Differentiate between fluorescence, phosphorescence and chemiluminescence.
a. Fluorescence and phosphorescence are observed when molecules are excited by a powerful source of radiation. On the contrary, chemiluminescence does not require a source of radiation where excitation takes place as a result of chemical energy produced from a chemical reaction.
b. Fluorescence is obtained when a molecule relaxes from $S_1$ to $S_0$. The fluorescence lifetime is in the range from $10^{-7}$-$10^{-9}$s and occurs at shorter wavelength than phosphorescence.
c. Phosphorescence is obtained when a molecule relaxes from $T_1$ to $S_0$. The phosphorescence lifetime is in the range from $10^{-4}$– few seconds and occurs at longer wavelength than phosphorescence.

A $\pi-\pi^*$ is the most useful transition that results in fluorescence and phosphorescence? Why?
A $\pi-\pi^*$ Transition is the most convenient and useful transition in both fluorescence and phosphorescence. However, n-$\pi^*$ transitions promote intersystem crossing and improves phosphorescence. Excitation is a prerequisite step to emission, therefore, $\pi-\pi^*$ transitions are very important due to the following reasons:
1. The molar absorptivity for the $\pi-\pi^*$ transition is high allowing good excitation.
2. The energy required is moderate, far less than dissociation energy.
3. In presence of the most convenient solvent (water), the energy required for a $\pi-\pi^*$ transition is usually smaller.
The lifetime of the $\pi-\pi^*$ transition is rather short ($10^{-7}$-$10^{-9}$ s), which results in less collisional and radiationless deactivation.

Explain the difference between a fluorescence emission spectrum and a fluorescence excitation spectrum. Which more closely resembles an absorption spectrum?
An emission spectrum should be a mirror image of an excitation spectrum because they originate from opposite processes. The excitation spectrum more closely resembles an absorption spectrum because both originate from the same process of absorption of radiation by molecules. The emission spectrum is obtained at higher wavelengths and is usually different from excitation or absorption spectra due to instrumental artifacts.

Define the following terms: (a) fluorescence. (b) phosphorescence. (c) resonancefluorescence. (d) singlet state. (e) triplet state. (f) vibrational relaxation, (g) internal conversion, (h) external conversion, (i) intersystem crossing, (j) predissociation, (k) dissociation, (l) quantum yield, (m) chemiluminescence.
a. Fluorescence: a process in which an excited molecule in the singlet state emits a photon and relaxes to ground state.
b. Phosphorescence: a process in which an excited molecule in triplet state emits a photon and relaxes to ground state; accompanied by a flip in spin.

c. Resonance Fluorescence: a process in which the wavelength of fluorescence is the same as the excitation wavelength.

d. Singlet State: a state where the two electrons are paired (total spin = 0).

e. Triplet State: a state in which the two electrons are unpaired (total spin = 1).

f. Vibrational Relaxation: a process describing relaxation of molecules excited to high energy vibrational levels to lower vibrational energy levels or main electronic levels of the same energy level. This is a non radiative process characterized by a very short time (~ $10^{-12}$ s).

g. Internal Conversion: the most important, but unfortunately least defined and understood, mechanism for radiationless deactivation from excited to ground state. This has to do with the nature of the molecular energy levels of the molecule where internal conversion is more pronounced in molecules with excited states of close proximity to ground state and where vibrational levels in ground state extends and overlaps with the excited state.

h. External Conversion: a process of radiationless deactivation where excited molecules lose their energy and relax to ground state by transfer of their energy to other molecules or solvent in the sample matrix.

i. Intersystem Crossing: a process where an excited molecule in the singlet state is transferred to the triplet state or vice versa; accompanied by a flip in spin.

j. Predissociation: a process where an excited molecule at a higher electronic level relaxes to an upper vibrational energy level of a lower electronic level. Bond rupture occurs due to very high vibrational energy.

k. Dissociation: bond rupture due to direct excitation of a molecule to an upper vibrational energy level of an excited state.

l. Quantum Yield: an expression describing the efficiency of molecules to produce fluorescence or phosphorescence (takes a value from 0 to 1).

m. Chemiluminescence: a process where generated energy from a chemical reaction cause excitation of molecules which, in turn, relax to ground state with emission of photons called chemiluminescence.

**Why are fluorescence measurements far more sensitive than UV-Vis absorption measurements?**

Fluorescence measurements are potentially more sensitive than spectrophotometry due to following reasons:
a. Possibility of increasing fluorescence by simply increasing the source radiant power.

b. In most cases, measurement takes place against zero background.

c. Fluorescence is directly proportional with concentration where fluorescence does not require measurement of a ratio of $P_o$ and $P$ and it is not a logarithmic term like absorbance.

d. Unlike absorbance measurements, there are several factors that can be controlled and optimized to improve fluorescence signals.

Which compound in each of the pairs below would you expect to have a greater fluorescence quantum yield? Explain.

![Phenolphthalein and Fluorescein](image)

Fluorescein has greater fluorescence quantum yield than phenolphthalein because fluorescein is structurally more rigid. The same argument can be said about the better fluorescence obtained from o,o'-dihydroxyazobenzene when compared to respective hydrazine structure.

In which solvent would the florescence of naphthalene be expected to be greatest: a. 1-chloropropane, b. 1-bromoopropane, or c. 1-iodopropane?

The fluorescence of naphthalene is expected to be greater in 1-chloropropane because of two reasons:

a. 1-chloropropane is more polar than the bromo or iodo derivatives which enhances the $\pi-\pi^*$ transition.
b. The presence of the heavy atoms (bromo and iodo derivatives) enhances intersystem crossing.

**Why do some absorbing compounds fluoresce but others do not?**

For a molecule to show fluorescence it should have some properties:

a. should have a good \( \pi \) system, in order to absorb well, as described above
b. should be structurally rigid
c. should not have heavy atoms or paramagnetic atoms
d. should preferentially be in solution that is oxygen free, has a high viscosity and contains no heavy atoms

**Discuss the major reasons why molecular phosphorescence spectrometry has not been as widely used as molecular fluorescence spectrometry.**

This is mainly because phosphorescence is more susceptible to radiationless deactivation mechanisms due to its relatively long lifetimes. In addition, not so many applications on phosphorescence are available, when compared to fluorescence. Also, the phosphorescence signal is usually less than fluorescence.

The reduced form of nicotinamide adenine dinucleotide (NADH) is an important and highly fluorescent coenzyme. It has an absorption maximum of 340 nm and an emission maximum at 465 nm. Standard solutions of NADH gave the following fluorescence intensities:

<table>
<thead>
<tr>
<th>Conc. NADH, ( \mu \text{mol/L} )</th>
<th>Relative Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.100</td>
<td>2.24</td>
</tr>
<tr>
<td>0.200</td>
<td>4.52</td>
</tr>
<tr>
<td>0.300</td>
<td>6.63</td>
</tr>
<tr>
<td>0.400</td>
<td>9.01</td>
</tr>
<tr>
<td>0.500</td>
<td>10.94</td>
</tr>
<tr>
<td>0.600</td>
<td>13.71</td>
</tr>
<tr>
<td>0.700</td>
<td>15.49</td>
</tr>
<tr>
<td>0.800</td>
<td>17.91</td>
</tr>
</tbody>
</table>

(a) Construct a spreadsheet and use it to draw a calibration curve for NADH.
(b) An unknown exhibits a relative fluorescence intensity of 12.16. Use the spreadsheet to calculate the concentration of NADH.
(c) If an unknown concentration of NADH resulted in a fluorescence signal of 14.28, find its concentration

a. Plot the data using a software like excel or statview; you get:
b. The least square equation of the plot is:

\[ F = 0.031 + 22.43 \, C_{\text{NADH}} \]

c. \( 14.28 = 0.031 + 22.43 \, C_{\text{NADH}} \)

\[ C_{\text{NADH}} = 0.631 \, \mu\text{M} \]

**What is the difference between dissociation and predissociation?**

In predissociation, a molecule relaxes from a higher electronic state to an upper vibrational energy of a lower electronic state. When the vibrational energy is large enough and is greater than the bond energy, bond rupture occurs in a process called predissociation.

Dissociation, on the other hand, involves absorption of high energy so that the molecule is directly promoted to a high energy vibrational level where bond rupture directly occurs.

**External conversion is an important radiationless deactivation process. Explain. What affects external conversion?**

External conversion (EC) is a process whereby excited molecules lose their energy due to collisions with other molecules or by transfer of their energy to solvent or other unexcited molecules. Therefore, external conversion is influenced by temperature, solvent viscosity, as well as solvent composition.

**What are the factors affecting fluorescence and phosphorescence? Explain how both techniques are affected.**

1. Structure

Bst luminescence is observed for molecules with π bonds and preferably those having aromatic rings due to presence of low energy π−π*.
2. Structural rigidity
The nature of the chemical structure of a molecule in terms of flexibility and rigidity is of major influence on the fluorescence and phosphorescence signal. Molecules that have high degree of flexibility will tend to decrease fluorescence due to higher collisional probability. However, more rigid structures have lower probability of collisions and thus have more fluorescence potential.

3. Solvent Nature
Solvents characteristics have important effects on luminescent behavior of molecules. Three main effects can be recognized:

a. Solvent Polarity
A polar solvent is preferred as the energy required for the p-p* is lowered.

b. Solvent Viscosity
More viscous solvents are preferred since collisional deactivation will be lowered at higher viscosities.

c. Heavy Atoms Effect
If solvents contain heavy atoms, fluorescence quantum efficiency will decrease and phosphorescence will increase.

4. Temperature
Higher temperatures result in larger collisional deactivation due to increased movement and velocity of molecules. Therefore, lower temperatures are preferred.

5. pH
The pH of the solution is a very important factor that influences luminescence.

6. Dissolved Oxygen
Dissolved oxygen largely limits fluorescence since it promotes intersystem crossing because it is paramagnetic. However, dissolved oxygen affects phosphorescence more than it does to fluorescence. Although one would think that as far as intersystem crossing is increased in presence of oxygen, phosphorescence is expected to increase. On the contrary, phosphorescence is completely eliminated and quenched in presence of dissolved oxygen. This may be explained on the basis that the ground state of oxygen is the triplet state and it is easier for an electron in the triplet state to transfer its energy to triplet oxygen rather than performing a flip in spin and relax to singlet state.

7. Concentration
Fluorescence is directly proportional to concentration: \( S = K \varepsilon b c \)

8. Radiant power
Fluorescence and phosphorescence are directly proportional to source radiant power: \( S = K \varepsilon b c \)

The relation: \( S = K \varepsilon b c \) suggests a direct relation between fluorescence and concentration. State the factors that lead to deviations from linearity.

a. At absorbances higher than 0.05.
b. Self-quenching whereby excited molecules lose their energies by collision with other molecules or solvent

c. Self-absorption which occurs when an emission band overlaps with an excitation (absorption) band. In this case, emitted photons excite other molecules in the ground state which results in no net emission.

**Why a xenon arc lamp is most frequently used in fluorescence instruments?**

Since scence signal is proportional to the radiant power of the source. a xenon arc lamp is usually the source of choice due to its high radiant power

**From Notes:**

1. Draw a schematic of a fluorometer, a single beam spectrofluorometer, and a dual beam spectrofluorometer. Define all components and comment on the advantages and disadvantages of each instrument.
2. Synchronous fluorescence have distinct advantages. What is meant by synchronous fluorescence and what is it used for?
3. How do you obtain the excitation and emission spectra of a compound (colorless or colored)?
4. Fluorescence instruments are not widely spread, as fluorescence. Why? What can you do to make your spectrofluorometer work as a phosphorimeter? Draw a schematic of the suggested instrumental modification.
5. Describe Three applications of chemiluminescence.
6. Suggest a structure of a usable luminescent stick. Carefully choose your reaction components. What can you do to change your luminescent color?