SYNOPSIS

OF THE THESIS ENTITLED

Fluorescence Studies on Biologically Relevant Heterocyclic Systems in Homogeneous Medium and Supramolecular Assemblies

BY

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Introduction

In the current trend of modern science, chemistry is not only a science of synthesis and structural manipulations of molecules but it has gradually undertaken the more challenging task of biology-oriented field. Modern science craves to a deeper understanding of life phenomena particularly molecular network in living systems. Understanding interface between chemistry and biology has great role in development of fluorescent probes. Surrounding environment determines the fluorescence of organic molecules. Fluorescent dyes are used to determine micro-environmental parameters, such as the polarity of media, as well as their relocation and distribution dynamics in micro-heterogeneous systems such as membranes, micelles, and cellular media as well as interfaces, polymers, and discrete supramolecular systems. Heterocyclic rings are one of the fundamental components in the skeleton of the biologically active compounds produced by nature [1, 2]. A new family of heterocyclic systems opens the possibility of finding further types of biologically active units for medicinal uses and generates potential functional materials to construct molecular devices. Therefore, developing new methodologies to increase the structural complexity in heterocyclic systems is a main area of research [3-8].

Scope of the Thesis & Layout Description

The present work, proposed to be submitted to North-Eastern Hill University (NEHU) for the award of Ph.D degree, deals with a systematic study on the photochemistry of some selected biologically relevant systems in homogenous medium. The structures of all the compounds are shown in figure 1. The systems were also
investigated for their fluorescence behavior in different biologically relevant heterogeneous environments. High level quantum chemical calculations were also performed to understand molecular mechanism of excited state interaction and mode of specific solvent effect. For the convenience of the presentation different aspects of the present work have been discussed in six different chapters of the thesis. Brief description of the arrangements of different chapters presented in the thesis is given below:

Chapter – 1: This is the introductory chapter of the proposed thesis and discusses the general aspects of fluorescence. Particular attention was paid to discuss the interface between fluorescence and biology with special importance to the fluorescent probes used to study the biological phenomena.

Chapter – 2: This chapter mainly describes the details of the experimental methodology. The details of the experimental techniques used in this study, viz. steady state absorption and fluorescence as well as time-resolved measurement technique were discussed in this chapter. Sample preparation, data collection and handling protocol were also discussed in detail. The details of synthesis and purification of the fluorescent probes were left to the subsequent individual chapters; however, the nature of different bio-mimetic micro-heterogeneous environments used in this study was incorporated in the present chapter.
Chapter – 3: The detailed fluorescence behavior and solvatochromism of ethidium bromide (1) was described in neat and mixed homogeneous solvents as well as in presence of natural surfactants like bile acid (BA) by steady state and time-resolved fluorescence spectroscopy. The observed solvatochromism and increase in fluorescence intensity of 1 in polar aprotic media was rationalized by multi-parametric approach using Kamlet-Taft equation. The total nonradiative decay rate was found to be strongly correlated with solvent properties and explains the dramatic decrease in either fluorescence yield or lifetime of 1 in polar protic environment. All the BAs bind 1 with moderate affinity and causes significant increase in fluorescence intensity by shielding the fluorophore from relatively more polar and hydrogen bonding aqueous phase [9].

![Figure 1: Structure of the compounds used in this study.](image)
Chapter-4: Photophysical properties of porphyrin appended substituted 1,3,4-oxadiazoles (2A) and thiazoles (2B) were described inhomogeneous medium as well as in presence of cationic surfactant cetyltrimethylammoniumbromide (CTAB) relative to the unsubstituted parent molecule H$_2$TPP. Binding of porphyrin derivatives with cationic micellar system was found to be associated with increase in fluorescence yield and life time, particularly in case of electron donor substituent. The unsymmetric systems were sequestered more preferentially in the micellar medium in comparison with the symmetric model compound resulting about two fold decrease in the total non-radiative rate [10-12]. Also, in this chapter, the photophysical behavior of substituted phthalazine derivatives (3) was incorporated in special relation to their use as fluorescent probes for studying biological environments [13].

Chapter-5: The photophysical behavior of different derivatives of substituted polyhydroquinolines (4) was studied in detail using steady state and time-resolved fluorescence spectroscopy in combination with density functional theory calculation. Interaction between the benzene and the quinoline rings increases with more electron donating substituent at the para-position resulting bathochromic shift both in the absorption and emission spectral peak position. N,N'-dimethylamino substituted system (4c) shows an additional largely-Stokes-shifted intramolecular charge transfer emission. (TD)DFT calculation results predict the formation of the pro-ICT structure in 4c, possibly resulting from 1,3-H migration in the excited state. The intensity of Stokes-shifted fluorescence decreases considerably in water and other polar protic solvents leading towards possible use of these systems as fluorescence assay for monitoring different bio-chemical systems [14].
Chapter 6: The photophysical behavior of differently substituted anil derivatives (5) of coumarinyl amine were studied in detail using steady state and time-resolved fluorescence spectroscopy in combination with density functional theory calculation. Efficient intramolecular charge transfer in the case of dimethylamino substituted derivative (5b) is confirmed from the high dipole moment as well as analysis of electron density distribution in the excited state. Linear regression of solvent dependent spectral shift data using Kamlet-Taft model indicates the importance of solvent hydrogen bonding on the excited state behavior of 5b. A combination of several factors like (i) excited state planarization and subsequent isomerization; (ii) efficient hydrogen bond formation at the immine nitrogen atom in protic medium triggered by intramolecular charge transfer in case of 5b leads to the formation of a new product. The process seems to be instantaneous in the excited state; however, involves a barrier of ~50 kJ mol\(^{-1}\) in the groundstate. The rapid formation of the new fluorescence band in dimethylamino substituted coumarin compound can be effectively manipulated to use as a “turn-on” fluorescent sensor for monitoring the protic environment in biological samples [15].

Conclusion

The work presented in this thesis consists of detailed fluorescence investigation on a series of biologically relevant heterocyclic systems. Steady state and time-resolved fluorescence experiments in different homogeneous as well as several heterogeneous environments ranging from cyclodextrin, natural & synthetic surfactants and model water soluble proteins were done to understand the photophysical behavior of the probes. Adequate theoretical calculation results based on time dependent density functional
theory (TDDFT) were also presented. The major conclusions obtained from the study in different systems can be highlighted as follows:

The observed solvatochromism and increase in fluorescence intensity of ethidium bromide (EB) in polar aprotic media is rationalized by multi-parametric approach using Kamlet-Taft equation. Water assisted fluorescence quenching of EB either in acetonitrile or methanol follows simple Stern-Volmer (SV) mechanism; however, strongly basic solvent DMSO quenches the fluorescence in an entirely different mechanism. All the bile acids bind EB with moderate affinity and causes significant increase in fluorescence intensity by shielding the fluorophore from relatively more polar and hydrogen bonding aqueous phase. The heterogeneous distribution of fluorophore in micellar sub-domain is evident from additional slower fluorescence decay component. The sharp break point observed in the population ratio of long to short decay component can efficiently monitor the onset of primary aggregation pattern in BA micelles.

Quenching of fluorescence for porphyrin appended substituted 1, 3, 4-oxadiazoles and thiazoles in presence of substituted acceptors was indicative of donor-spacer-acceptor type of photoinduced electron transfer system. Binding of porphyrin derivatives with cationic micellar system is associated with increase in fluorescence yield and life time, particularly in case of electron donor substituent. The unsymmetric systems were found to be sequestered more preferentially in the micellar medium in comparison with the symmetric model compound resulting about two fold decreases in the total non-radiative rate. These results lead us to believe that unsymmetrical porphyrin derivatives appended with electron donating oxadiazole or thiazole substituent can act as efficient fluorescence assay to monitor the interaction with endogenous carrier proteins leading toward potent
photodynamic therapeutic agent. Further, a simple and efficient, high yielding and relatively green protocol was developed for the synthesis of 2H-indazolo [2,1-b] phthalazine-trione under solvent free conditions using Ni-NPs properties as are usable catalyst. Screening for optical properties of the synthesized compounds in homogeneous and different heterogeneous media showed that some of these products may have a good future as new luminescence materials or fluorescence probes.

Interaction between the benzene and the quinoline rings in disubstituted polyhydroquinolines increases with more electron donating substituent at the para-position resulting bathochromic shift both in the absorption and emission spectral peak position. N,N′-dimethylamino substituted system shows an additional largely-Stokes-shifted intramolecular charge transfer emission. The formation of the pro-ICT structure is believed to be due to 1,3-H migration in the excited state. The intensity of Stokes-shifted fluorescence decreases considerably in water and other polar protic solvents leading towards possible use of these systems as fluorescence assay for monitoring different bio-chemical systems.

Efficient intramolecular charge transfer in the case of dimethylamino substituted coumarin derivative was confirmed from the high dipole moment as well as analysis of electron density distribution in the excited state. Linear regression of solvent dependent spectral shift data using Kamlet-Taft model indicates the importance of solvent hydrogen bonding on the excited state behavior in this system. A combination of several factors like (i) excited state planarization and subsequent isomerization; (ii) efficient hydrogen bond formation at the imine nitrogen atom in protic medium triggered by intramolecular charge transfer leads to the formation of a new product in
dimethylaminoderivative. The process seems to be instantaneous in the excited state; however, involves a barrier of ~50 kJ mol\(^{-1}\) in the ground state. The rapid formation of the new fluorescence band in dimethylamino substituted coumarin compound can be effectively manipulated to use as a “turn-on” fluorescent sensor for monitoring the protic environment in biological samples.

References

