CHAPTER I
INTRODUCTION

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1.1. General introduction

The study of interaction of radiation of light with organic molecules and subsequent changes that take place at molecular level has received a great deal of attention for over the past several years. It is well known that the electronic ground state of most organic molecules is a singlet state \((S_0)\), which spans a range of energies determined by quantized vibrational and rotational energies of the molecule. The rotational levels provide almost a continuum state between vibrational levels. After light absorption, organic molecules (within \(10^{-15}\) sec) are excited to higher vibrational levels of the first excited singlet state \((S_1)\), called Franck-Condon (FC) state and return to the lowest vibrational levels of the same state \((S_1)\) rapidly within a few picoseconds (ps) through a non-radiative process known as internal conversion (IC). Molecules from zero vibrational level of \(S_1\) state then jump to various vibrational levels of \(S_0\) state in accordance with the Franck-Condon principle through radiative and non-radiative
processes. The spontaneous emission of radiation from $S_1 \rightarrow S_0$ transition is referred to as fluorescence, since singlet-singlet transition is spin allowed, strong and broad fluorescence band is observed for organic molecules in liquids. The average lifetime of the first excited singlet state is short varying from 0.01 ns to 100 ns, because its decay to the ground state is highly probable. At low temperature, generally in non-polar media, some molecules show vibronic structure. The non-radiative process (IC) from $S_1 \rightarrow S_0$ always competes with fluorescence. The molecules in the $S_1$ state may also relax to a low lying first triplet state ($T_1$) through a non-radiative process called inter-system crossing (ISC) within a few microseconds (μs) or less. The triplet state is thus populated via $S_1$ state and the direct excitation of molecules to triplet ($T_1$) states from ground state ($S_0$) state is a rare phenomena. The radiation emitted from transition $T_1 \rightarrow S_0$ is termed as phosphorescence. Since the $T_1 \rightarrow S_0$ transition is spin forbidden, and the emission due to this transition is weak and long lived. In rigid media at low temperature, the triplet lifetime is lengthened and ranges from 10 ms to few seconds being dependent on the medium [1]. Owing to its relatively long lifetime, the triplet state acts as a trap for excited molecules. Several elementary processes that control the deactivation of electronically excited singlet state of the dye molecule in solution after photoexcitation are shown in Fig.1.1. Although the lifetime of first excited singlet, ($S_1$), state is shorter than that of the first triplet state, according to Hund's rule the former possesses higher energy and hence is more reactive than the latter of the same configuration. Since the total energy of a molecule depends primarily on the
Fig. 1.1. Photophysical processes of dye molecule:
Ab: electronic absorption, Fl: Fluorescence,
IC: Internal conversion, ISC: Intersystem crossing
Ph: Phosphorescence
spatial distribution of its electrons, the arrangement of electrons in the singlet and triplet state of the same configuration would be different. The nature and energy of electronically excited states of organic molecules are important in determining their photophysical and photochemical properties. When a molecule is surrounded by a liquid solvent or solid matrix, each state, i.e., the initial state and final state (FC) is stabilized or destabilized as the case may be, by the energy called the solvation energy. The difference in the solvation energy of the final and initial states in various solvents is taken as the solvatochromic shift and hence the solvatochromic shifts are the experimental evidences about these energy changes. The emission spectra are in this respect often more informative than the absorption spectra, since they are related to the energy of the relaxed excited states. There are various theories of solvatochromic shifts which try to relate the solvatochromic shifts to the electron distribution of the molecule in its excited states, especially to the total dipole moment and the average polarizability; these quantities are important for the description of intra-molecular charge transfer in the molecular excited state and of intermolecular charge transfer in exciplexes. In fact, Stoke shift measurements measure differences in the behaviour of ground and excited states. This differentiates the fluorescence of both environmental FC state and environmentally relaxed state. The medium can affect the solute molecule by its viscosity as well as by its solvation properties. In solvents of low viscosity, the orientational relaxation of solvent molecule becomes fast and takes place within the lifetime of the excited states. The emission can then come from the relaxed state. In viscous solvents,
because of slow solvent relaxation process, the emission originates from excited
states, which are nearly FC states, rather than from the relaxed states.

For understanding the photochemical reactions, it is necessary to know
the identity of the excited states and the unstable intermediates formed on
photoexcitation. In more polar molecules, the excited state generally becomes
more polar than the ground state. Hence an increase in the polarity of the
solvent produces a great stabilization of the excited state than of the ground
state. Consequently, a shift in both absorbance and fluorescence spectra to
lower energy or longer wavelength (red shift), is usually observed as the
dielectric constant of the solvent increases. But the magnitude of the shift
measured as a function of solvent polarity is substantially larger in fluorescence
than in absorption, thus indicating the nature of the transition as of \( \pi - \pi^* \) type.

It has been established that interaction between solute and solvent greatly
change absorption and emission properties of solute molecules. Solute-solvent
interactions are of two types: (1). Universal interaction, and (2). Specific
interaction. Universal interaction is due to the collective influence of the solvent
as a dielectric medium. It depends on the dielectric constant, refractive index
of the solvent and the dipole moment of the solute. Specific interactions are
short range interactions and involve hydrogen-bonding, charge transfer or
exciplex formation. H-bonding ability may change on excitation specially for
\( n - \pi^* \) transitions. As reported for quinoline and isoquinolines, H-bonding can
lead to the inversion of \( \pi - \pi^* \) and \( n - \pi^* \) states. Fluorescence spectroscopic
parameters are also sensitive functions of various phenomena that occur during
the lifetime of the excited molecule. Within the lifetime, there could be translational and rotational differences of fluorophore, collisions with the quenchers, formation of complexes with the solvents or solutes and solvent reorganization about the excited dipole of the solute etc. All such dynamical processes can affect the fluorescence spectra, fluorescence lifetime, fluorescence anisotropies and quantum yields, probability of transition, radiation polarization, etc. Therefore, their investigation under variety of experimental conditions gives important information on the microstructure of condensed media and the mechanisms of intermolecular and intra-molecular interactions.

Lifetimes of the excited states are important parameters since the reactivities of these energy states depend on them. A flash photolysis technique first developed by Porter and Norrish [2], gave new quantitative approach to such studies. They demonstrated successfully the application of milliseconds (ms) pulses from flash lamps in the study of transient species in chemical reactions by absorption method. The experimental probes of primary processes consisted both in the production of short optical pulses at suitable wavelength and sensitivity of the detection system. Although, further improvements occurred with the advent of nanosecond flash lamps, their limited repetition rates and low intensities were not adequate enough for fluorescence decay measurements. However, a break-through in the field of ultrafast spectroscopy occurred in 1966 [3]. Since the development of mode locked lasers capable of generating pulses of picosecond duration, several workers explored the use of pulsed and continuous-wave lasers and ways of studying fluorescence decays under various
experimental conditions. The duration of light pulses derived from ultrafast dye lasers was brought down from 1 picosecond (ps) to 6 femtosecond (fs) [4], and such dye lasers became routine tools for spectroscopic applications in the photochemistry and photobiology.

The various molecular dynamics that take place within the lifetime of the sample can only be studied quantitatively by time-resolved emission and absorption spectroscopy. At present, ultrafast picosecond and femtosecond pulses generated by mode-locked lasers can be effectively used to initiate and monitor the successive events that occur after the absorption of light by the molecule. The commonly used popular techniques to study the time-resolved emission studies are streak camera, time-correlated single photon counting and gating techniques (Kerr gating technique and upconversion). In particular, the time-correlated single photon counting technique provides more information as compared to other techniques [5]. This technique is becoming popular for measuring low light intensities as it is necessary in luminescence studies. The great advantage of this method is that it eliminates disturbance due to noise and stray light. This technique measures the time of emission of individual fluorescence photons, the reference zero time being the initial rise of the flash lamp light / laser pulse. The lifetimes of the electronically excited states are very much affected by energy transfer processes, diffusion collision, and viscosity of the medium and the nature of the transitions and various emitting species in the solutions. Molecular motions can also affect the lifetime. Therefore, the measurements of lifetime of
Si state would help to reveal the nature of the emitting species and electronic states and to indicate the involvement of interaction between solute and solvent molecule.

According to Kasha's rule [6], the luminescence of organic molecules always occur from the lowest electronic excited states of a given multiplicity, so that the fluorescence, \((S_1 \rightarrow S_0)\) and phosphorescence \((T_1 \rightarrow S_0)\) are the only two molecular luminescence to be expected e.g., the fluorescence and phosphorescence spectra do not depend on excitation energies. However, dual luminescence, though infrequent has been reported from a number of molecules [7-13], and the observed emission bands show excitation energy dependence. The multifluorescence generated from organic molecules have been attributed to the following variety of phenomena:

1. Excimers

At low concentrations of solution, many molecular systems show normal fluorescence. However, as concentration increases, these molecules display new structureless emission band on the long wavelength side of the normal fluorescence. Such new bands have been ascribed to excimer formation [14-17], by intermolecular process.

\[ ^1M^* + ^1M \rightarrow ^1D^* \]

The excimers are stable only in the excited states.
II. Complexes

An unexcited aromatic molecule $^1M$ in fluid solution may interact with an unexcited molecule $^1Q$ of a species to form a donor acceptor complex in the ground state,

$$^1M + ^1Q \rightarrow ^1E$$

If the ground state complex ($^1E$) is stable, then upon photoexcitation, it produces its fluorescence, which differs from normal fluorescence. If the complex $^1E$ is dissociative in the ground state but associated at the excited state, the excited state complex $^1E^*$ or exciplex may be formed in the solution by the process

$$^1M^* + ^1Q \rightarrow ^1E^*$$

The dual fluorescence resulting from complex or exciplexes does not conflict with Kasha's rule since $^1E^*$ differs from $^1M^*$. The complex between solute and solvent model leads to significant changes in its excited state behavior. That, the charge transfer singlet state is lower in energy than the locally excited singlet of solute is clear since its fluorescence appears as a broad featureless band at longer wavelength compared to the normal structured fluorescence. The formation of Electron-Donor-Acceptor (EDA) complex, can lead to significant changes in the ordering of electronic levels, thereby affecting fluorescence [18].
III. Molecular aggregates (Dimers or higher aggregates)

Many organic molecules form aggregates as dimers, closely spaced pairs and higher aggregates / associates, generally, at high concentration and at low temperature. Their fluorescence spectra are separated in monomer and dimer contributions and normal fluorescence and their occurrence depends on excitation energy and concentration. If emission components are not resolved, the emission due to dimer or any sort of molecular aggregates, gives rise to red shift with increasing excitation wavelengths. The superposition of monomer and aggregates is also supported by the concentration effect and lifetime measurements. The dimers are less strongly fluorescent than the monomer and can quench monomer emission by either radiative, or non-radiative energy transfer [19].

IV. Twisted intramolecular charge transfer state (TICT state)

The most frequently studied types of dual fluorescence involved a twisted intramolecular charge transfer [TICT] state emission [7-8], and an excited state intramolecular proton transfer [11]. The TICT phenomenon was first observed in case of p-(N,N-Dimethylamino) benzonitril (DMABN) in polar solvents, and to account for this, the TICT model was first suggested by Rotkiewicz and Grabowski [20]. According to this model a planer molecule upon photoexcitation is converted into another molecule with a
strong charge transfer (CT) character. This CT character is accompanied by a twist about the donor-acceptor bond. The resulting TICT state has nearly perpendicular geometry and has a large dipole moment. The TICT processes are found to be controlled exclusively by the polarity of the medium and the formation of the TICT state is favoured in hydrogen bonded solvents. The fluorescence from TICT is generally weak and occurs at higher wavelength relative to that of locally excited (LE) state. Upon excitation, the dye molecules are excited to different interchangeable states depending on the substitution pattern and microscopic solvent environment. In many cases dual fluorescence observed may be due to interconversion of one state into another [21], or interconversion of one kind of species into different species, or to the co-existence of two distinctly different species with a different conformation.

V. Protonation and H-bonding

The excited state intramolecular proton transfer [ESIPT] involves the transfer of proton, originally attached covalently to an atom A, to a neighboring hydrogen bonded atom B to produce a phototautomer. This process is extremely fast and occurs in the subpicosecond time scale especially in rigid media and at temperature 4K [43,44]. The tautomer emission is red shifted with respect to normal emission [43,44]. Tautomer emission along with a strong normal emission reported for HPBI, is the best example[42]. Interconversion
between the intramolecularly H-bonded rotator has been reported for HPBI [42]. The spectra are structured in non-polar solvents and the vibrational structure is lost as the polarity or hydrogen bond formation tendency of the solvent increases. The tautomerization depends on the acid and base properties of groups of solute. Intermolecular proton transfer between solvent and the solute may also result in the formation of cationic species.

When dipolar organic molecules in polar solvents are excited, generally, redistribution of charge takes place in the excited state. If a molecule contains donor-acceptor fragments in the molecular structure, the functional characteristics of these groups depend on the electron-charge-density distribution in the molecular system. Therefore, the relative contribution of intermolecular H-bonding to solvation may be different for the ground and the excited state of the molecule. When such molecules are found in protic solvents such as alcohols and water, there is always a tendency of forming H-bonding between the solute molecule and the solvent. The strength of H-bonding depends very much on the polarity of the solvent and the distance between H and active site of the chromophore. Depending on the O-H distance, the molecules can show different emission spectra [23], corresponding to two ensembles of molecules i.e. H-bonded species and protonated species.

Fluorescence / absorption spectra of some dye molecules are observed to depend on the pH of the solution and on the excitation wavelength. For example, the fluorescence maximum of proflavin is red shifted by ~1000 cm⁻¹ in going from acid to alkaline conditions in fluid solution and its
effect is attributed to the formation of different ionic forms of the dye molecule with a different fluorescence spectra. Some molecules display two partially overlapping bands corresponding to two ionic species, when studied as a function of pH and excitation wavelength.

Fluorescence properties of some organic molecule are modified greatly when solute molecules are embedded in more orderly medium such as Nile red, acridine orange and rhodamine-6G incorporated in porous glass [24], in lead-tin fluoride glass, silica gels, membranes and Langmuir Bladgett [Lb films]. Fluorescence polarization studies have helped to identify the presence of dimer on glasses, and the structure of dimers was found to be consistent with sample exciton theory [1994]. Exciton splitting of electric level of molecules with two or more interacting chromophores is known to produce dual emitting states. In a perfect aromatic crystal each excited electronic state of the molecule is split into two or more exciton states due to interaction with translationally inequivalent neighbour. For example, in crystals of naphthalene and anthracene, two singlet exciton states $1\alpha$ and $1\beta$ originating from the $S_1$ molecular state differs in energy by $\sim 200$ cm$^{-1}$. The magnitude and polarization of the transition moments to and from exciton states differ, so that under appropriate conditions two fluorescence originating from $1\alpha$ and $1\beta$, respectively and differing in spectrum, lifetime and polarization may be observed.

The molecular fluorescence spectrum of an aromatic polymer is a characteristic of the individual monomer segments. Intra and inter-molecular
interactions of excited aromatic segments can yield excimers, so that a structureless (excimer) fluorescence band is observed in polymers emission spectrum.

Fluorescence polarization spectroscopy plays important role in the study of molecular dynamics. As the polarization of light emitted by fluorescence species depends on the orientation of the emission dipole, the fluorescence polarization measurements yield information on the orientation of molecule. This information can be used to obtain rotational diffusion rates and the environmental parameter such as viscosity of the medium. The quantities most commonly measured in fluorescence polarization measurements with the linearly polarized light are the degree of fluorescence polarization $P$, and the fluorescence anisotropy, $r$. These parameters are defined as

$$ P = \frac{(l_{\parallel} - l_{\perp})}{(l_{\parallel} + l_{\perp})} \quad 1 $$

$$ r = \frac{(l_{\parallel} - 2l_{\perp})}{(l_{\parallel} + 2l_{\perp})} \quad 2 $$

where $l_{\perp}$ and $l_{\parallel}$ refer to the vertical and horizontal intensity components of the light relative to the observer. The physical significance of fluorescence polarization and anisotropies lie of course, in the mechanisms by which fluorophores emit light with a different polarization from that of absorbed light. The requirement for such mechanisms is that the emission transition dipole must lie along an axis that is different from the axis along which the absorbed light is polarized. This can be accomplished in several different ways. The probability of light absorption is generally proportional to the factor $\cos^2 \theta$, where $\theta$ is the angle between
the absorption transition dipole and the electric field of the light. Consequently, if an assembly of molecules, randomly oriented (e.g. in fluid solution), are excited with plane polarized light, a particular orientational distribution will be obtained in the excited state due to the dependence of the absorption probability on the angle between the transition dipole moment and the electronic vector. Brownian motion will randomize the excited state orientation ultimately resulting in a homogeneous population distribution. As the fluorescence polarization (with respect to some external co-ordinate system) depends on the orientation of the transition moment, the fluorescence will, with time, becomes depolarized due to the decay of the inhomogeneity of the excited orientation population distribution; this assumes that the excited state is sufficiently long lived for the randomization to occur. The rate of fluorescence depolarization is related to the orientational correlation function of the excited state molecule. This orientational relaxation can be a very sensitive probe of the microenvironment of a fluorescent species due to its dependence on the size and shape of the rotating molecule and, its interaction with the environment.

1.2. Proposed work

In the present thesis, our interest has been concentrated mainly on the steady state absorption, excitation, fluorescence emission, fluorescence polarization, fluorescence lifetime, orientational relaxation and dual laser emission characteristics studies of some heterocyclic molecules.
The organic molecules chosen for the present studies are 6-methoxy quinoline, 8-hydroxy quinoline, fluorescin-Na and eosin-B. Out of these compounds fluorescin-Na and eosin-B are efficient laser dyes [25], and can act as probes to study biological systems [26]. These two dipolar molecules are important as they can be used as “antenna” chromophores to rare earth ions to produce emitting chelates in the near IR region. These chelates find applications as luminescent makers in fluorimmuneassys and time resolved microscopy [27]. Despite, a large number of study-state absorption and emission studies on fluorescin-Na and eosin-B [28], excited state dipole moment, dual fluorescence and dual laser emission have not been reported.

There are very few reports on steady state measurements and time resolved emission studies on 6-methoxy quinoline [12], and 8-hydroxy quinoline [30], at room and at low temperature. However, for these molecules there are no reports on excited state dipole moment, dual fluorescence and anisotropy decay measurements. Various substituted quinolines are reported to possess a wide range of biological activities including antimicrobial [31], antitumor [32], anticonvulsant [33], antidepressant [34], antimalaria [35], antihistamine etc. Hydroxy quinoline and their derivatives deserve special attention from both fundamental and practical point of view [30]. In particular, 8-hydroxy quinoline (or oxine) and many of its derivatives have been extensively studied because they are very well known chelating and fluorogenic reagents used in analytical chemistry [37], although their fluorogenic effect is
not yet fully understood. Another interesting feature is that some 8-hydroxy quinoline derivatives are expected to exhibit non-linear optical properties [38].

In view of these important features of the organic molecules, the following aspects of the mentioned dyes are investigated in the present study.

1. Steady state absorption/excitation, fluorescence emission, fluorescence lifetime and polarization characteristics of 6-methoxy quinoline and 8-hydroxy quinoline.

2. Rotational reorientation of 6-methoxy quinoline.

3. Dual laser emission characteristics of Fluorescin-Na and Eosin-B and Steady state absorption, emission and lifetime measurements.
1.3. References


