Spectrofluorimetry

Principle

- The interaction of photons with molecules results in the promotion of valence electrons from ground state orbitals to higher energy level orbitals.
- The molecules are said to be in an excited state.
- Some excited molecules, however, return to the ground state by emitting the excess energy as light. This process is called fluorescence.
- This relaxation process, which is very rapid, leaves the molecule in the lowest vibrational level.

Two important characteristics of the emitted light should be noted:

- *The emitted light is of longer wavelength (lower energy) than the excitation light. This is because part of the energy initially associated with the **S** state is lost as heat energy.*
- *The emitted light is composed of many wavelengths, which results in a fluorescence spectrum. This is due to the fact that fluorescence from any particular excited molecule may return the molecule to one of many vibrational levels in the ground state.*

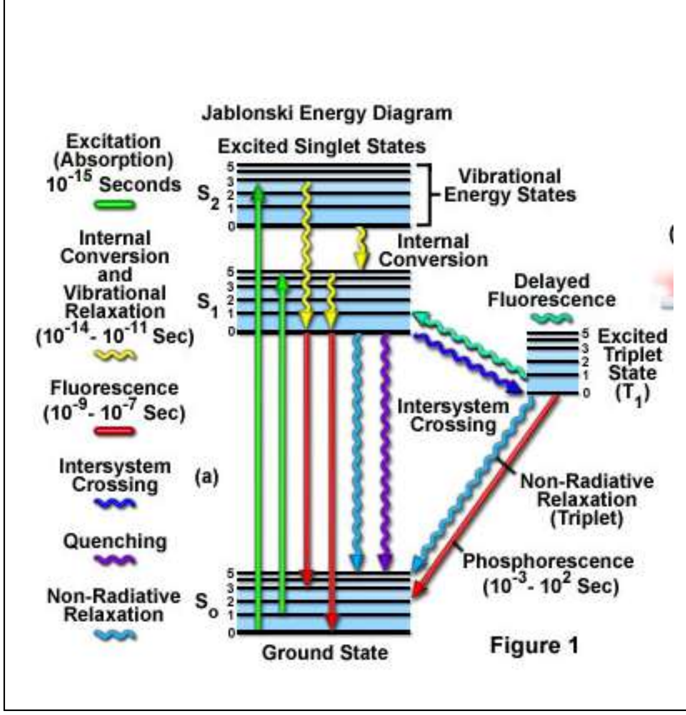
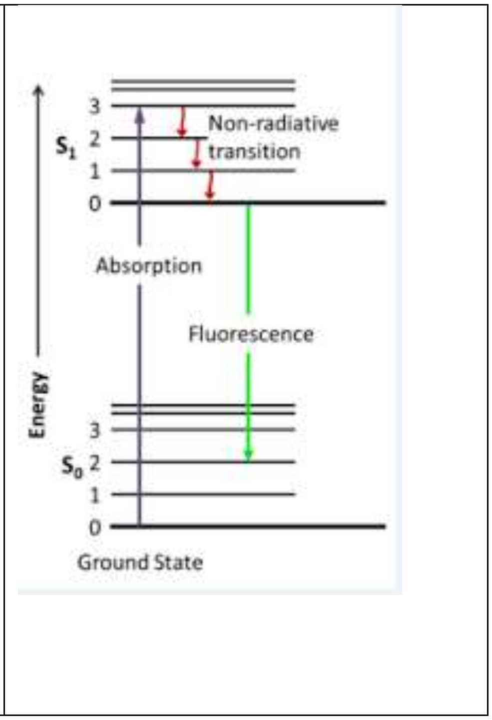


Figure 1



Quantum Yield

- A molecule in the excited state can return to lower energy levels by collisional transfer or by light emission.
- The fluorescence intensity is often defined in terms of quantum yield, represented by **Q**.
- By definition, **Q** is the ratio of the number of photons emitted to the number of photons absorbed.

$$\boldsymbol{Q = \frac{number\ of\ photons\ emitted}{number\ of\ photons\ absorbed}}$$

- Measurement of quantum yield is often the goal in fluorescence spectroscopy experiments.
- Q is of interest because it may reveal important characteristics of the fluorescing system.
- Two types of factors affect the intensity of fluorescence, internal and external (environmental) influences.
  - Internal factors, such as the number of vibrational levels available for transition and the rigidity of the molecules, are associated with properties of the fluorescent molecules themselves.
  - External factors include macromolecule conformation and molecular interactions between small molecules (ligands) and larger biomolecules (proteins, nucleic acids).
- Of special value is the study of experimental conditions that result in quenching or enhancement of the quantum yield.
- Quenching in biochemical systems can be caused by chemical reactions of the fluorescent species with added molecules, transfer of energy to other molecules by collision (actual contact between molecules), and transfer of energy over a distance (no contact, resonance energy transfer).
- The reverse of quenching, enhancement of fluorescent intensity, is also observed in some situations.
- Several fluorescent dye molecules are quenched in aqueous solution, but their fluorescence is greatly enhanced in a nonpolar or rigidly bound environment (the interior of a protein, for example).
- This is a convenient method for characterizing ligand binding. Both fluorescence quenching and fluorescence enhancement studies can yield important information about biomolecular structure and function.