# Molecular Luminescence Spectroscopy

In Molecular Luminescence Spectrometry ( MLS ) , molecules of the analyte in solution are excited to give a species whose emission spectrum provides information for qualitative or quantitative analysis .
There are three types of MLS :
Fluorescence: excitation by absorption of photon, short-lived excited state (singlet), emission of photon.
Phosphorescence: excitation by absorption of photon, long-lived excited state (triplet), emission of photon.
These two types are called photoluminescence where molecules are excited by absorption of electromagnetic radiation
Chemiluminescence: no excitation source – chemical reaction energy to excite molecule which emit radiation .
These three methods will be the subject of this unit .

Fluorescence is important due to its inherent sensitivity which can be several orders of magnitude more sensitive than absorption methods. Another reason which adds to its importance is the specificity of fluorescence methods since, relatively, small margin of molecules fluoresce.
When radiation of an appropriate wavelength is used to irradiate molecules in a sample, certain electronic transitions take place. As excited molecules return to the ground state they emit radiation of longer wavelength. The emitted radiation is referred to as fluorescence. The following Figure shows a description of absorption and emission phenomena and energy levels associated with these electronic transitions.

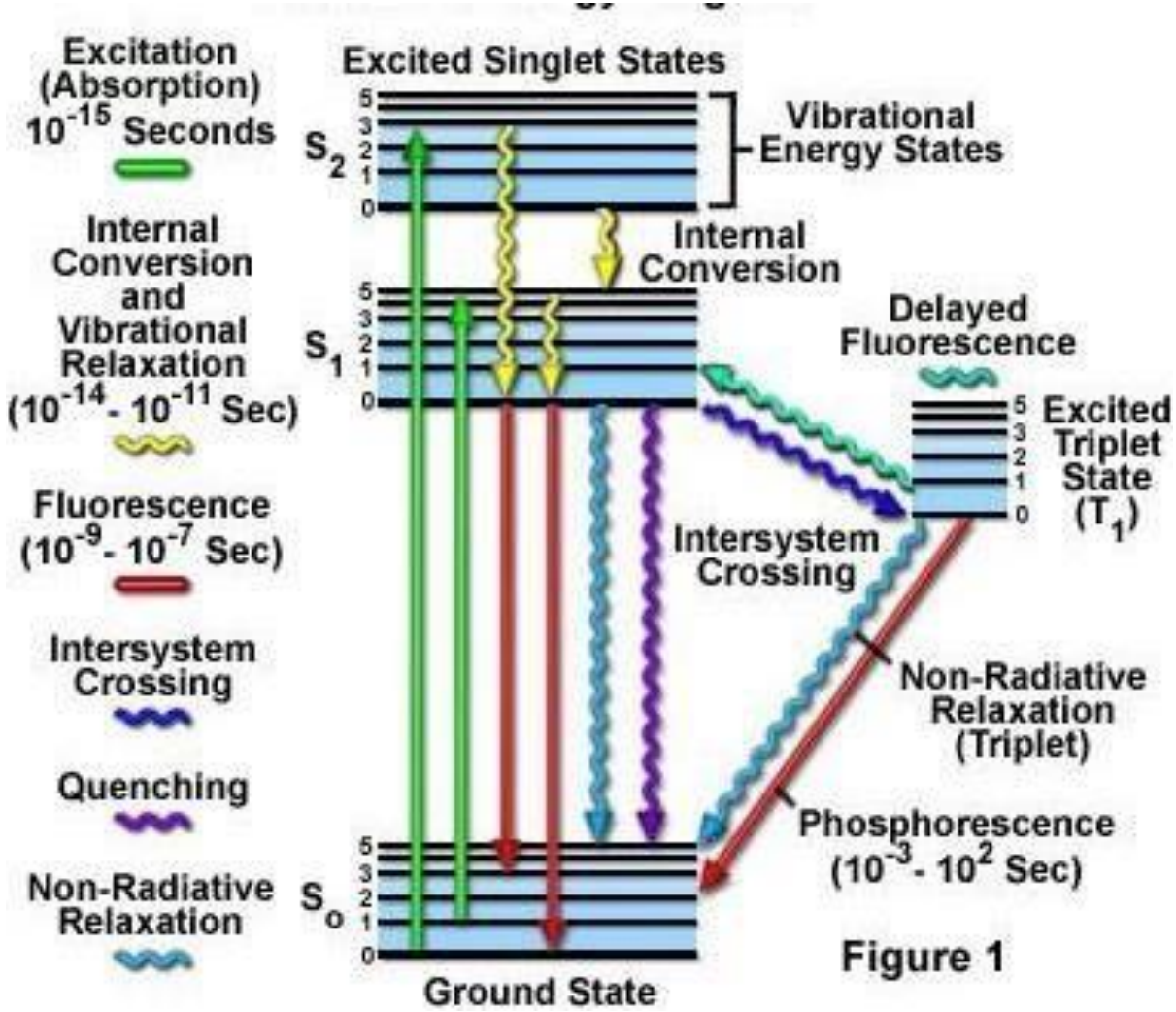

Excitation (Absorption) $10^{-15}$ Seconds
Internal Conversion and Vibrational Relaxation ($10^{-14}$ - $10^{-11}$ Sec)
Fluorescence ($10^{-9}$ - $10^{-7}$ Sec)
Intersystem Crossing
Quenching
Non-Radiative Relaxation
Excited Singlet States
$S_2$
$S_1$
$S_o$
5
3
2
1
0
Vibrational Energy States
Internal Conversion
Delayed Fluorescence
Excited Triplet State ($T_1$)
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Phosphorescence ($10^{-3}$ - $10^{2}$ Sec)
Ground State

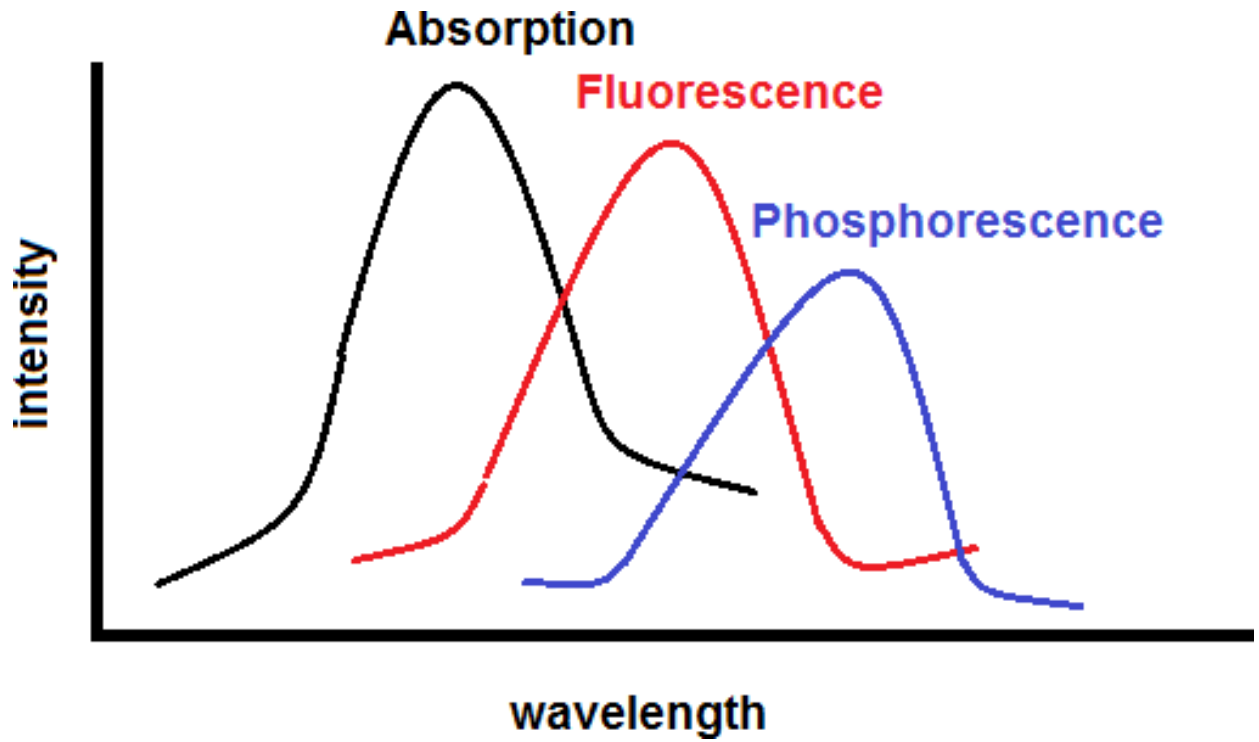
Figure 1

Electrons in the ground state (So) absorb energy from incident radiation and are excited to S1 or S2 excited states. Vibrational relaxation results in the return of the electron to lower vibrational level. Vibrational relaxation is a nonradiative deactivation process where excess energy is consumed as kinetic energy or heat. A second collisional deactivation process can take place and the electron returns to the ground state by a nonradiative deactivation process to So. The other possibility involves direct transition of the electron from the S1 excited state to So ground state and excess energy is emitted as photons at specific wavelengths called fluorescence.

Electrons in the excited state can follow a third approach by changing their spin and transferring to the triplet state through a process known as intersystem crossing.

The path of the excited electron depends on several factors which will be mentioned shortly . An electron in the triplet state will surely exhibit a vibrational relaxation to the main triplet energy level. A second flip in spin is necessary for such an electron to transfer to the ground state by one of two mechanisms. The first involves emitting a photon at a specific wavelength (phosphorescence) or non-radiative deactivation. Some molecules show a different behavior where an electron in the triplet state can experience a flip in spin and return to the first excited singlet state followed by vibrational relaxation then either non-radiative deactivation or fluorescence.

Fluorescence of this type can be observed after some time and is referred to as delayed fluorescence. It should be noticed that the life time of an electron in the excited state is about $10^{-9}$ second which is the time needed to observe fluorescence. Phosphorescence or delayed fluorescence can be observed after some time, usually exceeding $10^{-4}$ second. As all processes of deactivation and phosphorescence are possible, precautions should be considered in order to inforce circumstances that prefer fluorescence route.


Absorption
Fluorescence
Phosphorescence
intensity
wavelength

# Molecular Fluorescence Spectroscopy

Molecular Fluorescence is a photoluminescence process in which molecules in solution are excited by absorption of electromagnetic radiation. The excited molecules then relax to the ground state, giving up their excess energy as photons ,typically, but not necessarily in, visible radiation.

One of the most attractive features of molecular fluorescence is its inherent sensitivity ( F, 0 ) which is often one to three orders of magnitude better than absorption spectroscopy ( P , P0 ).

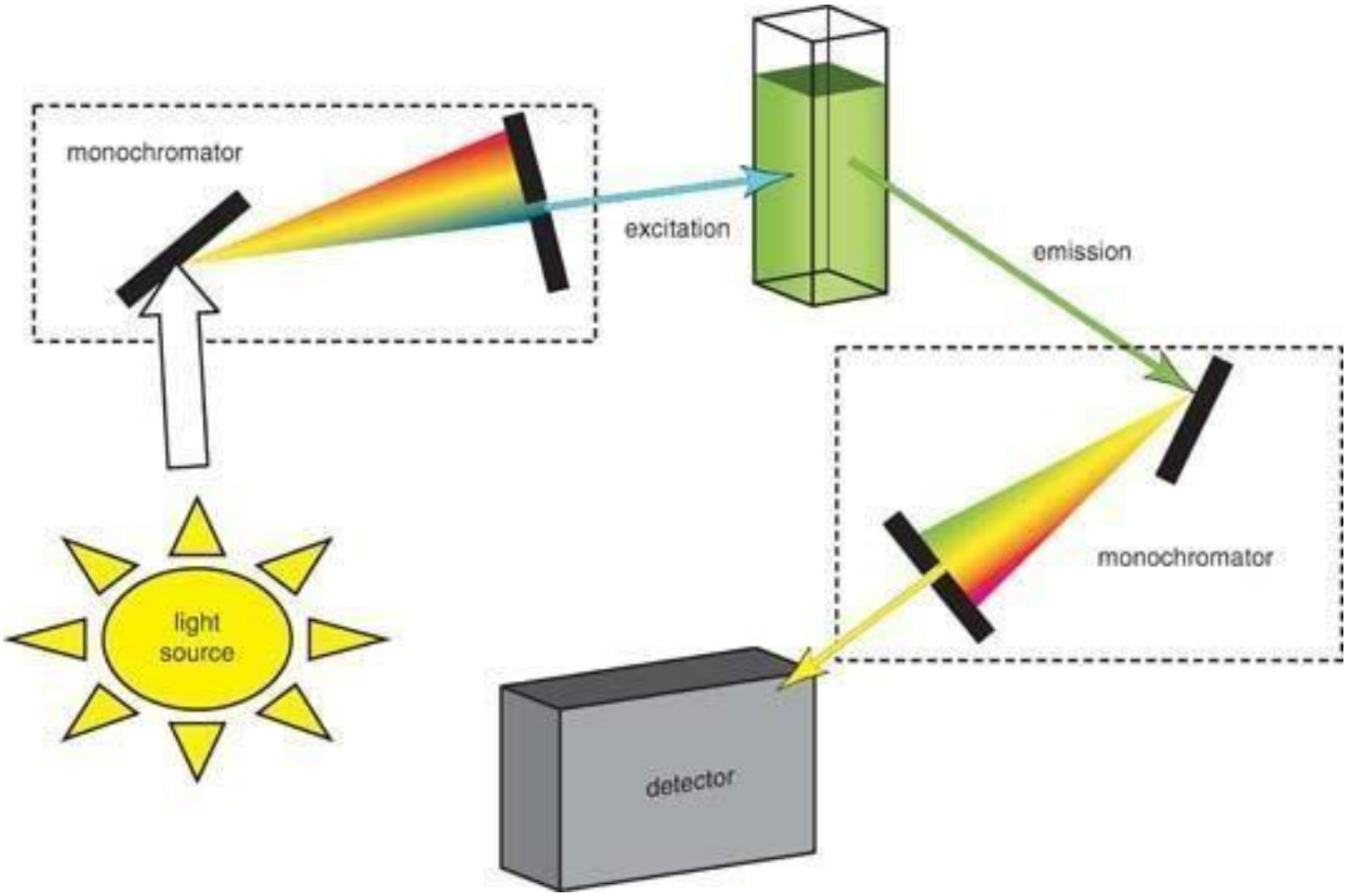
Another advantage is the large linear concentration range of fluorescence methods, which is significantly greater than those encountered in absorption spectroscopy.

Most compounds absorb UV , little absorb visible , few fluoresce and very few phosphoresce so the order of the selectivity is :

Phosphorescence > fluorescence > absorption

. Fluorescence methods are, however, much less widely applicable than absorption methods because of the relatively limited number of chemical compounds that show appreciable fluorescence. This can be advantage due to minimizing interferences .

Molecular fluorescence is measured by exciting the sample at the absorption wavelength, also called *excitation wavelength*, and measuring the emission at a longer wavelength called the emission or fluorescence wavelength. Usually, fluorescence emission is measured at right angles to the incident beam so as to avoid measuring the transmitted radiation. As we mentioned, the short-lived emission that occurs is called *fluorescence*, whereas luminescence that is much longer lasting is called phosphorescence.


monochromator
excitation
emission
monochromator
light source
detector

Relative rates of relaxation depends on the molecule, the solvent, temperature, pressure, etc. Energy of Phosphorescence is less than the energy of fluorescence . Phosphorescence occurs at a longer wavelengths than fluorescence . Lifetime of Fluorescence ($10^{-8}$ to $10^{-4}$ s) is very short compared to phosphorescence (10-4 to 102 s) because T S is forbidden and involves change of spin.

Following absorption of radiation , the molecule can lose the absorbed energy by several pathways :

1- Vibrational relaxation which involves transfer of energy to neighboring molecules in solution . This termed external conversion .

2- non-radiative transitions from higher vibrational excited level to lower vibrational excited level , this called internal conversion.

3- The molecule after losing some of it's energy in non radiative transitions can return to the singlet ground state and emit radiation with wavelength longer than the absorption wavelength . This is termed fluorescence .

4- The molecule can return from the highest excited singlet state to the ground singlet state and emits radiation with wavelength equal to the wavelength of the absorption radiation . This is termed resonance fluorescence .

5- The molecule can undergo intersystem crossing which involves transfer and electron spin flip from the excited singlet state into a triplet state . Following this the molecule may return to the singlet ground state emitting radiation called phosphorescence with wavelength longer than fluorescence .

**Notes :**

Two Possible Transitions in Excited State
Single state – electron spins opposed
Triplet state – electron spins are parallel
In general, triplet state has lower energy than singlet state.
Singlet to Triplet transition has a very low probability
Singlet to Singlet Transition are more probable

# Excitation Spectra and Fluorescence Spectra

Because the energy differences between vibrational states is about the same for both ground and excited states, the absorption, or *excitation spectrum*, and the fluorescence spectrum for a compound often appear as approximate mirror images of one another .

When measuring fluorescence spectra, the wavelength of the excitation ( absorption ) is kept constant, preferably at a wavelength of high absorption $\lambda_{max}$ , and the emission monochromator scans the spectrum. For measuring excitation spectra, the wavelength passing through the emission monochromator ( fluorescenc wavelength ) is kept constant preferably at $\lambda_{max}$ and the excitation monochromator is scanning. The excitation spectrum generally is identical to the absorption spectrum as the fluorescence intensity is proportional to the absorption .

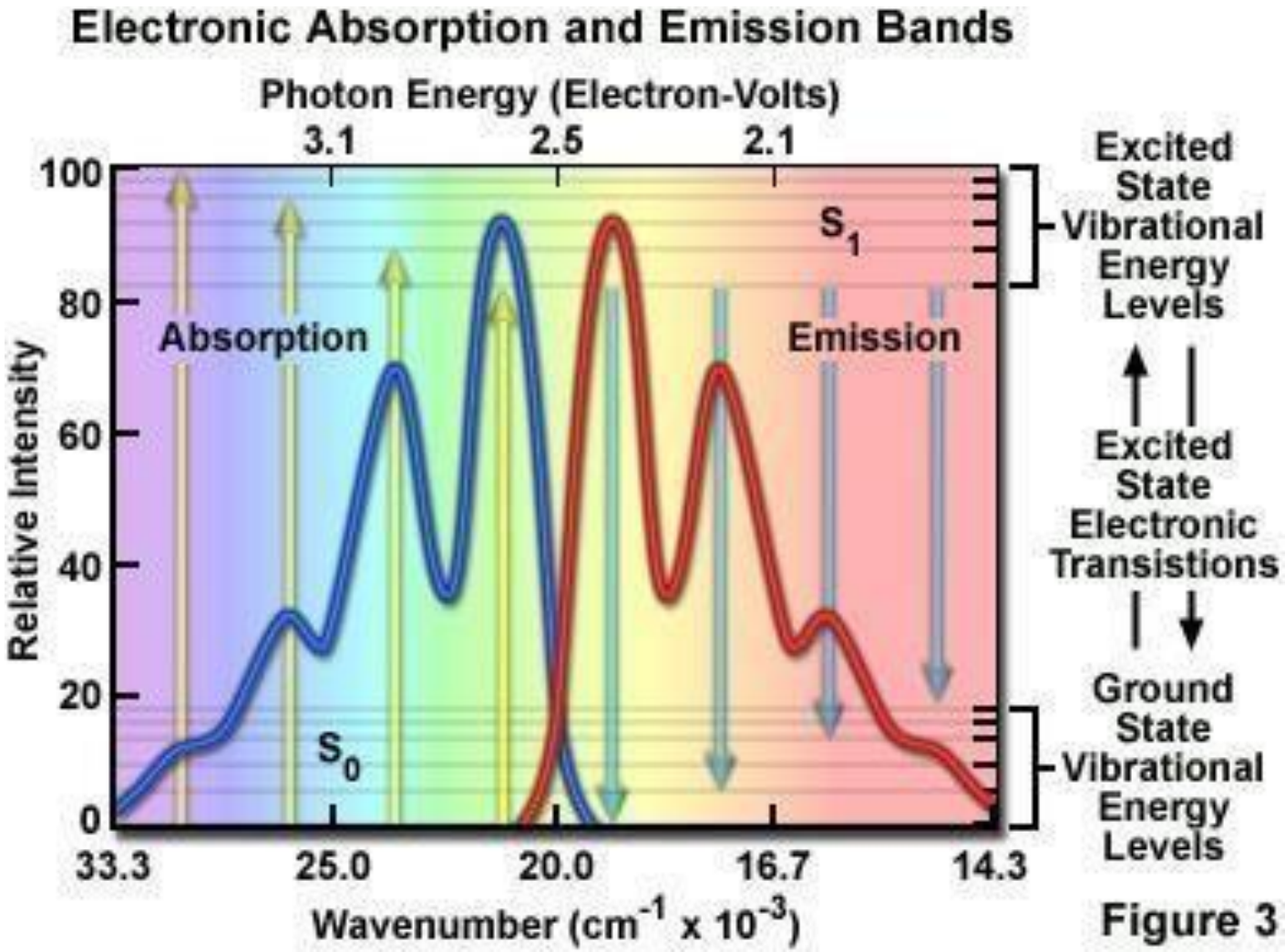

Electronic Absorption and Emission Bands
Photon Energy (Electron-Volts)
3.1
2.5
2.1
100
80
60
40
20
0
Relative Intensity
Absorption
Emission
$S_1$
$S_0$
33.3
25.0
20.0
16.7
14.3
Wavenumber ($cm^{-1}$ x $10^{-3}$)
Excited State Vibrational Energy Levels
Excited State Electronic Transistions
Ground State Vibrational Energy Levels

Figure 3

## Concentration and Fluorescence Intensity

The radiant power of fluorescence F is proportional to the radiant power of the excitation beam absorbed:

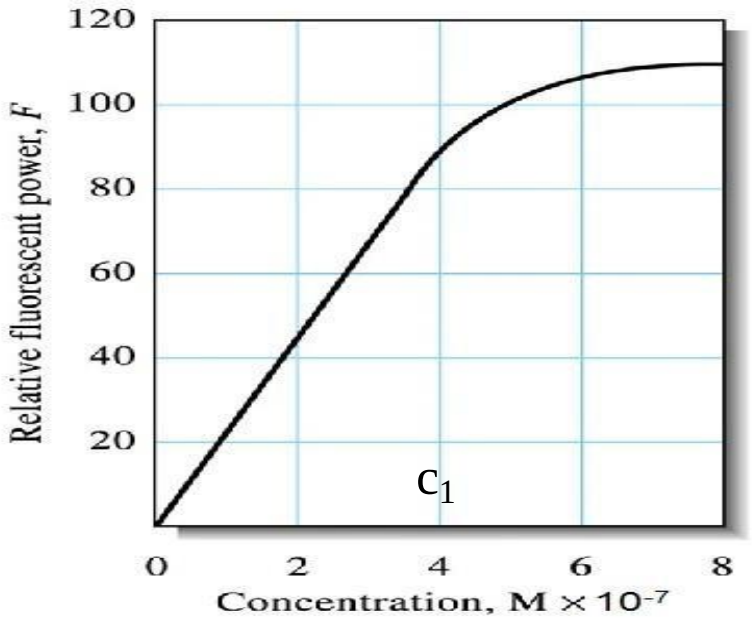
$$\mathbf{F = k(P_0 - P)}$$

where, $P_0$ is the radiant power of the beam incident on the sample and P is the radiant power after it traverses a path length b of the sample solution . At low concentrations where fluorescence is most often employed

$$\mathbf{F = K\, P_0\, C}$$

where, c is the concentration of the fluorescent species ( analyte ) and K is a new proportionality constant. F is directly proportional to analyte concentration. Thus, a plot of the fluorescent radiant power versus the concentration of the emitting species should be, and ordinarily is, linear at low concentrations.

When c becomes great enough that the absorbance is larger than about 0.05 M , linearity is lost and F begins to reach a plateau with concentratior




For a concentration above C1 the calibration is no longer linear .

The constant K depends on several factors :

1- The intensity of the incident radiation $P_0$ , $K \alpha P_0$ . So to increase F you increase $P_0$ by choosing the suitable source .

2- Quantum effeciency of fluorescence ϕ which is equal to

$$Quantum\ efficiency = \frac{\text{int}ensity\ of\ fluorescence}{\text{int}ensity\ of\ absorption}$$

$$\phi = F/(P0 - P)$$

Because not all the absorbed radiation is emitted as fluorescence F as mentioned before , $K \alpha \phi$

$$\phi \leq 1$$

3- Absorptivity a of the analyte as in Beer's law : $K \alpha a$ .

4- Cell bath length b , $K \alpha b$

## Factors affecting fluorescence intensity

Quenching excited molecule returns to the ground state by non-radiative transitions as a result of a collision with other molecules in solution . Also other molecules may absorb the fluorescence from the analyte .

Temperature : Decreasing temperature will decrease collisions and non-radiative transitions , and as a result will increase fluorescence . So some methods performed in cold medium .

pH :The fluorescence of many analytes depends on the pH of the solution .Some of these analytes are used as acid – base indicators.

Conjugation : molecules must have conjugation ( $\pi$ electrons ) so that UV-Vis radiation can be absorbed and preferably have an aromatic rings .

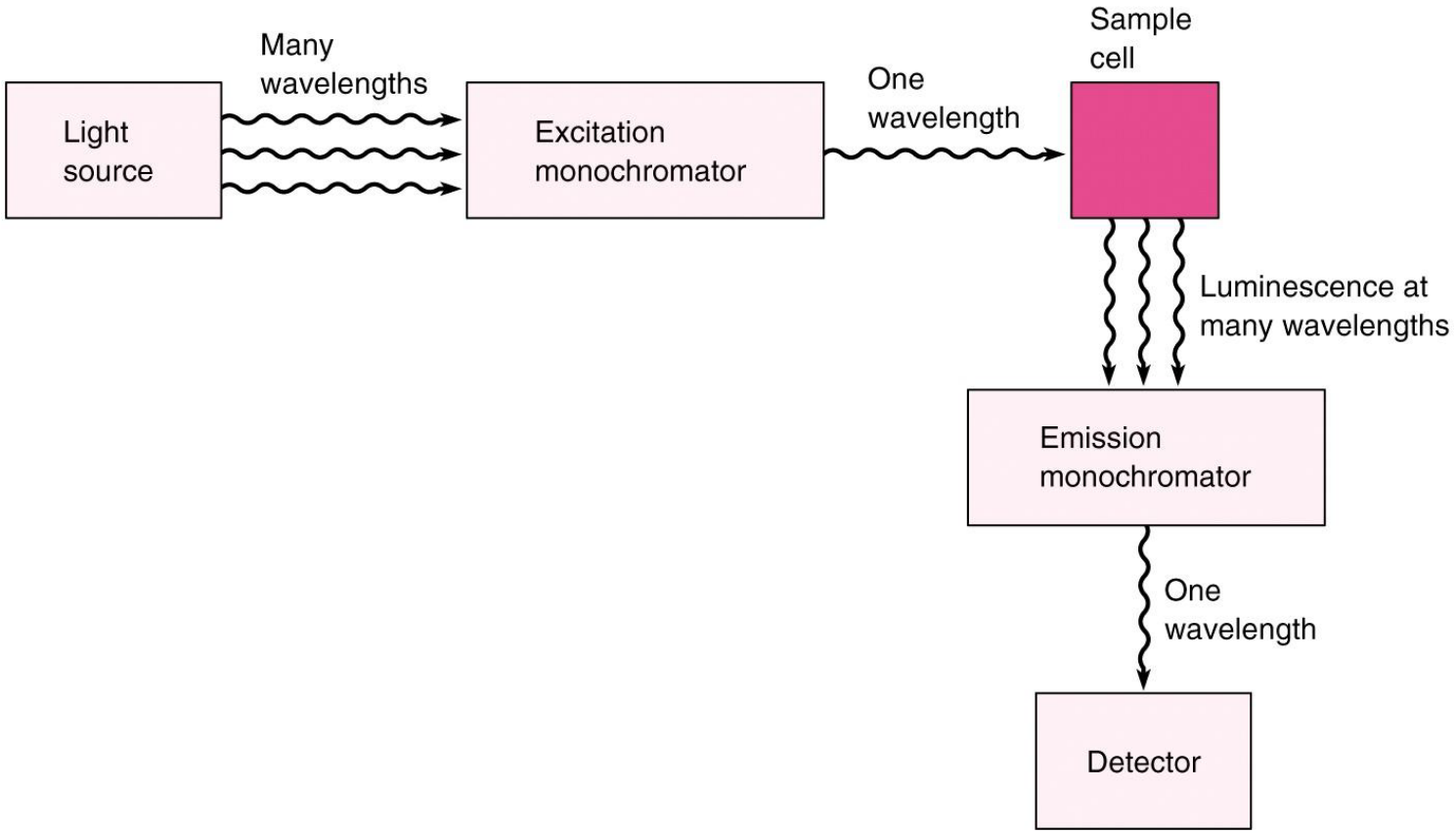
Nature of substitution groups :
Electron donating like NH2 , OH enhance fluorescence .
Electron withdrawing like NO2 , COOH reduce fluorescence.
More rigid the structure of the molecule more the intensity the fluorescence .
Presence of $O_2$ and some metals decreases the fluorescence because both increase intersystem crossing so deaerate solution .
Solvent : Decreased of viscosity of solvent leads to decreased fluorescnce due to increased collisions and thus increased external conversition processes .

The most important transition that contribute to good fluorescence characteristics is the $\pi \rightarrow \pi^*$ transition since Ɛ for this transition is the largest. The $n \rightarrow \pi^*$ transition is damaging to fluorescence because it facilitate and increase the possibility of intersystem crossing.

## Instrumentation

Two general types of instruments exist:
fluorometer use filters spectrofluorometr use diffraction grating to isolate the incident light and fluorescent light.
Both types use the following scheme: The light from an excitation source passes through a filter or monochromator, and strikes the sample. A proportion of the incident light is absorbed by the sample, and some of the molecules in the sample fluoresce. The fluorescent light is emitted in all directions. Some of this fluorescent light passes through a second filter or monochromator and reaches a detector, which is usually placed at 90° to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the detector

# Spectrofluorometer components



Various light sources may be used as excitation sources. Commonly a xenon lamp which has a continuous emission spectrum with nearly constant intensity in the range from 300-800 nm is used .

Two monochromators are used , emission and excitation .The most common type of monochromator utilizes a diffraction grating As mentioned before.

The detector can either be single-channel or multichannel. The single-channel detector can only detect the intensity of one wavelength at a time, while the multichannel detects the intensity of all wavelengths simultaneously, making the emission monochromator unnecessary

Sample Cell **:** cylindrical and rectangular cell fabricated of glass or silica are employed for fluorescence measurements.

# Methods of fluorescence determination

Direct methods - natural fluorescence of the fluorecent sample is measured .

Indirect (derivatisation) methods - the nonfluorescent compound is converted into a fluorescent derivative by specific reaction or marked with fluorescent dye by attaching dye to the studied substance .

Quenching methods - The reduction in the intensity of some fluorescent dye due to the quenching action of the measured analyte e.g. metal ions .

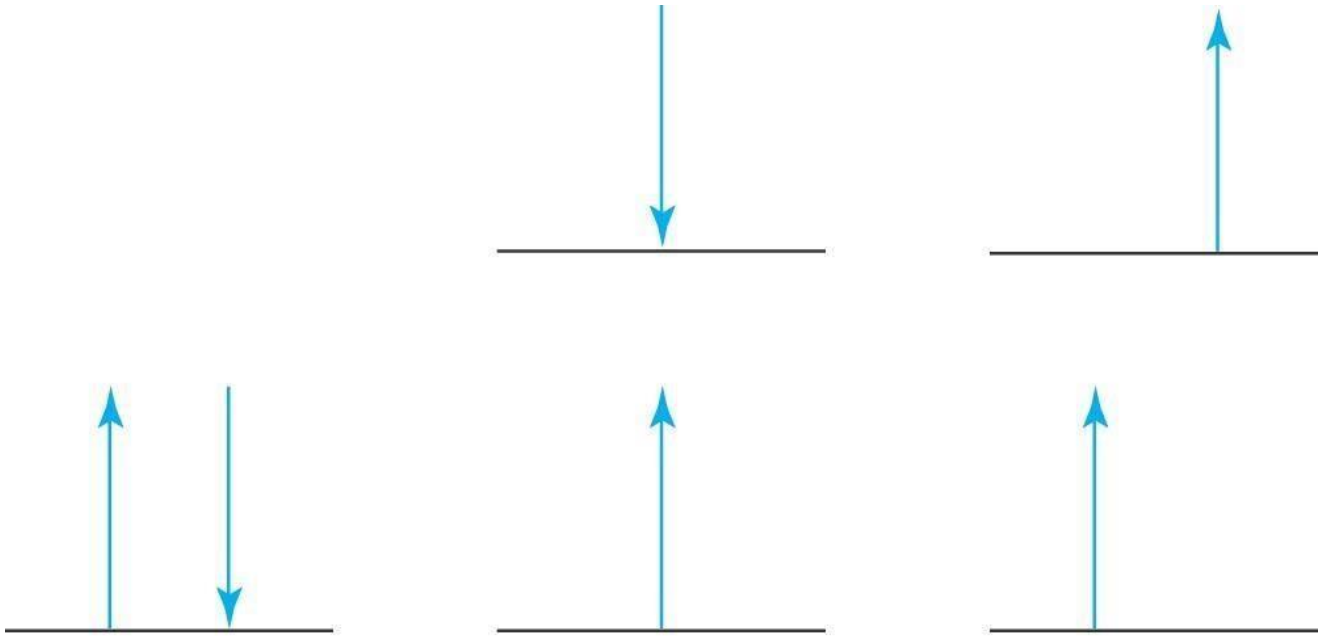
## Applications

Luminescence methods are amongst some of the most sensitive and selective methods of analysis available . Detection limits are in the ppm levels for absorption spectrophotometry and in the ppb levels for luminescence methods . Fluorescence spectroscopy is used in , biochemical, medical, food products , pharmaceuticals , natural products and chemical research fields for analyzing organic compounds. In analytical chemistry, fluorescence detectors are used with HPLC. Fluorescence is most commonly observed in compounds containing aromatic functional groups .Quantum efficiency increases with the number of rings .

# Molecular Phosphorescence Spectroscopy

Phosphorescence is a photoluminescence phenomenon that is quite similar to fluorescence. Understanding the difference between these two phenomena requires understanding of electron spins and the difference between a singlet state and a triplet state. Ordinary molecules exist in the ground state with their electron spins paired. A molecular electronic state in which all electron spins are paired is said to be a singlet state.

When one of a pair of electrons in a molecule is excited to a higher-energy level, a singlet or a triplet state can be produced. In the excited singlet state the spin of the promoted electron is still opposite that of the remaining electron. In the triplet state, however, the spins of the two electrons become unpaired and are thus parallel. The excited triplet state is less energetic than the corresponding excited singlet state . One of the disadvantages of phosphorimetry is that it requires liquid nitrogen to cool the sample in order to minimize the unradiative transitions



Ground
singlet state
(a)

Excited
singlet state
(b)

Excited
triplet state
(c)

## Chemiluminescence Methods

Chemiluminescence is produced when a chemical reaction ( mostly exothermic oxidation ) yields an excited molecule, which emits light as it returns to the ground state. One attractive feature of chemiluminescence for analytical uses, is the very simple instrumentation. Since no external source of radiation is needed for excitation, the instrument may consist of only a reaction vessel and a photomultiplier tube. Generally, no wavelength selection device is needed because the only source of radiant is the chemical reaction. Chemiluminescence differs from fluorescence or phosphorescence in that the electronic excited state is the product of an exothermic chemical reaction rather than of the absorption of radiation . A standard example of chemiluminescence in the laboratory setting is the luminol test. Here, blood is indicated by luminescence upon contact with iron in hemoglobin.

A trail of blood made visible with the use of the reagent luminol.

$$Luminol + H_2O_2 \xrightarrow{Fe} chemiluminescence$$

## Advantages of chemiluminesence :

1- High sensitivity .

2- Inexpensive .

3- Chemiluminescence has been used as a detector in many flow-injection analysis methods and in HPLC ,,,, etc .

## Applications :

Many analytes could be directly or indirectly quantified by chemiluminescence . The indirect methods can be performed either by a chemical reaction producing product that emit luminescent or by measuring the compounds quenching effect.

Analysis of nitrogen oxides in the atmosphere :

$$NO + O_3 \rightarrow NO_2^* + O_2$$

$$NO_2^* \rightarrow NO_2 + \text{light}$$

$$\text{Luminol} + H_2O_2 \xrightarrow{\textbf{peroxidase}} \text{3-aminoftalate} + \text{light}$$