Chapter 6

Basic Principles of Absorption and Emission in Dye Molecules

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6.1 Introduction

Fluorescence spectroscopy deals with photophysical studies viz. steady-state emission, time resolved fluorescence, single molecule detection and so on of various organic dye molecules and is one of the major realm of research and extensively applied in medical diagnostics, biotechnology, metal sensing, bio-imaging, in situ quantification of proteins, DNA sequencing, flow cyctometry, forensics, genetic analysis etc. [89–93]. The ease of applicability, non-destructive methodology, fast response, fast data acquisition, high sensitivity make fluorescence technique a blessing to the scientific field [89–93]. The use of fluorescence method exploiting organic fluorescent dyes allowed can prevent the usage of radio tracers for biological imaging purposes, making the technique more safe, inexpensive. Dye is a chemical substance that generates colour upon absorption of light. Dyes are divided into two main categories:

1) Non-fluorescent dyes, which do not emit light after absorption process (e.g., Crystal violet, brilliant green, methyl orange etc.) and
2) Fluorescent dyes, which possess characteristic emission after absorbing suitable radiation (Rhodamine, Coumarin, Cyanine, pyrromethenes etc.)

Depending upon the energy of the absorbed light (Fig. 6.1), three possible electronic transitions can take place in an organic molecule which can be arranged energetically as $n \rightarrow \pi^*$ $< \pi \rightarrow \pi^* < \sigma \rightarrow \sigma^*$. Due to very high energy associated with the $\sigma \rightarrow \sigma^*$ transitions, these are rare. On the other hand, although $n \rightarrow \pi^*$ transition is of low energy, symmetry consideration makes this transition of very weak in intensity, whereas, the $\pi \rightarrow \pi^*$ transition is of higher energy compared to $n \rightarrow \pi^*$ but symmetry consideration makes the $\pi \rightarrow \pi^*$

transitions highly allowed and so the intensity this transition is quite high in nature. In most of the organic molecules whatever spectra one encounters is mainly due to $\pi \rightarrow \pi^*$ transition, which lies in the visible region of the molecular spectrum.

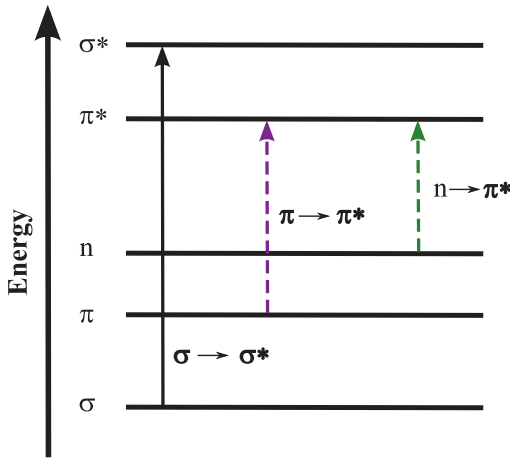

Figure 6.1: Energy level diagram of the molecular orbitals and different types of electronic transitions taking place in an organic molecule.

6.2 Jablonski Diagram

To understand the basics of the absorption and emission of the organic dye molecules, it is imperative to have an idea about the Jablonski diagram, which explains the different processes related to the electronic transitions during absorption and emission in a dye molecule. When an organic dye absorbs light, electrons are promoted from the ground state (S_0) to excited states (S_1, S_2,... and so on). After reaching the excited state, the excited electrons come down to the ground state through various mechanisms, which form the basis of emission. The probability of these various processes depends upon various factors, the structure of the dye molecules, its state (whether in solution or in the solid state), nature of the solvent (in solution state), temperature of the medium, presence of any interfering agents and so on. The emission process of a dye, however, is mainly governed by the two factors: one is the rate of radiative decay, which is an intrinsic property of the dye molecule and the other one is the rate of non-radiative decay, which can be tuned by various mechanisms one of which is supramolecular encapsulation.

Fluorescence is a process where de-excitation takes place via emission of light and this occurs through the electronic states of same multiplicity, i.e., from S_1 to S_0, and takes place within the time scale of few nanoseconds. However, in addition to fluorescence, there are many non-radiative processes that can take place simultaneously in the excited S_1 state and those are vibrational relaxation (time scale: 10^{-11} s to 10^{-9} s), internal conversion (10^{-12} s or less) and intersystem (S_1 to T_1) crossing (10^{-10} s to few seconds). The rate of these non-radiative processes especially can vary depending upon the surrounding environment of the fluorophore. It is to be noted that there are certain luminescent molecules that can show emission due to the electronic transition from the first triplet state (T_1) to the singlet ground state (S_0) of the molecules. This phenomenon is known as phosphorescence. Phosphorescence is a spin forbidden transition and hence, occurs generally in the time scale of microseconds or longer.

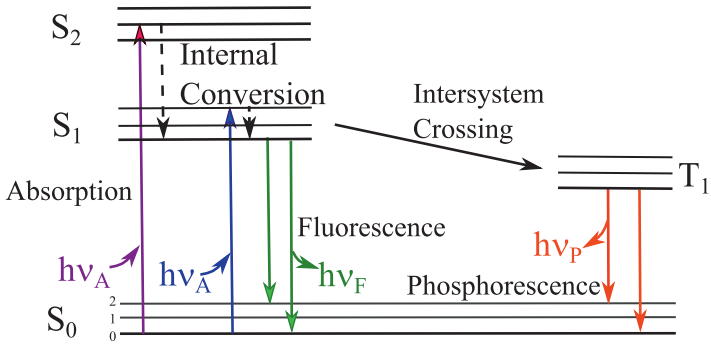

Figure 6.2: Jablonski diagram showing different energy states and de-excitation processes following light absorption by a molecular system.

6.3 Photophysical Properties of the Excited States of Dye Molecules

When a molecule absorbs a light photon, it is promoted to a higher electronic state and the molecule is then called as the excited molecule (M*), which can behave differently than its ground state, participating in different photophysical and photochemical processes. The properties of the chromophoric molecules in regard to their absorption spectra, emission spectra, emission (fluorescence) quantum yield (ϕ), excited state lifetime (τ), etc. are as a whole considered as the photophysical properties of the chromophoric systems. The details of these properties are discussed below:

6.4 Ground State Absorption and Steady State Emission Spectra

Ground state absorption spectra and steady state emission spectra provide us the information about the relative absorption and emission of light of different wavelengths by the chromophoric system under consideration (Fig. 6.2). Absorption spectra are obtained by plotting the optical density (O.D.) or absorbance (A) as a function of wavelength of the incident radiation, while the emission spectra are obtained by plotting the emission intensity against wavelengths of the emitted light. It is to be noted that while the intensity of the emitted light from the molecules depends on the wavelength of excitation; however, the position of peak emission or the overall shape/feature of the emission spectra usually remain unchanged with the excitation wavelength. In order to carry out any photophysical or photochemical study, it is always the first step to know the absorption and emission spectra of the dye molecules. Although absorption is a universal phenomenon for dye molecules, their emission is not. There are many examples where dye molecules show only very weak emission or no emission though their absorption spectra are quite strong.

6.5 Fluorescence Quantum Yield

The efficiency of a dye molecules undergoing emission process is expressed by the parameter fluorescence quantum yield, which can be defined as the number of the photons emitted per

number of photons absorbed by the molecules. Hence, mathematically ϕ can be written as,

$$\phi = \frac{\text{total number of photons emitted}}{\text{total number of photons absorbed}} \tag{6.1}$$

Alternatively, the emission quantum yield can also be defined as the rate of de-excitation of the excited molecules via radiative process over the sum of rates of all the de-excitation processes. Thus,

$$\phi = \frac{k_r}{k_{\mathrm{r}} + \Sigma k_{\mathrm{nr}}} \tag{6.2}$$

where k_r is the rate of radiative process and Σk_{nr} is the sum of all the non-radiative decay processes.

6.6 Excited State Lifetime

Excited state lifetime is defined as the average time spent by the excited molecules before they undergo de-excitation via all possible de-excitation pathways. Since, both radiative and non-radiative processes in general follow the first order kinetics, the de-excitation rate for the excited state molecules can be expressed as,

$$\frac{dn(t)}{dt} = n(t)(k_r + \Sigma\ k_{nr}) \tag{6.3}$$

$$\text{or,} \frac{dn(t)}{n(t)} = (k_r + \Sigma\ k_{nr})dt \tag{6.4}$$

Integrating both the sides,

$$n(t) = n_0 \exp\left(-\frac{t}{\tau}\right) \tag{6.5}$$

where n(t) and n_0 are the concentration of the excited species at times t and 0, respectively and $\tau = \frac{1}{(k_r + \Sigma k_{nr})}$ is the lifetime of the excited state molecules. When $t = \tau$, $\frac{n(t)}{n_0} = \frac{1}{e} = 0.37$. Thus, from the mathematical point of view; fluorescence lifetime, τ of an excited molecule is the lifetime by which 63% of the initial population has already undergone de-excitation. The radiative decay rate constant k_r is the intrinsic property of an excited state molecule which does not depend significantly on the surrounding environment. On the other hand the sum of the rate constants of the non-radiative de-excitation processes, which is the combination of rate constants of internal conversion, intersystem crossing, vibrational- rotational relaxation, solvent effect and also on, is the extrinsic property and can be modulated by tuning the surrounding environment by several means [94].

6.7 Solvent and Environmental Effects on Absorption and Emission Spectra

The local micro-environment and solvent polarity substantially modulate the electronic distribution in the chromophoric molecules which results in the changes of the absorption spectra, emission spectra and lifetime of the fluorophores to a significant extent. Interaction of solvent with the chromophoric molecule can also change the rates of the non-radiative de-excitation processes making the fluorophores either more or less fluorescent in nature on changing the solvent properties.

6.8 Effect of Solvent Polarity

Emission spectra of any molecule always appear at the longer wavelength region as compared to the absorption spectra. This is due to the fact that prior to emission a fraction of the excited state energy of the molecules is lost by different non-radiative processes, one of which is the solvent relaxation process. When a molecule is elevated to the first singlet state (S_1), it undergoes a very fast (within few picoseconds) vibrational relaxation to the lowest vibrational level of the S_1 state wherefrom the emission radiative transition to different vibrational levels of S_0 state takes place. However, if the molecule is excited to the higher electronic level, e.g. S_2, the fluorophore rapidly comes to the first excited state via non-radiative internal conversion (IC, t = 10^{-12} s or less) prior to undergo emission process. Solvent polarity generally lowers the energy difference between excited and ground states by stabilizing the excited state quite significantly than the ground state. Typically the fluorophores which have higher dipole moment in the excited state (μ_E) than that of the ground state (μ_G), red shift in the emission maximum with increasing solvent polarity is observed. This is due to the gradually larger stabilization of the excited state of the molecule than the ground state [94]. On the other hand, there are some fluorophores having higher ground state dipole moment (μ_G) than the dipole moment in the excited state (μ_E). In these cases, the increase in the solvent polarity leads to a hypsochromic shift of the emission peak. This is because the greater stabilization of the ground state than that of the excited state results in the increase in the energy gap between the two states involved in the electronic transition during emission. The fluorophore those are non-polar in nature, show only nominal changes in the emission characteristic with the changing solvent polarity. Unsubstituted aromatic hydrocarbons, the fluorophores having very less polarity difference between excited and ground states (e.g. BODIPY dyes) fall in this category [95]. The influence of solvent polarity on the energies

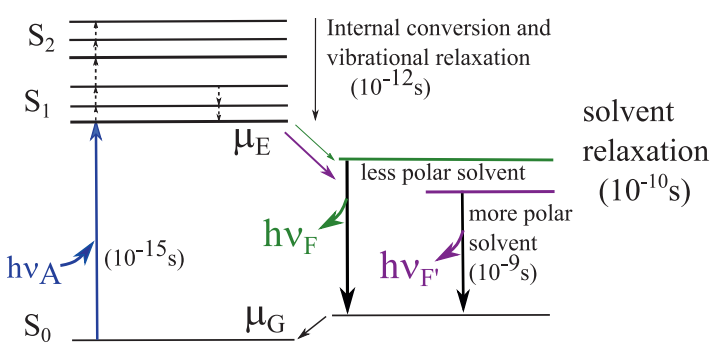

Figure 6.3: Absorption of light by the sample solution.

of the electronic states is quantitatively shown in Fig. 6.3. It is clear from the above figure that the absorption process takes place within the time scale of femtoseconds and hence, the position of the fluorophore or the solvent molecules in the ground state and the excited state remains unchanged during absorption. This indicates that the fluorophore experiences the same local environment in the ground state and in the excited state immediately following excitation. This explains why the absorption spectra are insensitive to solvent polarity. Subsequently however, excited energy level undergoes substantial solvent relaxation, resulting a solvent polarity dependent shift in the emission spectra especially.

6.9 Supramolecular Encapsulation

Fluorescence quantum yield of a dye can also be increased by trapping the dye molecules inside a macrocyclic cavity which is commonly known as supramolecular entrapment of the dye molecules. Macrocyclic hosts are commonly known as supramolecular hosts that can encapsulate a dye molecule depending on its size and geometry. Various macrocyclic hosts are reported in the literature among which cucurbiturils, calixarenes, cyclodextrins, pillarenes are the most commonly used host molecules.

Frequently Asked Questions

Q1. What are the processes take place during emission of a dye molecule?

Q2. How does solvent lead to blue shift or red shift of the absorption spectrum of a dye?

Q3. What are the factors, which govern the excited state lifetime of a dye?

Q4. When does a dye molecule become non-fluorescent in nature?

Q5. How can fluorescence quantum yield of a dye be increased?