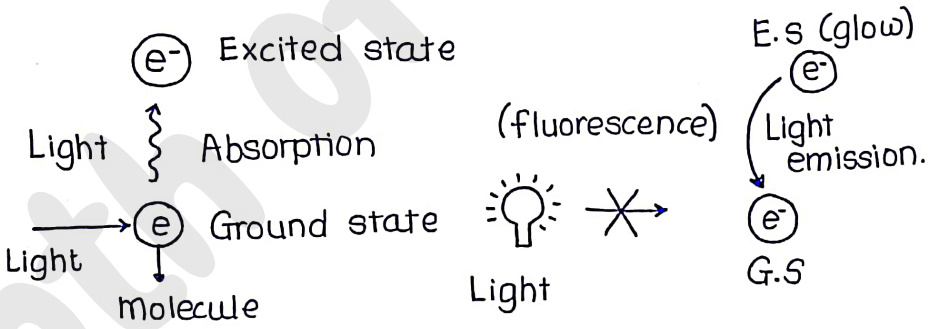
Fluorimetry

- It is the measurement of fluorescence intensity at a particular wavelength with the help of spectrofluorimeter.
- It is the process in which we can easily find out the concentration of a solution by measuring the intensity of a fluorescence or Phosphorescence.

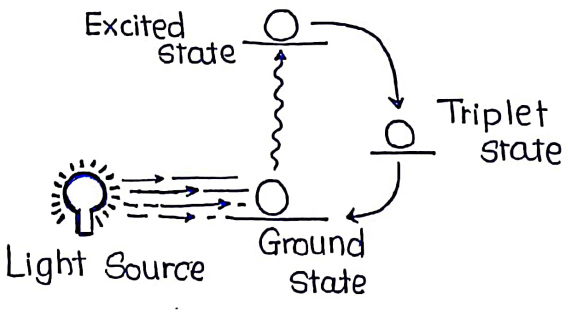


When a beam of light or incident ray is fall on sample. The molecule of the sample get excited from G.S to E.S (electron) and start's to glow or emit visible light. (So, we can say molecule absorb the light and goes into excited State. When incident light is cut off then e- again moves from ES to G.S by the emission of Light and this emission is known as fluorescence.

* The lifetime of fluorescence is short or very short approx. 10^{-8} second.

Phosphorescence → change in electron spin, endure for several second.

- Also called as Delayed fluorescence.
- Emission of light continuously for sometime (seconds) even after we cut the incident light (or source of light).
- Molecule electron get excited when a indicient light falls on it due to this e⁻ are shifted from GS to E.S.
- When light source is removed the e⁻ emit the light and come back to normal state (Ground state). But in case of phosphorescence e⁻ shift from excited state to Triplet State then in Ground State.
- The lifetime of Triplet State is long 10^{-2} - 100 second approx.

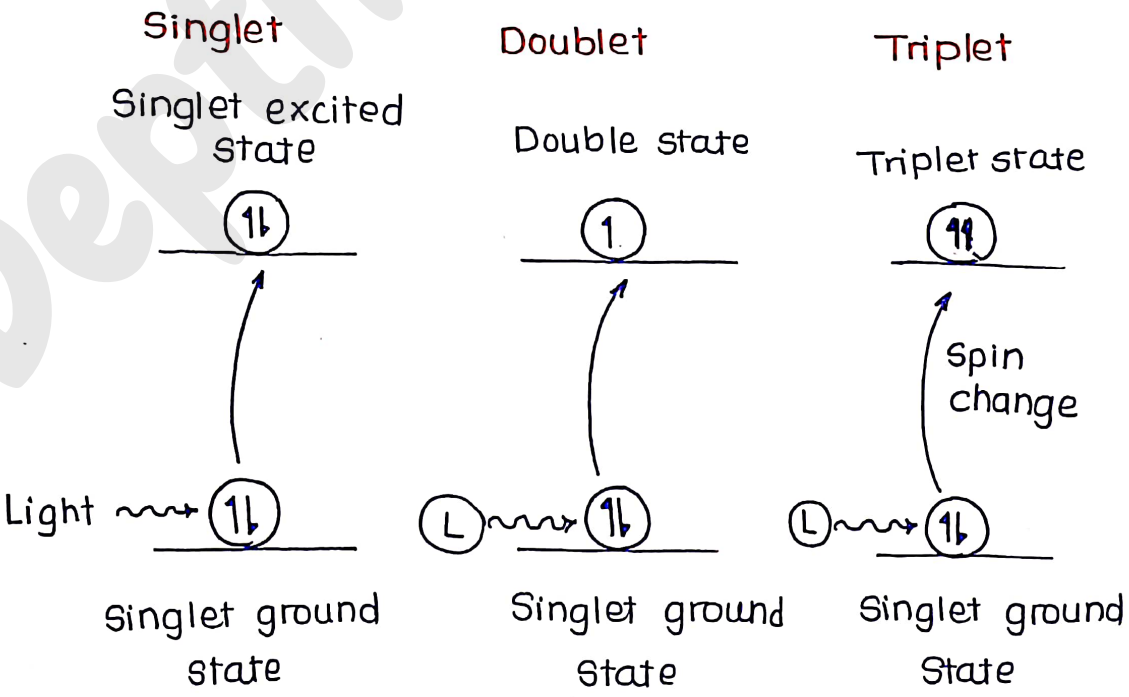


- Concept of Singlet, Doublet, Triplet State.
- These types of electronic state describe the amount of unpaired e- present in a molecule.

i. Single State → In which electron is present in paired form. (↿⇂)

ii. Double State → In which atleast one unpaired electron is present. (↿) or (⇂)

iii. Triplet State → In which e- of a molecule are present in same spin.
(↿↿) (⇂⇂).



Formula ⟶ 2(s) + 1

1 ↓
+½ −½

a. Singlet ⟶ 1↓ → +½ −½

+½ −½

2 (s) + 1
∴ = 2 (0) + 1
∴ = 1

b. Doublet State ⟶ 1 → +½

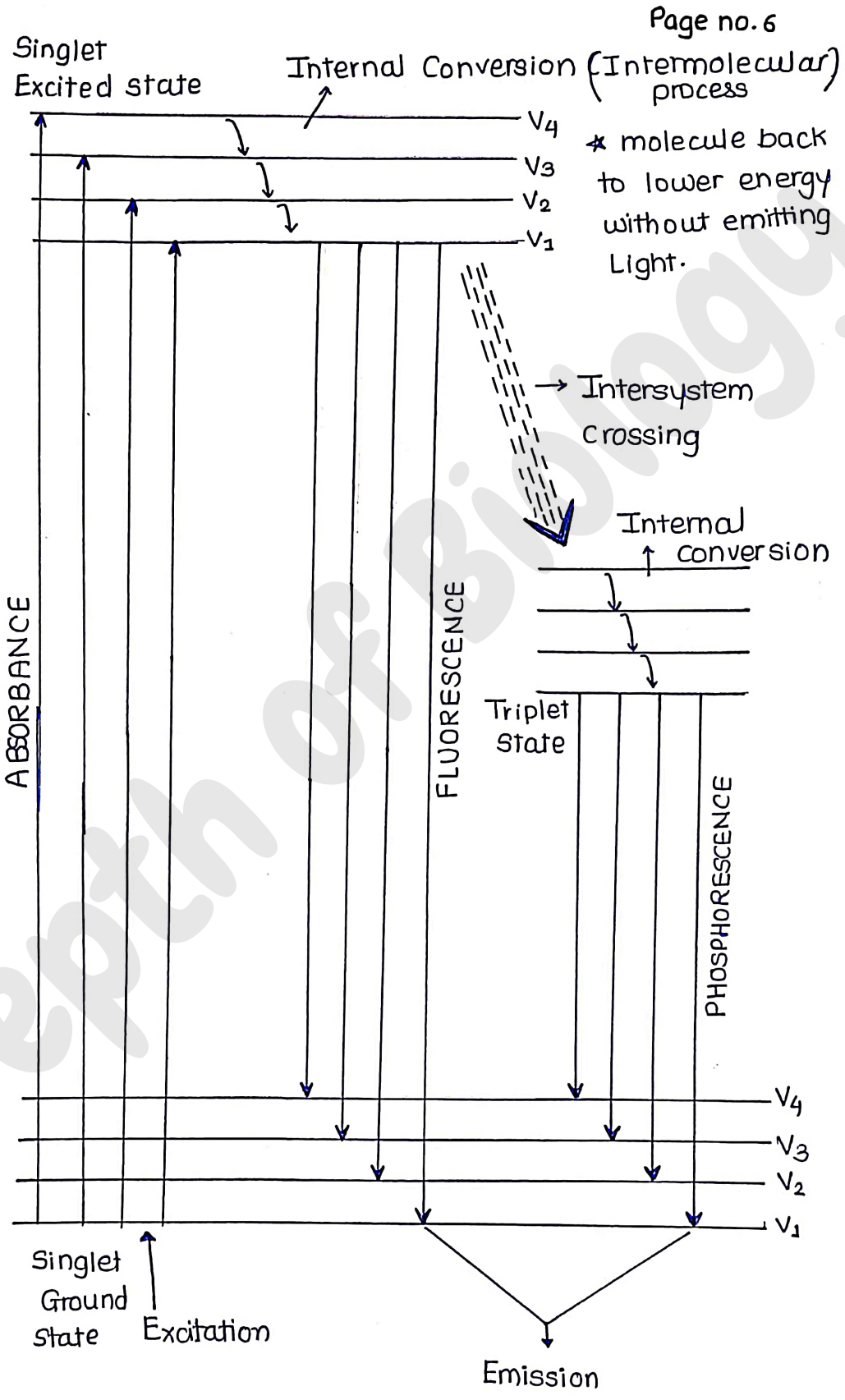
2 (s) + 1
= 2 (½) + 1
= 2

c. Triplet state ⟶ 11 → +½ +½

2 (s) + 1
= 2 (½ + ½) + 1
= 2 + 1
= 3

Principle

- When a beam of light is generated from a light source and fall on sample solution. Then the sample solution molecule absorb the light and the electron get excited from Ground State to Excited State.
- When the source of light is cut e- will be back to the ground state from excited state by emitting the radiation / Light known as fluorescence.
- Now with the help of the fluorescence we can easily find out the concentration of molecule present in sample. More will be the sample more will be the fluoroscence, Less will be the sample (concentration) less will be the fluorescence. And there is another term called Delayed fluorescence or Phosphorescence, in which e- are shifted from excited State to Triplet State and then back to the ground state.



• Factors affecting Fluorescence

1. Nature of Molecule

- All of the molecule cannot show the Phenomenon of fluorescence.

- Fluorescence only showed by those molecule who can absorb UV/visible radiation.
- Conjugation (π bond) $\propto$ Fluorescence
  or
- Unsaturation $\propto$ Fluorescence
- Lone pair $\propto$ Fluorescence
- Greater the Absorbancy of molecule $\rightsquigarrow$ The more intense it's fluorescence.

2. Nature of Substituents

a. Electron withdrawing group

b. Electron donating group.

- Substituent also affect the fluorescence of molecule.

a. Electron withdrawing group

↳ $\left[EWG \propto \frac{1}{\text{Fluorescence}} \right]$

- Electron withdrawing group can decrease or diminished or destroy fluorescence

  eg. COOH, NO_2, N=N.

b. Electron Donating Group

↳ [EDG ∝ Fluorescence]

- Some Electron donating Group like -OH, $-NH_2$ enhance fluorescence.

  But some EDG like $-SO_3H$, NH_4 and Alkyl group not have much effect on fluorescence.

c. If we introduce high Atomic number into πe^- system then it decrease fluorescence and enhance phosphorescene.

3. Effect of Concentration

- Fluorescence is directly proportional to conc. of molecule.

$[F \propto C]$

F = Fluorescence
C = Concentration.

$[F \propto A \propto C]$

A = Absorbance.

- But in fluorimetry analysis if we increase the concentration of molecule then chances of collision between molecule is increased.
- So, in this case low concentration sample is used (0.02%)

$[F = I_0 \phi]$.

- So, according to this we can say that fluorescence is independent of concentration & it is dependent on intensity of incident radiation.

4. Adsorption

- To enhance the sensitivity of fluorimetry we required or we used a very dilute solution (to avoid collision between molecule) & to obtain accurate reading.
- Adsorption of molecule on the container wall creates a serious problems. [So, solution must be diluted to avoid this].

5. Light

- Monochromatic light is essential for fluorescence.

6. Oxygen

- Oxygen interfere with fluoroscence by direct oxidation of fluorescent substance to non-fluorescent or by Quenching of fluorescence.

  eg. Anthracene is susceptible to O_2.

7. pH

- Alteration of pH of solution will have / leads to significant effect on fluorescence.

  Example : Phenol show fluorescence in both forms dissociated and undissociated form.

8. Temperature and Viscosity

- Temperature and viscosity also affect fluorescence.

  T ↑ Viscosity ↓ ⟶ Collison ↑ ⟶ fluorescence ↓

  T ↓ Viscosity ↑ ⟶ Collison ↓ ⟶ fluorescence ↑.

- **Quenching**

  It is a process by which intensity of fluorescence is reduced.

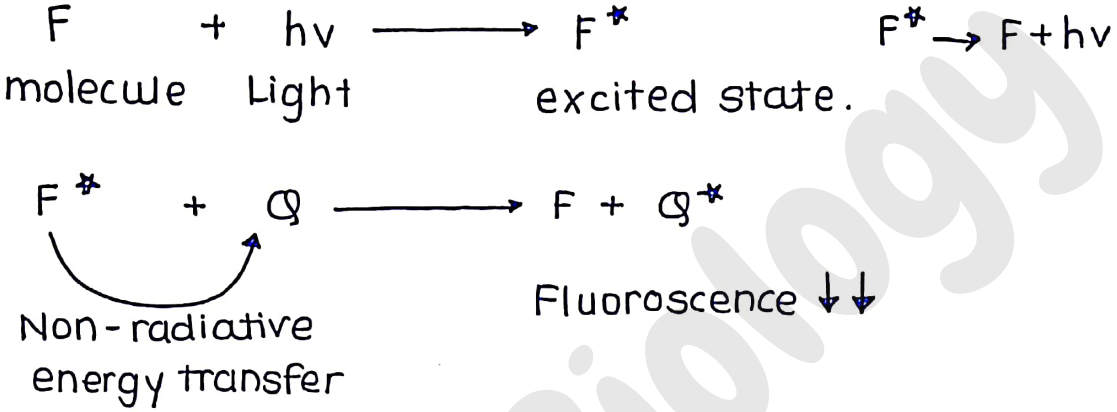
- Substance which cause Quenching called Quenchers.
- This may occur due to various factors like pH, Temperature, Concentration, Viscosity, presence of O_2, Heavy metal.

Quenching
- a. Collisional Quenching
- b. Static Quenching
- c. Concentration Quenching
- d. Chemical Quenching.

a. Collisional Quenching

(occurs when number of collision increased).

- This type of Quenching occured by the interaction of a Quencher molecule with excited molecule of fluorescent substance.
- This type of collision occur due to [T↑ V↓, Halides, Heavy metals].



b. Static Quenching

- Static Quenching occurs at Ground state.
- A complex formation occurs between (F) fluorescent molecule and Quencher molecule (Q) through a strong coupling.
- Such complex may not undergo excitation or excited to a little extent. i.e reducing the intensity of fluorescence molecule.

$[F + Q \longrightarrow F:Q]$

$F:Q + h\nu \longrightarrow Q^* + F$

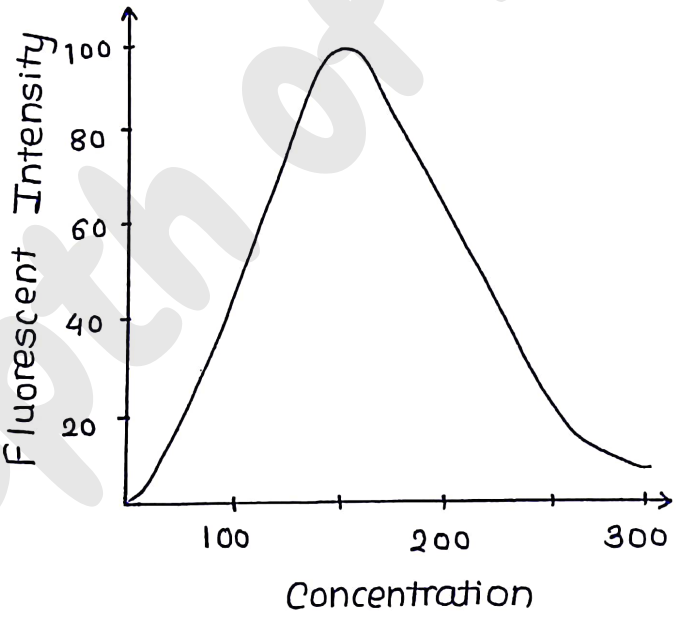
$Q^* \longrightarrow Q$ + Energy (Heat).

c. Concentration Quenching

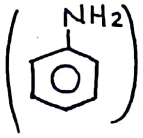
- Also known as self Quenching.
- A low concentration linearity observed (0.02 sample).

↓

But increase the concentration then it lead to decrease in the fluorescence intensity.



d. Chemical Quenching

i. pH ⟶ Aniline  at pH 5-13 gives blue Fluorescence when excited at 290 nm.

(but pH < 5 or > 13) ⟶ does not show fluorescence.

ii. Oxygen → Cause oxidation of flourescent substance

↓

To Non-flourescent Substance → Cause Quenching.

iii. Halides and EWG

- Halides like Cholride and Iodide ions cause Quenching.
- EWG like NO_2, COOH, CHO cause Quenching.

iv. Heavy metal

- Heavy metals forms complex with flourescent substance and lead to the complex formation & cause Quenching.

- Factors affecting Quenching

1. pH
2. oxygen
3. Temperature
4. Viscosity
5. Heavy metals
6. Halides
7. Electron withdrawing Group (EWG).

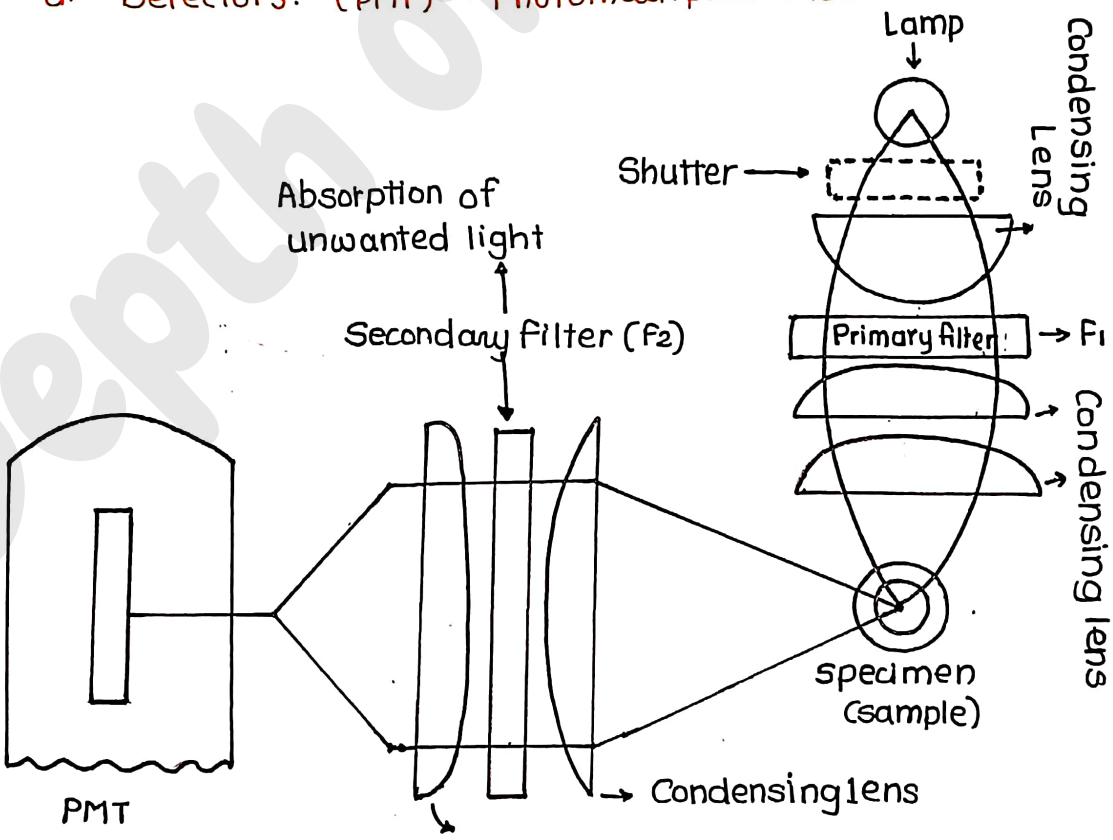
Instrumentation and Application.

Fluorimeter

- Instrument which are used to measure the intensity of fluorescence.

Fluorimeter is also known as Spectrofluorimeter.

* There are mainly 4 components of fluorimeter.
  a. Source of light
  b. Filters and monochromator
  c. Sample cell / Cuvettes
  d. Detectors. (PMT) → Photomultiplier Tube.



- Lamp is the source of light and this light is condensed by condensing Lens and then after condensation the light is passed on specimen/ sample.
- Sample molecule absorb this light and electron are shifted from ground state to Excited state.
- When molecules (electron) get back from excited state to Ground State they emit Light/Radiation.
- This emitted light/radiation again pass through condensing lens and condensed this radiation on PMT.

1. Source of Light

a. Mercury Arc Lamp → Mercury vapours
   - At low pressure gives 254 nm.
   - At High Pressure gives 350 nm.

b. Xenon Arc Lamp → Produce Intense Radiation
   Range between 250-600 nm.

c. Tungsten Lamp → Low intensity Lamp
   - Produce radiation of visible region.
   - does not offer UV radiation.

2. Filters and Monochromator

- These are optical filters work on principle of absorption of unwanted light and transmitting the required wavelength of Light.

a. **Primary filters** : Absorb visible radiation and transmit UV radiation.

b. **Secondary filters** : Absorb UV radiation and Transmit visible Radiation.

Monochromator

- They convert polychromatic Light into monochromatic Light.
- They isolate a specific or particular range of wavelength.

a. **Excitation Monochromator.**

- Provide suitable radiation for → Excitation of molecule.

b. **Emission Monochromator.**

- It isolate those radiation which is emitted by fluorescent Molecule.

3. Sample Cell / Cuvettes

- Made up of Quartz or Glass

- These are used for Holding Liquid samples.
- Cylindrical or Rectangular in shape.
  (Path length 10 mm or 1 cm).

4. Detectors

a. Photovoltic cell
b. Photo tube
c. PMT → Best and accurate.

Applications of Fluorimetry

1. Used extensively in the field of Nuclear research.
2. Generally Inorganic ions do not show fluorescence but some of them react with Non-fluorescent Organic Molecule and show flourescence.
3. Used in the determination of Uranium
Eg. a. Used in the estimation of Traces of Boron in steel.

↓

- By complex formation with Benzoin.

b. Used in the determination of Ruthenium ion.
This ion form complex with 5-methyl 1-1,10 Phenonthroline forms the complex ion which show fluorescence at pH 6.

4. Fluorescent Indicator

Intensity and colour of fluorescence

↓

Depends on pH of solution.

- So, it can be used in acid-base titration.

5. Flurometric Reagent

- Flurometric reagent used in cation analysis.

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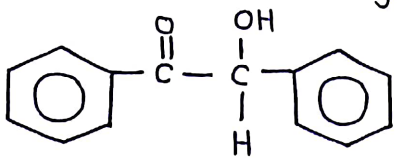
- Must have fluorometric structure.
- With 2 or more donor functional group, then it form chelate with metal ion.

example of Flurometric reagent →

8-Hydroxy Quinoline

and



Benzoin

6. Determination of Vitamin B_1

- Vitamin B_1 (Thiamine) is non-fluroscent (Not show fluorescence).

- But it's oxidation product Thiochrome fluorescence with Blue colour.

* This property is used for determination of Vitamin B_1 in food sample like Cereal, Meat, etc.

7. Determination of Vitamin B_2

- To detect the impurity in Vitamin B_2 (Riboflavin) or to know the exact condition we check fluroscence power of Vitamin B_2.

It is altered with Impurities and condition.

Vitamin B_{12} (Riboflavin) $\xrightarrow{\text{oxidation}}$ Non-fluroscent Substance.

8. Organic Analysis

Fluroscence is used in the Quantitative as well as Qualitative analysis of many Aromatic compound in Cigratte smoke, Air pollutant and automobile exhaust.