CHAPTER 4
CONCLUSIONS

C6 formed a 1:1 inclusion complex with β-CD and a 1:2 complex with C-HPA. C6 molecule was complexed by the narrower rim of β-CD with the preferred encapsulation of chromone moiety. There are two prototropic equilibria viz., neutral–monocation and monocation–dication. The pK\textsubscript{a} in the presence of β-CD differs in the formation of monocation–dication equilibria. The formation of the dication required more demanding acidic conditions in β-CD than in water and such acidic conditions would hydrolyze cyclodextrin. This adjoined to the conclusion that the chromone part was inside the cavity of β-CD. Contrary to that, encapsulation of both the benzothiazole and chromone parts by C-HPA was inferred in the C6/C-HPA complex. The pK\textsubscript{a} of C6–C-HPA was lower than the same equilibria in water and in β-CD. This could be due to the 1:2 stoichiometry inclusion complex and hence the more covered-up C6 which got shielded from easy protonation. The mode of inclusion of C6 with the hosts β-CD/C-HPA was supported by 2D ROESY NMR spectroscopy and the structure of the inclusion complexes are proposed.

C153 formed 1:2 and 1:1 inclusion complex in β-CD/C-HPA with the binding constant values $9.55 \times 10^4 \text{M}^{-2}$ and $8.44 \times 10^5 \text{M}^{-1}$ respectively. The effect of pH results showed that there was a complexity to protonation of C153 in presence of host molecules. This led to shift of pK\textsubscript{a} towards smaller value, in the presence of β-CD as this molecule offered a protective environment to C153 molecule from attack by protons. The same effect was found even more in presence of C-HPA. This change could not be observed in the excited state properly since the protonation occurred in the ground state itself. The formation of inclusion complex of C153 with the hosts β-CD and C-HPA was evidenced by 2D ROESY NMR spectroscopy. The mode of inclusion was supported by molecular docking studies.

β-Cyclodextrin encapsulation of C7 resulted in the enhancement in fluorescence intensity with a blue shift of 5 nm. The stoichiometry of the host–guest complex was 1:1 with the binding constant of $1.09 \times 10^2 \text{M}^{-1}$. The fluorescence
emission of C7 was quenched at the addition of C-HPA and the calculated quenching constant \( k_q \) is \( 2.37 \times 10^{14} \text{ M}^{-1}\text{s}^{-1} \). The fluorescence quenching observed for the inclusion complexation is quite opposed to the usual enhancement of fluorescence due to encapsulation of guest by the host. The inclusion of C7 with C-HPA was determined by the lifetime measurement, 2D ROESY NMR and the change of pK\(_a\) equilibrium. Hence, mode inclusion of C7 with C-HPA was substantiated as the weak inclusion occurred through the N–Et\(_2\) group.

The stoichiometry of the C334–β-CD complex was determined as 1:1 with the binding constant 99.11 M\(^{-1}\). C334 showed a fluorescence quenching when it interacted with β-CD. The quenching constant \( k_q \) \( 4.66 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \) was calculated. Quite contrarily, a fluorescence enhancement of C334 was observed for the interaction of C-HPA and it leads to a non-linear curve in the Benesi–Hildebrand plot. The stoichiometry of the host: guest complex was 1:1 with a binding constant of \( 9.5 \times 10^5 \text{ M}^{-1} \) determined by the non-linear curve fitting. 2D ROESY NMR spectral data implied that the host–guest interactions of C334 with β-CD/C-HPA through the quinolizidine ring of C334. The change in pK\(_a\) of C334 in absence and presence of hosts were determined and the monocation formation might be hindered due to the pronounced hydrogen bonding interaction between the hydroxyls of β-CD/C-HPA as the complexation of C334 molecule.

The UV-visible absorption spectrum of C314 was enhanced with significant red shift on the interaction of β-CD. The binding constant was \( 1.89 \times 10^3 \text{ M}^{-1} \) of the 1:1 complex of C314–β-CD. The fluorescence spectrum was unusually quenched. The Stern–Volmer plot for the quenching of fluorescence showed a downward curve. In such cases, the Stern–Volmer quenching constant of the accessible fraction was obtained and \( K_a \) was calculated as \( 2.57 \times 10^2 \text{ M}^{-1} \). The change in the relative amplitude and lifetime of C314 with addition of β-CD evidenced the complex formation. The pK\(_a\) for the neutral–monocation equilibrium of C314 in water and β-CD represented that C314 molecule became more basic in the excited state. With the ROESY NMR results suggested that the mode of inclusion occurred through the quinolizidine part of C314 to the β-CD molecule. The red shifted
absorption bands are due to the surfactant action of the hydroxyls of C-HPA acting on the ester part of C314 molecule. The fluorescence of C314 molecule was quenched by C-HPA with a calculated $k_q$ of $2.24 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$. The $pK_a$ value of C314 in C-HPA obtained was similar to that observed for the same equilibrium in water. The relative amplitudes in time-resolved fluorescence of C314 in C-HPA did not vary significantly. Hence, the inclusion complexation may not occur between C314 and C-HPA. Although C314 and C-HPA molecules come closer in solution and there are some alterations in the absorption and the emission spectra of C314 by C-HPA.

On inclusion of C343 with β-CD resulted with the fluorescence quenching. The quenching constant ($k_q$) $7.65 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ was calculated from the slope of the Stern–volmer plot and the lifetime of the C343. The fluorescence quenching of C343 may be due to the presence of carboxyl acid group. In presence of β-CD, the ground state $pK_a$ value of C343 did not change due to its weak complexation with β-CD. The excited state $pK_a$ for the neutral–monocation equilibrium was similar to that of ground state $pK_a$ showing that the protonation occurs in the ground state itself and that the excited state of C343 was unaltered by β-CD. The C-HPA forms 1:2 complex with C343 and the binding constant was calculated as $1.18 \times 10^{11} \text{ M}^{-2}$. The 1:2 stoichiometry was confirmed by time-resolved fluorescence and evidenced by 2D ROESY NMR spectroscopy. The ground state $pK_a$ of the neutral–monocation equilibrium of C343 in presence of C-HPA was significantly less than that in water which implies that the amino group of C343 molecule is fully inside the cavity of the host.

Two guest molecules, 6-methyl-3-[phenylimino]methyl-4H-chromen-4-one (MPIMC) and 6-methyl-3-[(4-methylphenyl)imino]methyl-4H-chromen-4-one (MMPIMC) were synthesized. The derivatives were characterized by NMR spectroscopy and the host–guest interactions with β-CD/C-HPA were studied.

A 1:2 stoichiometric inclusion complex of MPIMC with β-CD formed and the binding constant was derived as $4.14 \times 10^4 \text{ M}^{-2}$. The stoichiometry of the inclusion complex was 1:2. The binding constant was calculated as $5.34 \times 10^{10} \text{ M}^{-2}$.
The pKₐ value for the neutral–monocation equilibrium of MPIMC was 1.12 in water and 0.98 in presence of β-CD and this revealed that a restriction to protonation was offered by β-CD. The fluorescence quenching in the MPIMC–C-HPA complex was occurred upon acidification due to the complete encapsulation of MPIMC by the two C-HPA molecules and inaccessibility of the guest molecule to the protonation.

The stoichiometry was found to be 1:2 for the MMPIMC–β-CD/C-HPA complexes. The binding constants (K) of the MMPIMC–β-CD/C-HPA complexes were calculated as $1.90 \times 10^4\text{ M}^{-2}$ and $1.13 \times 10^{10}\text{ M}^{-2}$ respectively. The ground and excited state pKₐ values for the neutral–monocation equilibrium of MMPIMC were calculated to be −1.65 and 0.8 respectively. This was not clearly observed for the same equilibrium in presence of β-CD which suggested that the encapsulation by β-CD offered restriction for the access of MMPIMC by protons. There was no significant shift in the absorption and fluorescence bands on the acidification of MMPIMC in presence of C-HPA and hence the monocation formation was not found even at high acidic conditions. This was due to that the formed inclusion complex was sufficiently strong at acidic conditions and that the MMPIMC molecule was fully shielded from the protonation by C-HPA molecules. The structure of the inclusion complexes of MMPIMC with β-CD/C-HPA was proposed.

Baicalein (BCN) showed 1:1 stoichiometric complex with β-CD with a binding constant of $2.92 \times 10^3\text{ M}^{-1}$. The ground and excited state pKₐ value of the protonation equilibrium of BCN were not significantly altered by β-CD which might be due to a week binding between β-CD and BCN. The binding stoichiometry for the BCN–C-HPA complex was determined as 1:1 ratio and the binding constant (K) was $6.71 \times 10^4\text{ M}^{-1}$. Hence, the BCN forms a strong inclusion complex with C-HPA than in β-CD. The ground and excited state pKₐ of BCN in C-HPA showed the shift of equilibrium happens at higher acid concentration ($H_0$ −0.84) than that in water and β-CD. This is due to the influence of C-HPA on the acidification of BCN which involved in the host–guest interactions with C-HPA.
The stoichiometry of the SBN–β-CD/C-HPA inclusion complex was found as 1:1 from the Benesi–Hildebrand plot and the calculated binding constant was 42.85 M\(^{-1}\). The binding constant (K) was 4.90 \times 10^4 M\(^{-1}\) for the SBN–C-HPA inclusion complex. The mode of binding of SBN with β-CD/C-HPA was that the part of 3-methoxy substituted phenyl ring got encapsulated by the host molecules.

Inclusion complexation of FL with β-CD occurred with a stoichiometry of 1:1. The binding constant (K) value was calculated as 1.45 \times 10^3 M\(^{-1}\). Fluorescence quenching occurred at the formation of the FL–β-CD complex and the Stern–Volmer plot was non-linear. Hence, the fluorescence intensities were employed to derive the accessible fraction of fluorophore to the host and the K\(_a\) was calculated as 4.62 \times 10^2 M\(^{-1}\). The quenching constant (k\(_q\)) was calculated as 2.60 \times 10^{13} M\(^{-1}\)s\(^{-1}\) for the quenching of fluorescence in FL–C-HPA complexation. The structure of inclusion complex was proposed for the FL–β-CD/C-HPA.

A 1:1 inclusion complex formed between 7-AF and β-CD with a binding constant of 1.50 \times 10^2 M\(^{-1}\). Time-resolved fluorescence decay profile of 7-AF with the increasing concentrations of β-CD showed the change in lifetime and relative amplitude due to the inclusion complexation. 7-AF showed little difference in the ground state and the excited state pK\(_a\) values in water with those of 7-AF–β-CD complex. This led to the conclusion that the amino group of 7-AF might be outside the β-CD cavity in the complexed form of 7-AF. This was supported by 2D ROESY NMR spectra. 7-AF molecule was encapsulated by β-CD through the phenyl ring attached to chromen-4-one and it left out the amino group at position 7 of the benzopyran part free. The absorbance of 7-AF showed a hyperchromic shift whereas the quenching of fluorescence was observed on the addition of C-HPA. The calculated binding constant for the increased absorption was 2.06 \times 10^4 M\(^{-1}\) and the quenching constant (k\(_q\)) for the quenched of fluorescence was 4.90 \times 10^{14} M\(^{-1}\)s\(^{-1}\). Less significant difference in the pK\(_a\) values of 7-AF in C-HPA from that in water suggested that the change in the acidity did not affect the complexation and it leaving the amino group free for the protonation. This was supported by the cross correlation peaks found in the 2D ROESY spectra.
The Benesi–Hildebrand plot from the fluorescence intensities of MO–β-CD complex resulted in the 1:1 stoichiometry and the binding constant $K$ was calculated as $1.11 \times 10^2 \text{ M}^{-1}$. The enhancement in the intensities of fluorescence band of MO strongly enhanced with an increase in the concentration of C-HPA. It resulted in a non-linear curve and the calculated binding constant ($K_{11}$) $6.40 \times 10^4 \text{ M}^{-1}$ for the 1:1 stoichiometric ratio of MO–C-HPA complex. The change in lifetimes of MO and relative amplitudes of the β-CD/C-HPA hosts suggested that the occurrence of complex formation. The structure of the complex was proposed based on the 2D ROESY NMR results obtained.

The enhancement in the absorption and the fluorescence spectra of β-NF at the addition of β-CD ascribed to the inclusion of complexation. The stoichiometry of the β-NF–β-CD complex was 1:1 as inferred from the linearity of the Benesi–Hildebrand plot with the binding constant ($K$) of $1.45 \times 10^2 \text{ M}^{-1}$. The absorption and the fluorescence bands of β-NF highly shifted to hypsochromically with increase in the concentration of C-HPA due to the encapsulation of β-NF by the C-HPA cavity. The complex formation of β-NF with C-HPA resulted with the linearity in the Benesi–Hildebrand plot for the 1:1 stoichiometry. The binding constant ($K$) was calculated as $2.89 \times 10^9 \text{ M}^{-1}$. The structure of β-NF–β-CD/C-HPA complex was suggested by cross peaks obtained in the ROESY NMR spectra.

This detailed host–guest complexation study of the coumarins and flavones with β-CD and C-HPA lead to the conclusion that most of the selected guests (C7, C334, C314, BCN, SBN, FL, 7-AF, MO and β-NF) formed 1:1 inclusion complexes with both the host molecules (β-CD and C-HPA). MPIMC, MMPIMC yielded the 1:2 complexes with both the hosts. In addition, the 1:2 inclusion complexes formed in, (i) the host–guest complexation of C153 with β-CD, (ii) the inclusion complexation of C6 with C-HPA, (iii) the host–guest complexation of C343 with C-HPA.