

## Concluding remarks

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This thesis presents the spectroscopic investigations of the photophysical properties of fluorescent organic molecules and interactions of such molecules with other small molecules and biological macromolecules. Different spectroscopic techniques have been used, *viz.* absorption spectroscopy, fluorescence emission spectroscopy, fluorescence excitation spectroscopy, synchronous fluorescence spectroscopy, red edge excitation spectroscopy, fluorescence anisotropy, time resolved single photon counting, circular dichroism and laser flash photolysis. Theoretical calculations have also been performed to complement the experimental findings, such as the ground state optimization of the molecules and molecular docking analysis.

The emissive behaviour of KTHC-derivatives DMTCO, MDDCO and TDCO are considerably solvent sensitive, although their absorption spectra are relatively less sensitive to the nature of the solvents. The fluorescence decays of these molecules are single-exponential, except in long chain alcohols from HxOH to DdOH, due to the occurrence of differential solvation in the polar head groups and hydrophobic chains of the solvents. The solvatochromic emission of the KTHC-derivatives originates from significant changes in dipole moment on photoexcitation. The emissive behaviour of such molecules maintains better correlation with the empirical polarity parameters  $E_T(30)$ ,  $\alpha$ ,  $\beta$  and  $\pi^*$ , rather than the parameter  $f_{LM}(\epsilon, n)$  derived from the dielectric constant and refractive index. All the KTHC-derivatives, DMTCO, MDDCO and TDCO are capable of forming hydrogen bonds with the solvents, and the order of their hydrogen bonding ability is MDDCO > TDCO > DMTCO. The multiparameter analyses by Kamlet-Taft and Catalan suggest that the hydrogen bond donating ability of the solvents is primarily important in controlling the fluorescence spectral shifts of the KTHC-derivatives.

The excited state properties of DMTCO, MDDCO and TDCO are influenced by the presence of aromatic amines DMA and DEA. As a result of such molecular interactions, the fluorescence of the KTHC-derivatives quenches, their fluorescence lifetime reduces and also their transient absorption decreases. The appearance of the signatures of the radical cations of the amines in the transient absorption spectra is indicative of the occurrences of photoinduced electron transfer from the amines to the KTHC-derivatives.

The emissive properties of KTHC-derivatives KTHC-67 and MOTHCA are modified in presence of  $\beta$ -cyclodextrin, and to a greater extent compared to the changes in absorbance. The absorbance of the molecules reduces, although their fluorescence enhances. The appearances of isosbestic points in the absorption spectra and isoemissive points in emission spectra suggest the establishment of binding equilibrium. The binding interactions are weak in both cases, which have been suggested by the experimental as well as the molecular docking calculations. Theoretical calculations further attest the formation of inclusion complexes.

Photophysics of NAN in aprotic solvents reveal that their excited state properties are solvent sensitive, especially to the proton affinity of the solvents. The presence of aliphatic amines TEA, TBA and DIEA affects the emissive behaviour of NAN. Quenching of fluorescence intensity and lowering of fluorescence lifetime are the consequences of the molecular interactions. Transient absorption spectra of NAN in presence of the amines contain the signatures of the radical cations of the amines, as well as the radical anion of NAN, which proves that photoinduced electron transfer is liable for the molecular interactions.

The different handedness of R- and S-BINOL is responsible for their differing modes of interaction with the model transport protein HSA. Molecular docking calculations suggest that S-BINOL has the proper alignment to form hydrogen bond with Tyr138 residue in domain IA of HSA and as a consequence, the structural stability of HSA is enhanced as evident from the decrease in the extent of urea induced denaturation. Alternatively, R-BINOL cannot form similar hydrogen bond with the neighbouring residues and it prefers to bind to the domain IIIA of HSA despite of domain IA. However, the binding sites of these two BINOLs are equally polar and rigid. The formation of hydrogen bond assists deprotonation of S-BINOL and subsequent formation of its anion as exemplified by the formation of a new peak at 448 nm in the fluorescence spectra. As a consequence, the helicity of HSA is increased by 65.6% in presence of S-BINOL, whereas R-BINOL increases only 5.6% of helicity in the range of nanomolar concentration. Moreover, S-BINOL quenches HSA fluorescence to a greater extent than R-BINOL and consequently the energy transfer efficiency is higher in case of S-BINOL.

A better understanding of the photophysical properties of molecules and the various photoinduced phenomena is required for finding suitable usage of the molecules in analytical,

biological, therapeutical or industrial fields. Such basic experiments are the precursor of much more complicated studies, which would be relevant for the abovementioned different fields.

Apart from using the conventional spectroscopic techniques, we have devised some new techniques. The comparative hydrogen bonding abilities of the fluorophores have been evaluated by measuring the differences in the calculated values of dipole moments in aprotic and the protic solvents. The downward curvatures of the Stern-Volmer plots for the fluorescence quenching of HSA with BINOLs, which may be due to the differential accessibility of the BINOLs to the Trp and Tyr residues in HSA, have been supported by the linear Stern-Volmer plots for the quenching of synchronous fluorescence spectra of Trp and Tyr in presence of BINOLs. Moreover, the better quenching of BINOLs by the Trp residues than the Tyr residues has been explained by measuring the average distances of the BINOLs from the two residues.

### **Future plans**

Photophysics of the KTHC-derivatives have been interesting and so we plan to extend the studies on this class of fluorophores. The bi-exponential decays of KTHC derivatives in long chain alcohols will be resolved by carrying out the experiments in femtosecond up-conversion setup. The transient absorption measurements will be carried out in various micelles, reverse micelles in presence of magnetic fields, to find out the initial spin correlations during the photoinduced electron transfer from amines to the KTHC derivatives. We also plan to perform some in-depth theoretical calculations to explain the solvatochromic behaviour of this class of molecules and also the role of solvation on their interaction with other molecules. Moreover, we plan to carry out the experiments on several other KTHC-derivatives, which would help to make a comparative review of the photophysical properties of these molecules.