Chapter 15
Molecular Luminescence Spectrometry

- Two types of Luminescence methods are:

  1**) Photoluminescence,** Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called "photo-excitation." One way this excess energy can be dissipated by the sample is through the emission of light, or luminescence.

  (i) fluorescence

  (ii) phosphorescence

  2) **Chemiluminescence,** based on an excited species formed by a chemical reaction. - no excitation source –

- In each, molecules of the analyte are excited to give a species whose emission spectrum provides information for qualitative or quantitative analysis.
- Luminescence methods are used as detectors for HPLC & CE.

Theory Of Fluorescence And Phosphorescence

Types of Fluorescence:

- Resonance Fluorescence
  - (emitted λ = excitation λ; e.g., AF)
- Stokes shift
  - (emitted λ > excitation λ; e.g., molecular fluorescence)

Electron spin and excited states:

Excited, paired = excited singlet state → fluorescence

Excited, unpaired = excited triplet state → phosphorescence

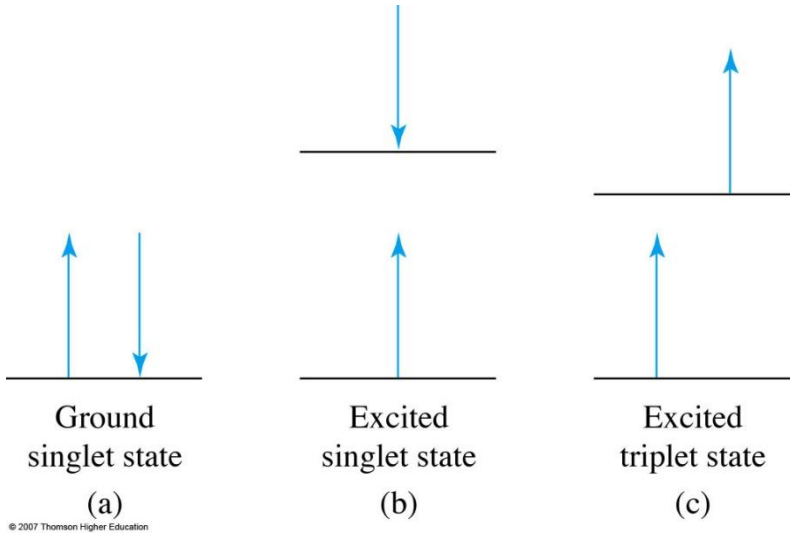


FIGURE 15-1 Electronic spin states of molecules. In (a) the ground electronic state is shown. In the lowest energy or ground state, the spins are always paired, and the state is said to be a singlet state.
In (b) and (c), excited electronic states are shown. If the spins remain paired in the excited state, the molecule is in an excited singlet state (b).
If the spins become unpaired, the molecule is in an excited triplet state (c).

Term Symbols

Example: Na ground state $1s^2\ 2s^2\ 2p^6\ 3s^1$

s=1/2, 2S+1=2, ground state doublet s electron written $3(^2S)$

Two spin states of equal energy (up/down)

→ Na 1st excited state $1s^2\ 2s^2\ 2p^6\ 3p^1$
Doublet written $3(^2P)$

BUT two spins states?

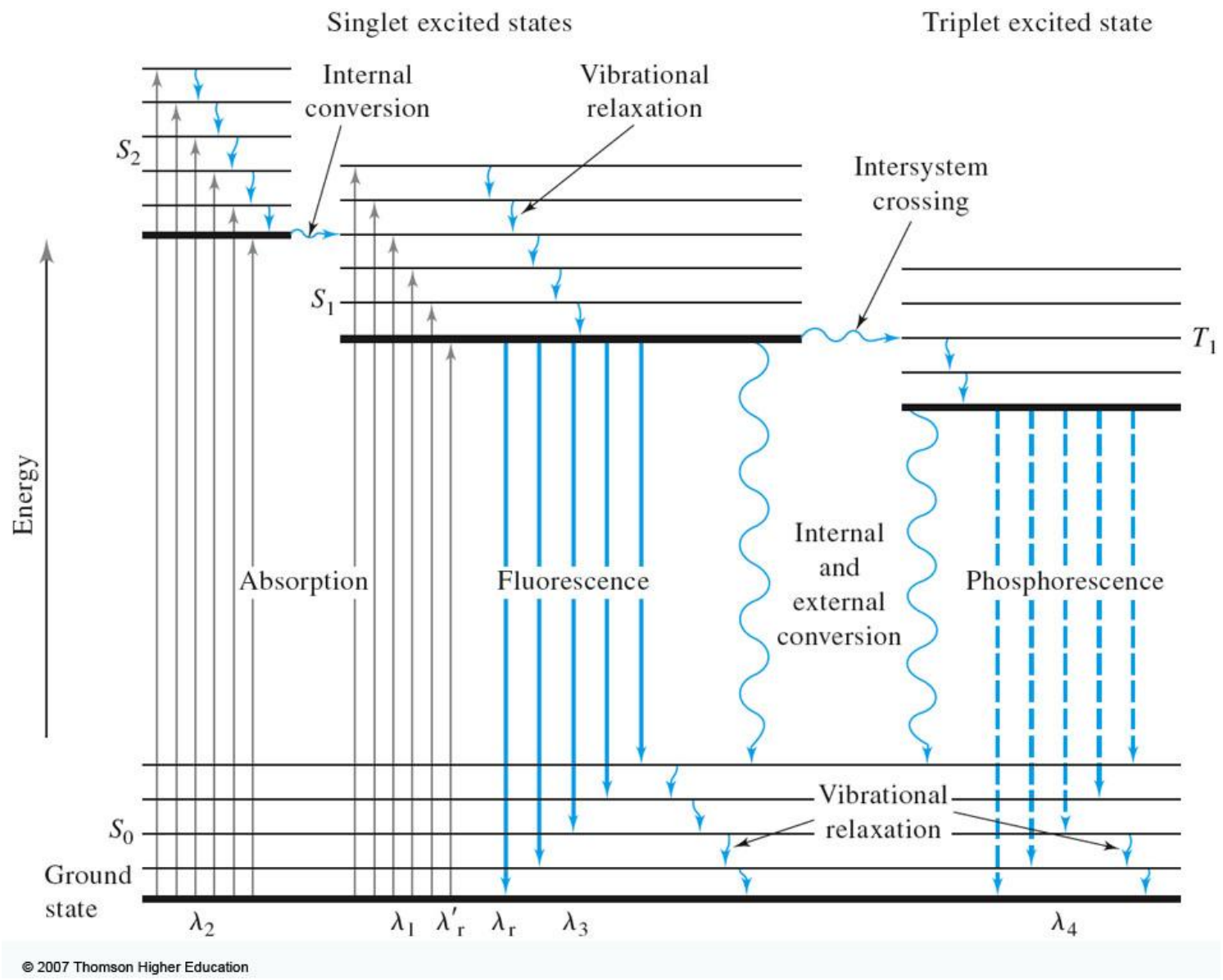
J (total ang. mom)=L+S or L-S

now $1s^2\ 2s^2\ 2p^6\ 3s^1 = 3(^2P_{1/2})$ and $3(^2P_{3/2})$

Term Symbol $^{2S+1}L_J$

Na 3p→3s fluorescence two lines : at 589.6 nm ($^2P_{3/2}$) and
at 589.0 nm ($^2P_{1/2}$)

Energy-level diagram for typical photoluminescent system



Deactivation Processes:

- **Fluorescence:** absorption of photon, short-lived excited state (singlet), emission of photon.
- **Phosphorescence:** absorption of photon, long-lived excited state (triplet), emission of photon.
- **Vibration Relaxation**
- **Internal Conversion**
- **External Conversion**
- **Intersystem Crossing**

Deactivation process by which an excited molecule returns to the ground state by minimizing lifetime of electronic state is preferred (i.e., the deactivation process with the faster rate constant will predominate)

Radiationless Deactivation

Without emission of a photon (i.e., without radiation)

Terms From Energy-level Diagram

Term: *Absorption* **Effect:** Excitation

Process: Analyte molecule absorbs photon (very fast ~ 10^{-14} – 10^{-15} s); electron is promoted to higher energy state. Slightly different wavelength → excitation into different vibrational energy levels.

Term: *Vibrational Relaxation* **Effect:** Radiationless Deactivation

Process: Collisions of excited state analyte molecules with other molecules → loss of excess vibrational energy and relaxation to lower vibrational levels (within the excited electronic state)

Term: *Internal conversion* **Effect:**RadiationlessDeactivation

Process: Molecule passes to a lower energy state – vibrational energy levels of the two electronic states overlap (see diagram) and molecules passes from one electronic state to the other.

Term: *Intersystem Crossing* **Effect:** Radiationless Deactivation

Process: Spin of electron is reversed leading to change from singlet to triplet state. Occurs more readily if vibrational levels of the two states overlap. Common in molecules with heavy atoms (e.g., I or Br)

Term: *External Conversion* **Effect:** Radiationless Deactivation

Process: Collisions of excited state analyte molecules with other molecules → molecule relaxes to the ground state without emission of a photon.

Term: *Fluorescence* **Effect:** Raditive Deactivation

Process: Emission of a photon via a singlet to singlet transition

(short – lived excited state ~10^{-5} – 10^{-10} s).

Term: *Phosphorescence* **Effect:** Radiative Deactivation

Process: Emission of a photon via a triplet to single transition

(long–lived excited state ~ 10^{-4} – 10^{1}s)

Quantum Efficiency or Quantum Yield:

- The quantum yield or quantum efficiency for fluorescence or phosphorescence is the ratio of the number of molecules that luminesce to the total number of excited molecule.
- It gives a measure of how efficient a fluorophore (i.e., fluorescing molecule) is.
- A quantum yield = 1 means that every excited molecules deactivates by emitting a photon – such a molecule is considered a very good fluorophore.
- We can express quantum yield as a function of rate constants

$$\text{Quantum Yield, } \phi = \frac{\text{total \# luminescing molecules}}{\text{total \# of excited molecules}}$$

$$\phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d} \text{[k = rate constant]}$$

What Factors Affect fluorescence ?

1) Excitation wavelength

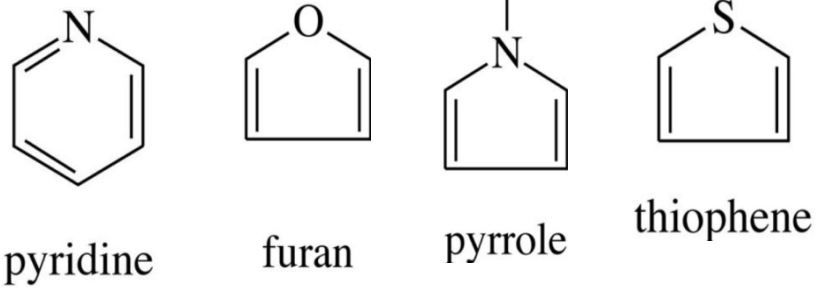
- Short λs break bonds, increase $k_{pre\text{-}dis}$ and k_{dis}
  - σ → σ* photochemical decomposition (seldom observed)
- Transitions mostly occur from
  - n → π* or Low–energy π → π* (aromatic, most intense fluorescence)

2) Molecular structure

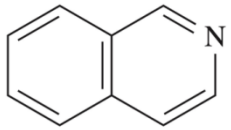
➢ Conjugated double bond structures exhibit fluorescence.

➢ Most unsubstituted aromatic hydrocarbons fluoresce in solution, the quantum efficiency usually increasing with the number of rings and their degree of condensation.

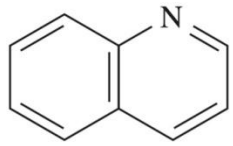
➢ The simple heterocyclics *such as pyridine, furan, thiophene, and pyrrole* do not fluoresce; heterocyclics fused to other rings fluoresce. Heteroatom increases ISC then ϕ_f decreases. (pyridine-quinoline)



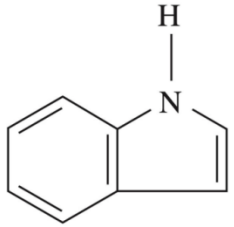
pyridine furan pyrrole thiophene



isoquinoline



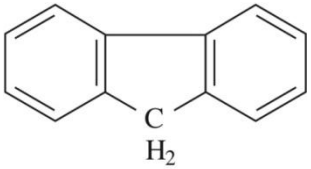
quinoline



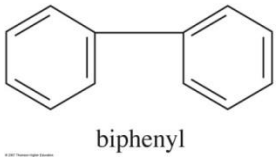
indole

3) Structural rigidity

- fluorescence is particularly favored in molecules with rigid structures.(e.g., fluorene vs biphenyl). If flexibility increases, ϕ_f decreases.



fluorene


biphenyl

- Lack of rigidity in a molecule probably causes an enhanced internal conversion rate and a consequent increase in the likelihood for radiationless deactivation.

4) Temperature and Solvent effect

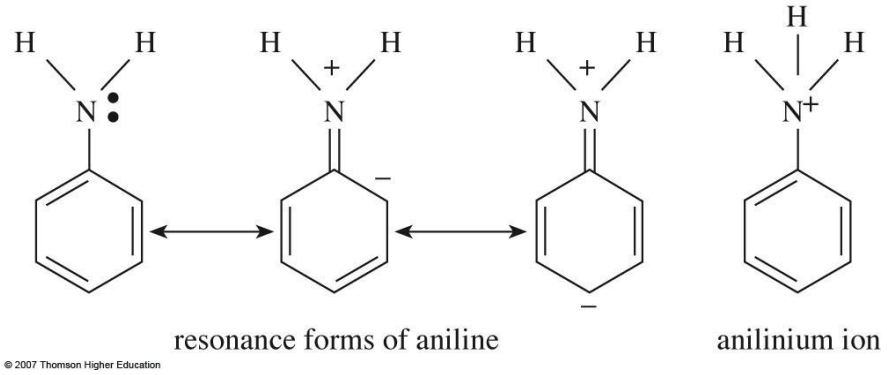
• The quantum efficiency *of fluorescence* in most molecules *decreases* with *increasing temperature* because the increased frequency of *collisions* at elevated temperatures improves the probability for deactivation by external conversion

• *increased fluorescence* with *increased viscosity* (decreased likelihood of external conversion – radiationless deactivation)

• The *fluorescence of a molecule is decreased* by solvents containing *heavy atoms* or other solutes with such atoms in their structure: Heavy atoms such as I, Br, Th increases ISC, as a consequence ϕ_f decreases. Compounds containing heavy atoms are frequently incorporated into solvents when enhanced phosphorescence is desired.

5) Effect of pH on Fluorescence

- The fluorescence of an aromatic compound with acidic or basic ring substituents is usually pH dependent.
- Both the wavelength and the emission intensity are likely to be different for the protonated and unprotonated forms of the compound.



Increased resonance structures (protonation or deprotonation) → stable excited state and greater quantum yield

- analytical procedures based on fluorescence frequently require close control of pH.

6) Dissolved oxygen;

- Presence of dissolved oxygen reduces fluorescence yield due to oxidation of the fluorescent specie. Also, paramagnetic properties of the oxygen promotes ISC and transition to triplet state.

Fluorescence Intensity And Concentration Of Analyte

- The power of fluorescence emission *F is proportional* to the radiant power of the excitation beam that is absorbed by the system.
- fluorescence intensity depends linearly on concentration. F = Kc
- Deviations occur at high concentrations
  - Self absorption: neighboring molecule absorbs emitted photon from other molecule – happens if there is overlap between the excitation and emission spectra
  - Quenching: collisions of excited state molecule with other excited state molecules → radiationless deactivation

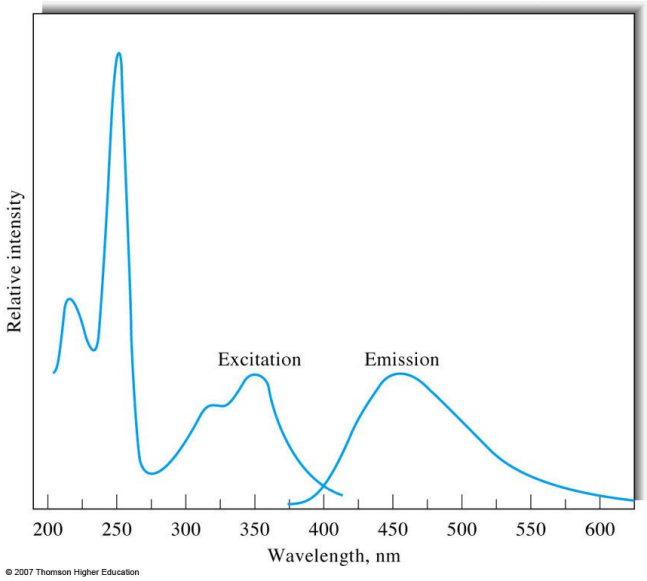
- Photobleaching, Photochemical Decomposition:

  Excited state molecule absorbs another photon and is destroyed → destroyed excited state molecule is not able to emit fluorescent photon

Excitation And Emission Spectra

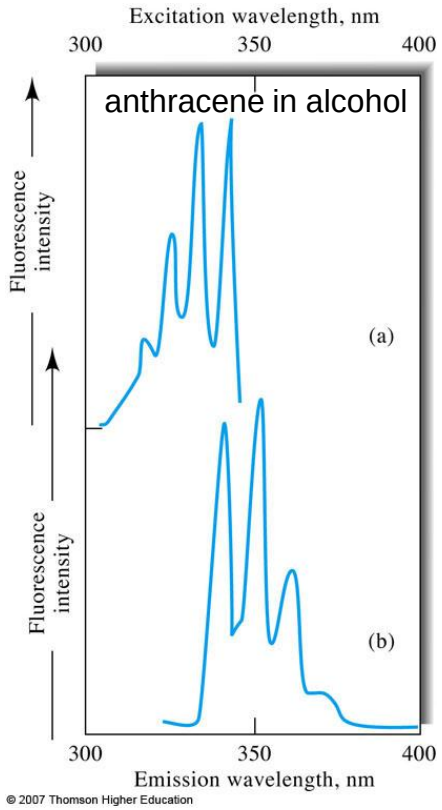
Excitation spectrum:

- Emission wavelength is fixed; excitation wavelength is scanned
- Monochromator or filters selected to allow only one λ of fluorescent light to pass through to the detector.
- Excitation wavelength is varied – at each excitation λ increment, fluorescent photons at the fixed emission λ are collected.
- The emission intensity (i.e., the number of fluorescent photons collected) at each λ increment varies as the excitation λ comes closer to or goes further from the λ of maximum absorption → this is why an excitation spectrum looks like an absorption spectrum.



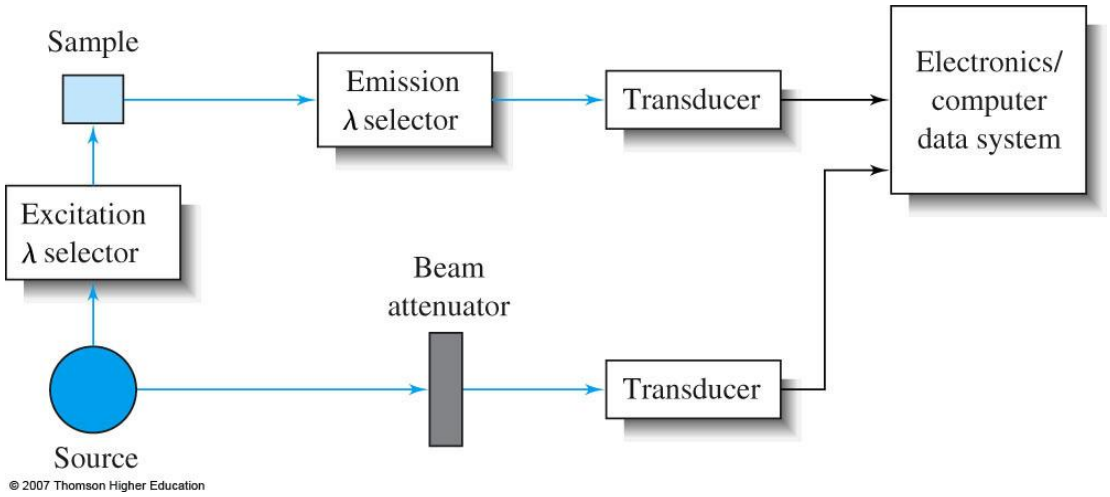
Emission spectrum:

- Excitation wavelength is fixed; emission wavelength is scanned
- Monochromator or filter is selected to allow only one λ of excitation light to pass onto the sample.
- Emission λ is varied → fluorescent photons are collected at each incremental emission λ.
- The emission intensity (i.e., the number of fluorescent photons collected) at each λ increment varies as the emission λ is changed.
- Spectrum shows at what λ the fluorescence intensity is a maximum for a given excitation λ.



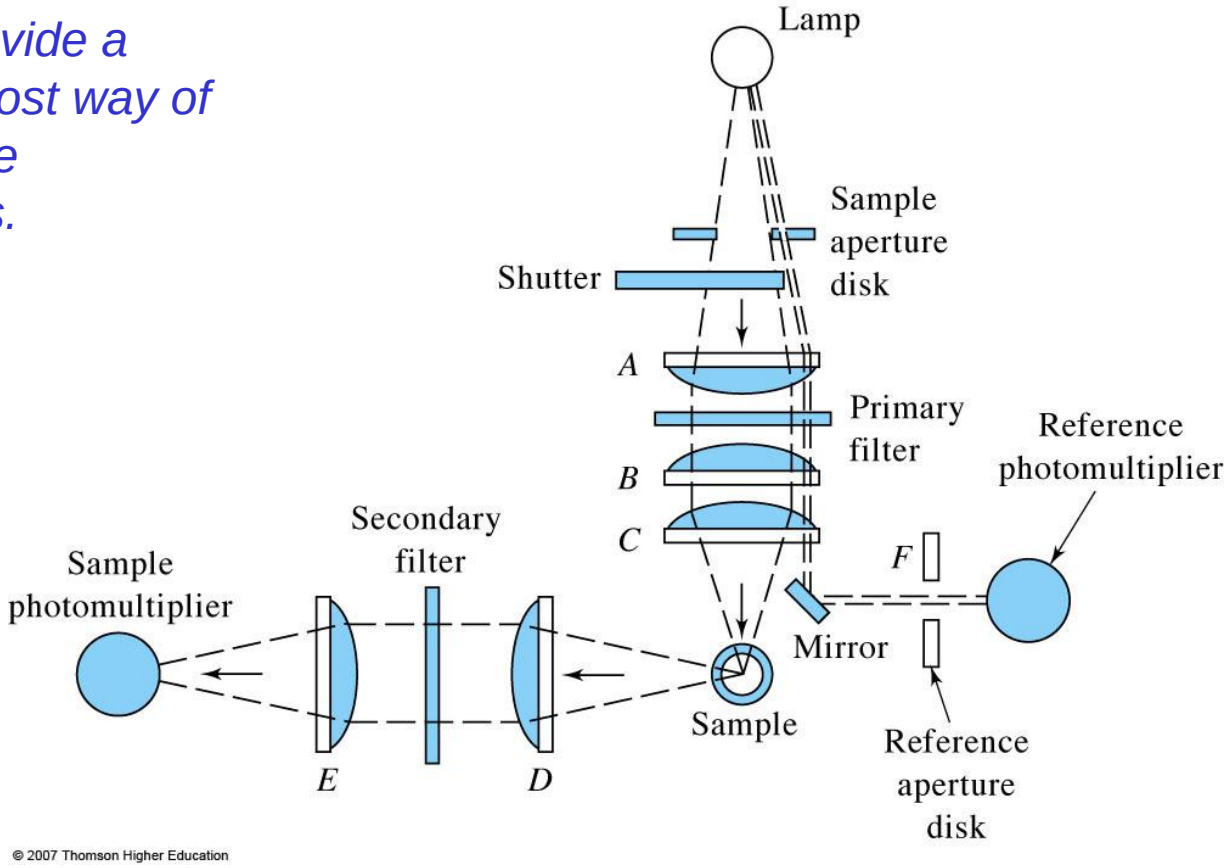
INSTRUMENTATION

- Sources
  - Low pressure Hg lamp (254, 302, 313, 546, 578 nm) line source
  - Xe lamp (300 – 1300 nm) continum source
  - Lasers (tunable dye lasers)
- Filter/monochromator
  - Isolate excitation λ
  - Scan excitation λ
  - Isolate emission λ from excitation λ
  - Scan emission λ
- Both cylindrical and rectangular cells fabricated of glass or silica are employed for fluorescence measurements. Care must be taken in the design of the cell compartment to reduce the amount of scattered radiation reaching the detector. Baffles are often introduced into the compartment for this purpose. Even more than in absorbance measurements, it is important to avoid fingerprints on cells because skin oils often fluoresce,
- Detector
  - Usually PMT: very low light levels are measured. Transducers are sometimes cooled to improve signal-to-noise ratios.
  - Charge-transfer devices such as charge-coupled devices (CCDs), are also used for spectrofluorometry. This type of transducer permits the rapid recording of both excitation and emission spectra and is particularly useful in chromatography and electrophoresis.



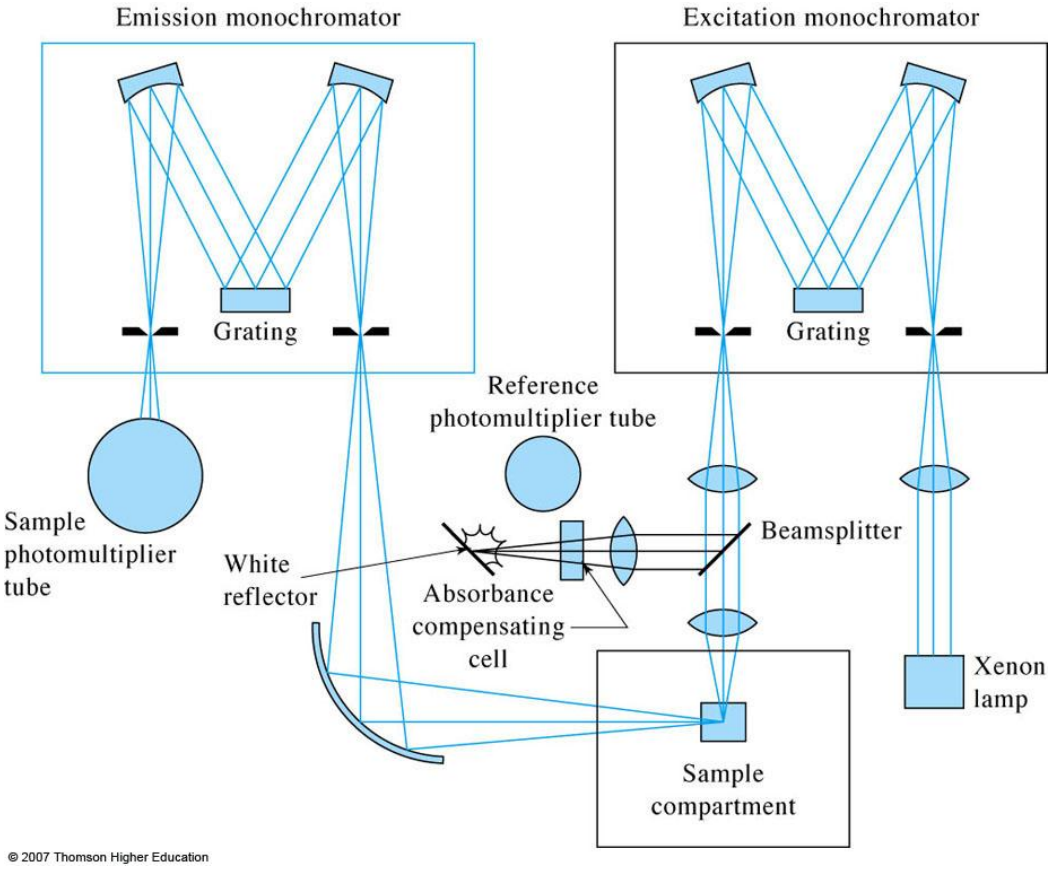
Fluorometer:

Filter fluorometers provide a relatively simple, lowcost way of performing quantitative fluorescence analyses.



Spectrofluorometer

- *Several instrument manufacturers offer spectrofluorometers capable of providing both excitation and emission spectra.*
- *The optical design of these instruments employs two grating monochromators.*
- *Radiation from the excitation monochromator is split, part passing to a reference photomultiplier and part to the sample.*
- *The resulting fluorescence radiation, after dispersion by the emission monochromator is detected by a second photomultiplier.*



Phosphorescence Instrumentation

• Similar in design to the fluorometers and spectrofluorometers except that two additional components are required.
• The first is a device that alternately irradiates the sample and, after a suitable time delay, measures the intensity of phosphorescence.
•The time delay is required to differentiate between long-lived phosphorescence emission and short-lived fluorescence emission, both of which would originate from the same sample.
• Both mechanical and electronic devices are used, and many commercial fluorescence instruments have accessories for phosphorescence measurements. Many of the current instruments use a gated scheme for the delay. A pulsed xenon arc lamp is often used to excite the sample. After a delay time, specified by the user, the data-acquisition system is activated to obtain the phosphorescence signal. Often, the signal is integrated during this period when the lamp is off and fluorescence has decayed to a very small value.
• The second new component is needed because phosphorescence measurements are usually performed at liquid nitrogen tcmperature in a rigid medium to minimize collisional deactivation of the long-lived triplet state.
• Usually, a Dewar flask with quartz windows, as shown in Figure 15-13, is a part of a phosphorimeter. At the temperature used, the analyte exists as a solute in a glass or solid solvent. A common solvent for this purpose is a mixture of diethylether, pentane, and ethanol.

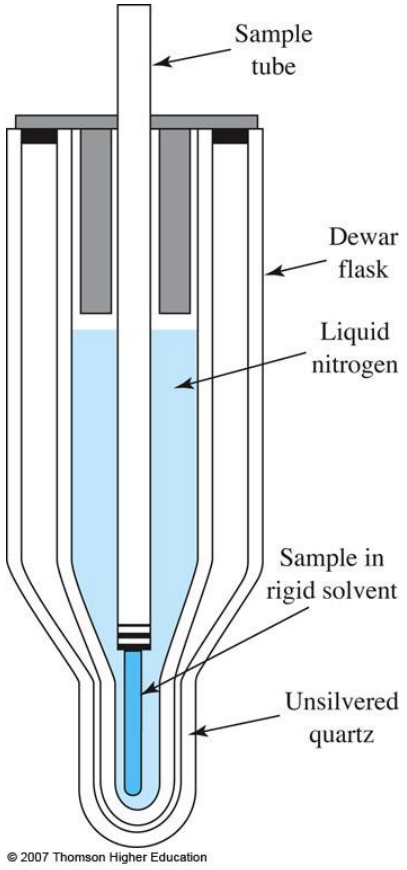
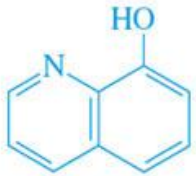
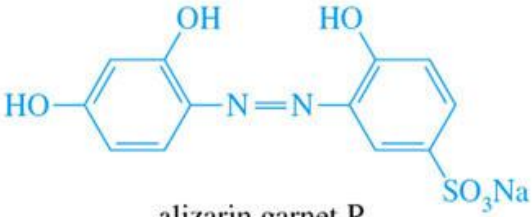


TABLE 15-2 Selected Fluorometric Methods for Inorganic Species

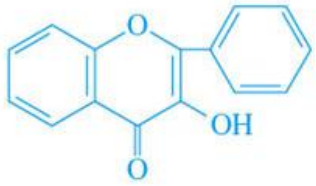
Ion	Reagent	Wavelength, nm		LOD, μg/mL	Interferences
		Absorption	Fluorescence		
Al^{3+}	Alizarin garnet R	470	500	0.007	Be, Co, Cr, Cu, F^-, NO_3^-, Ni, PO_4^{3-}, Th, Zr
F^-	Quenching of Al^{3+} complex of alizarin garnet R	470	500	0.001	Be, Co, Cr, Cu, Fe, Ni, PO_4^{3-}, Th, Zr
$B_4O_7^{2-}$	Benzoin	370	450	0.04	Be, Sb
Cd^{2+}	2-(*o*-Hydroxyphenyl)-benzoxazole	365	Blue	2	NH_3
Li^+	8-Hydroxyquinoline	370	580	0.2	Mg
Sn^{4+}	Flavanol	400	470	0.1	F^-, PO_4^{3-}, Zr
Zn^{2+}	Benzoin	—	Green	10	B, Be, Sb, colored ions



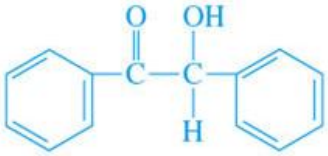
8-hydroxyquinoline
(reagent for Al, Be, and other metal ions)



alizarin garnet R
(reagent for Al, F^-)



flavanol
(reagent for Zr and Sn)



benzoin
(reagent for B, Zn, Ge, and Si)