Fluorescence and phosphorescence are types of molecular luminescence methods. A molecule of analyte absorbs a photon and excites a species. The emission spectrum can provide qualitative and quantitative analysis. The term fluorescence and phosphorescence are usually referred as photoluminescence because both are alike in excitation brought by absorption of a photon. Fluorescence differs from phosphorescence in that the electronic energy transition that is responsible for fluorescence does not change in electron spin, which results in short-live electrons (<10^{-5}\text{ s}) in the excited state of fluorescence. In phosphorescence, there is a change in electron spin, which results in a longer lifetime of the excited state (second to minutes). Fluorescence and phosphorescence occurs at longer wavelength than the excitation radiation.

**Introduction**

Fluorescence can occur in gaseous, liquid, and solid chemical systems. The simple kind of fluorescence is by dilute atomic vapors. A fluorescence example would be if a 3s electron of a vaporized sodium atom is excited to the 3p state by absorption of a radiation at wavelength 589.6 and 589.0 nm. After 10^{-8}\text{ s}, the electron returns to ground state and on its return it emits radiation of the two wavelengths in all directions. This type of fluorescence in which the absorbed radiation is remitted without a change in frequency is known as resonance fluorescence. Resonance fluorescence can also occur in molecular species. Molecular fluorescence band centers at wavelengths longer than resonance lines. The shift toward longer wavelength is referred to as the **Stokes Shift**.

**Singlet and Triplet Excited States**

Understanding the difference between fluorescence and phosphorescence requires the knowledge of electron spin and the differences between singlet and triplet states. **The Pauli Exclusion principle** states that two electrons in an atom cannot have the same four quantum numbers \((n, l, m_l, m_s)\) and only two electrons can occupy each orbital where they must have opposite spin states. These opposite spin states are called spin pairing. Because of this spin pairing, most molecules do not exhibit a magnetic field and are diamagnetic. In diamagnetic molecules, electrons are not attracted or repelled by the static electric field. Free radicals are paramagnetic because they contain unpaired electrons have magnetic moments that are attracted to the magnetic field.

Singlet state is defined when all the electron spins are paired in the molecular electronic state and the electronic energy levels do not split when the molecule is exposed into a magnetic field. A doublet state occurs when there is an unpaired electron that gives two possible orientations when exposed in a magnetic field and imparts different energy to the system. A singlet or a triplet can form when one electron is excited to a higher energy level. In an excited singlet state, the electron is promoted in the same spin orientation as it was in the ground state (i.e., paired). In a triplet excited stated, the electron that is promoted has the same spin orientation (parallel) to the other unpaired electron. The difference between the spins of ground singlet, excited singlet, and excited triplet is shown in Figure \(\PageIndex{1}\). Singlet, doublet and triplet is derived using the equation for multiplicity, \(2S+1\), where \(S\) is the total spin angular momentum (sum of all the electron spins). Individual spins are denoted as spin up \((s = +1/2)\) or spin down \((s = -1/2)\). If we were to calculated the \(S\) for the excited singlet state, the equation would be

\[
\[2(+1/2 + -1/2)+1 = 2(0)+1 = 1\]
\]
therefore making the center orbital in the figure a singlet state. If the spin multiplicity for the excited triplet state was calculated, we obtain

$$\left[2(\frac{1}{2} + \frac{1}{2}) + 1\right] = 2(1) + 1 = 3$$

which gives a triplet state as expected.

Figure \(\PageIndex{1}\): Spin in the ground and excited states

The difference between a molecule in the ground and excited state is that the electrons is diamagnetic in the ground state and paramagnetic in the triplet state. This difference in spin state makes the transition from singlet to triplet (or triplet to singlet) more improbable than the singlet-to-singlet transitions. This singlet to triplet (or reverse) transition involves a change in electronic state. For this reason, the lifetime of the triplet state is longer the singlet state by approximately \(10^4\) seconds fold difference. The radiation that induced the transition from ground to excited triplet state has a low probability of occurring, thus their absorption bands are less intense than singlet-singlet state absorption. The excited triplet state can be populated from the excited singlet state of certain molecules which results in phosphorescence. These spin multiplicities in ground and excited states can be used to explain transition in photoluminescence molecules by the Jablonski diagram.

Jablonski Diagrams

The Jablonski diagram that drawn below is a partial energy diagram that represents the energy of photoluminescent molecule in its different energy states. The lowest and darkest horizontal line represents the ground-state electronic energy of the molecule which is the singlet state labeled as \(S_{0}\). At room temperature, majority of the molecules in a solution are in this state.

Figure \(\PageIndex{2}\): Partial Jablonski Diagram for Absorption, Fluorescence, and Phosphorescence. Image used with permission from Bill Reusch.
The upper lines represent the energy state of the three excited electronic states: \( S_1 \) and \( S_2 \) represent the electronic singlet state (left) and \( T_1 \) represents the first electronic triplet state (right). The upper darkest line represents the ground vibrational state of the three excited electronic state. The energy of the triplet state is lower than the energy of the corresponding singlet state.

There are numerous vibrational levels that can be associated with each electronic state as denoted by the thinner lines. Absorption transitions (blues lines in Figure \( \PageIndex{2} \)) can occur from the ground singlet electronic state (\( S_0 \)) to various vibrational levels in the singlet excited vibrational states. It is unlikely that a transition from the ground singlet electronic state to the triplet electronic state because the electron spin is parallel to the spin in its ground state (Figure \( \PageIndex{1} \)). This transition leads to a change in multiplicity and thus has a low probability of occurring which is a forbidden transition. Molecules also go through vibration relaxation to lose any excess vibrational energy that remains when excited to the electronic states (\( S_{1*} \) and \( S_{2*} \)) as demonstrated in wavy lines in Figure \( \PageIndex{2} \). The knowledge of forbidden transition is used to explain and compare the peaks of absorption and emission.

### Absorption and Emission Rates

The table below compares the absorption and emission rates of fluorescence and phosphorescence. The rate of photon absorption is very rapid. Fluorescence emission occurs at a slower rate. Since the triplet to singlet (or reverse) is a forbidden transition, meaning it is less likely to occur than the singlet-to-singlet transition, the rate of triplet to singlet is typically slower. Therefore, phosphorescence emission requires more time than fluorescence.

<table>
<thead>
<tr>
<th>Process</th>
<th>Transition</th>
<th>Timescale (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Absorption (Excitation)</td>
<td>( S_0 \rightarrow S_n )</td>
<td>ca. (10^{-15}) (instantaneous)</td>
</tr>
<tr>
<td>Internal Conversion</td>
<td>( S_n \rightarrow S_1 )</td>
<td>(10^{-14}) to (10^{-11})</td>
</tr>
<tr>
<td>Vibrational Relaxation</td>
<td>( S_{n*} \rightarrow S_n )</td>
<td>(10^{-12}) to (10^{-10})</td>
</tr>
<tr>
<td>Intersystem Crossing</td>
<td>( S_1 \rightarrow T_1 )</td>
<td>(10^{-11}) to (10^{-6})</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>( S_1 \rightarrow S_0 )</td>
<td>(10^{-9}) to (10^{-6})</td>
</tr>
<tr>
<td>Phosphorescence</td>
<td>( T_1 \rightarrow S_0 )</td>
<td>(10^{-3}) to 100</td>
</tr>
<tr>
<td>Non-Radiative Decay</td>
<td>( S_1 \rightarrow S_0 ) ( T_1 \rightarrow S_0 )</td>
<td>(10^{-7}) to (10^{-5}) (10^{-3}) to 100</td>
</tr>
</tbody>
</table>

### Deactivation Processes

A molecule that is excited can return to the ground state by several combinations of mechanical steps that will be
The deactivation process of fluorescence and phosphorescence involves an emission of a photon radiation as shown by the straight arrow in Figure 2. The wiggly arrows in Figure 2 are deactivation processes without the use of radiation. The favored deactivation process is the route that is most rapid and spends less time in the excited state. If the rate constant for fluorescence is more favorable in the radiationless path, the fluorescence will be less intense or absent.

- **Vibrational Relaxation:** A molecule maybe promoted to several vibrational levels during the electronic excitation process. Collision of molecules with the excited species and solvent leads to rapid energy transfer and a slight increase in temperature of the solvent. Vibrational relaxation is so rapid that the lifetime of a vibrational excited molecule ($<10^{-12}$) is less than the lifetime of the electronically excited state. For this reason, fluorescence from a solution always involves the transition of the lowest vibrational level of the excited state. Since the space of the emission lines are so close together, the transition of the vibrational relaxation can terminate in any vibrational level of the ground state.

- **Internal Conversion:** Internal conversion is an intermolecular process of a molecule that passes to a lower electronic state without the emission of radiation. It is a crossover of two states with the same multiplicity meaning singlet-to-singlet or triplet-to-triplet states. The internal conversion is more efficient when two electronic energy levels are close enough that two vibrational energy levels can overlap as shown in between $S_1$ and $S_2$. Internal conversion can also occur between $S_0$ and $S_1$ from a loss of energy by fluorescence from a higher excited state, but it is less probable. The mechanism of internal conversion from $S_1$ to $S_0$ is poorly understood. For some molecules, the vibrational levels of the ground state overlap with the first excited electronic state, which leads to fast deactivation. These usually occur with aliphatic compounds (compound that do not contain ring structure), which would account for the compound is seldom fluorescing. Deactivation by energy transfer of these molecules occurs so rapidly that the molecule does not have time to fluoresce.

- **External Conversion:** Deactivation of the excited electronic state may also involve the interaction and energy transfer between the excited state and the solvent or solute in a process called external conversion. Low temperature and high viscosity leads to enhanced fluorescence because they reduce the number of collision between molecules, thus slowing down the deactivation process.

- **Intersystem Crossing:** Intersystem crossing is a process where there is a crossover between electronic states of different multiplicity as demonstrated in the singlet state to a triplet state ($S_1$ to $T_1$) on Figure 1. The probability of intersystem crossing is enhanced if the vibration levels of the two states overlap. Intersystem crossing is most commonly observed with molecules that contain heavy atom such as iodine or bromine. The spin and orbital interaction increase and the spin become more favorable. Paramagnetic species also enhances intersystem crossing, which consequently decreases fluorescence.

- **Phosphorescence:** Deactivation of the electronic excited state is also involved in phosphorescence. After the molecule transitions through intersystem crossing to the triplet state, further deactivation occurs through internal or external fluorescence or phosphorescence. A triplet-to-singlet transition is more probable than a singlet-to-singlet internal crossing. In phosphorescence, the excited state lifetime is inversely proportional to the probability that the molecule will transition back to the ground state. Since the lifetime of the molecule in the triplet state is large ($10^{-4}$ to 10 second or more), transition is less probable which suggest that it will persist for some time even after irradiation has stopped. Since the external and internal conversion compete so effectively with phosphorescence, the molecule has to be observed at lower temperature in highly viscous media to protect the triplet state.

**Variables that affect Fluorescence**

After discussing all the possible deactivation processes, variables that affect the emissions to occur. Molecular structure and its chemical environment influence whether a substance will fluoresce and the intensities of these emissions. The **quantum yield** or **quantum efficiency** is used to measure the probability that a molecule will fluoresce or
phosphoresce. For fluorescence and phosphorescence is the ratio of the number of molecules that luminescent to the total number of excited molecules. For highly fluoresce molecules, the quantum efficiency approaches to one. Molecules that do not fluoresce have quantum efficiencies that approach to zero.

Fluorescence quantum yield \( \langle \phi \rangle \) for a compound is determined by the relative rate constants \( k \) of various deactivation processes by which the lowest excited singlet state is deactivated to the ground state. The deactivation processes including fluorescence \( k_f \), intersystem crossing \( \langle (k_i) \rangle \), internal conversion \( k_{ic} \), predissociation \( k_{pd} \), dissociation \( k_d \), and external conversion \( k_{ec} \) allows one to qualitatively interpret the structural and environmental factors that influence the intensity of the fluorescence. They are related by the quantum yield equation given below:

\[
\frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d}
\]

Using this equation as an example to explain fluorescence, a high fluorescence rate \( k_f \) value and low values of the all the other relative rate constant terms \( k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d \) will give a large \( \langle \phi \rangle \), which suggest that fluorescence is enhanced. The magnitudes of \( k_f \), \( k_d \), and \( k_{pd} \) depend on the chemical structure, while the rest of the constants \( k_i \), \( k_{ec} \), and \( k_{ic} \) are strongly influenced by the environment.

Fluorescence rarely results from absorption of ultraviolet radiation of wavelength shorter than 250 nm because radiation at this wavelength has sufficient energy to deactivate the electron in the excited state by predissociation or dissociation. The bond of some organic molecules would rupture at 140 kcal/mol, which corresponds to 200-nm of radiation. For this reason, \( \langle \sigma \rightarrow \sigma^* \rangle \) transition in fluorescence are rarely observed. Instead, emissions from the less energetic transition will occur which are either \( \langle \pi^* \rightarrow \pi \rangle \) rightarrow \( \langle \pi \rangle \) or \( \langle \pi^* \rightarrow n \rangle \) rightarrow \( \langle n \rangle \) transition.

Molecules that are excited electronically will return to the lowest excited state by rapid vibrational relaxation and internal conversion, which produces no radiation emission. Fluorescence arises from a transition from the lowest vibrational level of the first excited electronic state to one of the vibrational levels in the electronic ground state. In most fluorescent compounds, radiation is produced by a \( \langle \pi^* \rightarrow \pi \rangle \) \( \langle \pi \rangle \) or \( \langle \pi^* \rightarrow n \rangle \) \( \langle n \rangle \) transition depending on which requires the least energy for the transition to occur.

Fluorescence is most commonly found in compounds in which the lowest energy transition is \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) (excited singlet state) than \( \langle n \rightarrow \pi^* \rangle \) which suggest that the quantum efficiency is greater for \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) transitions. The reason for this is that the molar absorbptivity, which measures the probability that a transition will occur, of the \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) transition is 100 to 1000 fold greater than \( \langle n \rightarrow \pi^* \rangle \) process. The lifetime of \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) (10^{-7} to 10^{-9} s) is shorter than the lifetime of \( \langle n \rightarrow \pi^* \rangle \) (10^{-5} to 10^{-7}).

Phosphorescent quantum efficiency is the opposite of fluorescence in that it occurs in the \( \langle n \rightarrow \pi^* \rangle \) excited state which tends to be short lived and less susceptible to deactivation than the \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) triplet state. Intersystem crossing is also more probable for \( \langle \pi \rangle \) rightarrow \( \langle \pi^* \rangle \) excited state than for the \( \langle n \rightarrow \pi^* \rangle \) state because the energy difference between the singlet and triplet state is large and spin-orbit coupling is less likely to occur.
References


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