexes.
- Complexation with cyclodextrins and calix[4]arene enhances the solubility, bio-availability, stability and bioactivity of various guests.