Instrumental Methods of Analysis

B-Pharmacy (7th Sem)

Unit - 1 → UV Visible spectroscopy

Lect - 1

- The method we can used for analysis in this unit and all units is Spectroscopy.

SPECTROSCOPY

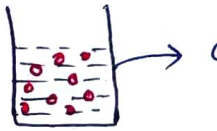
- Spectroscopy is made up by two words -

Spectroscopy
- Spectrum → Light
- Scopy → To determine

- Spectroscopy is a technique of analysis of Quantitative determination of any sample with help of

Basics

Analysis
- Qualitative → Quality of Product
- Quantitative → Quantity or amount



- How much Quantity or of drug in whole sample?

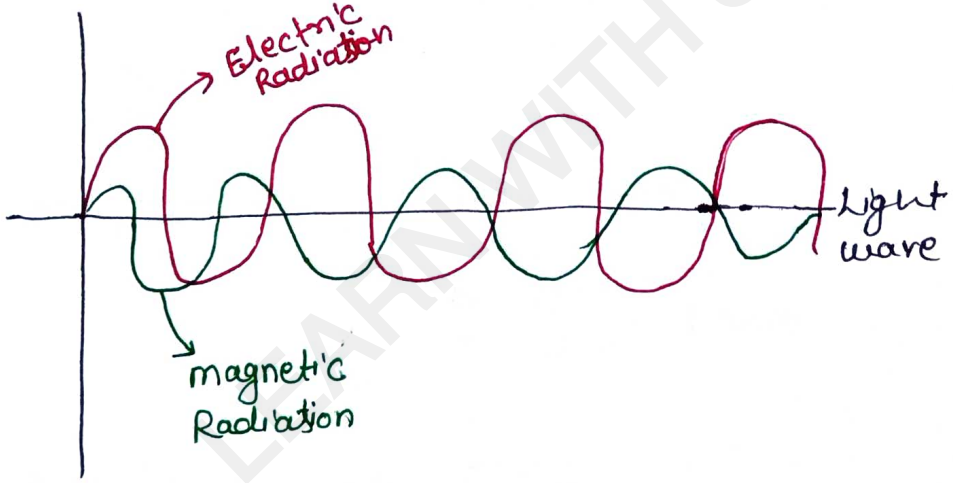
- we can study various methods before like titration, redox titration etc.

Light.

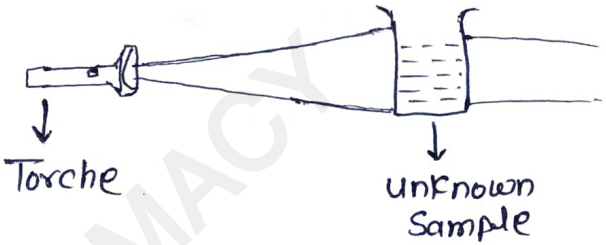
What is light?

- Light is a electromagnetic radiation.
- it contain packets of photons.
  - Packets of energy.

- Electromagnetic Radiation contains both electric and magnetic radiation.

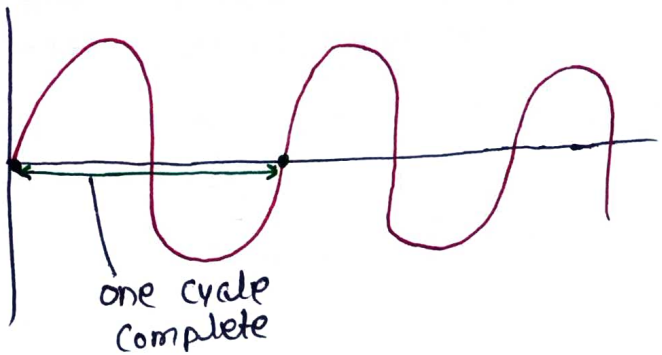


Travelling in Horizontal and vertical way.



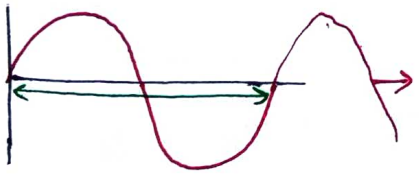
→ There are two imp. characteristics of light -
- frequency
- wavelength

Frequency

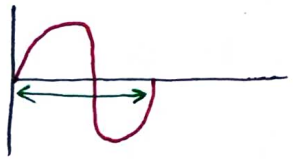


- The time taken to comp. 1 cycle is denoted by ν or η. (it is frequency)

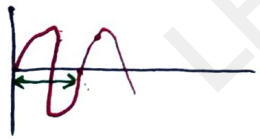
- if the frequency of light is strong then it takes less time to comp. 1 cycle.
- if the frequency of light is low then it takes more time to comp. 1 cycle.



Low freq.
↓
more time take to comp. 1 cycle.

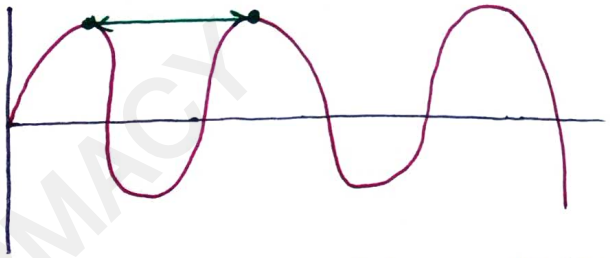


→ High freq.
↓
Less time taken to comp. 1 cycle



→ strong freq.
↓
Little bit time taken to comp. 1 cycle.

⇒ Wavelength,



- the distance b/w two peaks of a light wave is denoted by λ (lemda)
(it is wavelength)

Note → जिसकी wavelength ज्यादा उसकी frequency कम।

⇐ → जिसकी frequency ज्यादा उसकी wavelength कम.

$$\Rightarrow \boxed{V \text{ or } \eta \propto \frac{1}{\lambda}}$$

Did you know ?

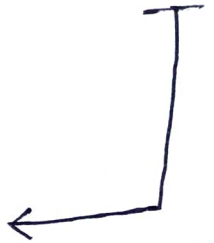
VIBGYOR

V – $\eta \uparrow$ (high freq.) $\lambda \downarrow$ (low wavelength)

R – $\eta \downarrow$ (low frequency) $\lambda \uparrow$ (high wavelength)
(तभी तो RED दूर से ही दिख जाता है)

- अगर खाना RED आग से बनाते हैं तो देर से बनता है because RED have low frequency or energy. [चूल्हा]
- और BLUE आग पर बनता है तो जल्दी because BLUE have high frequency or energy. [GAS]

This is Just for clear your Concept.



Principles of Spectroscopy

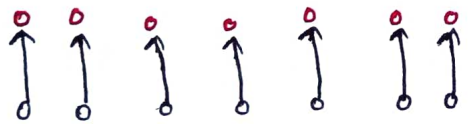
→ Spectroscopy is working on two principles –

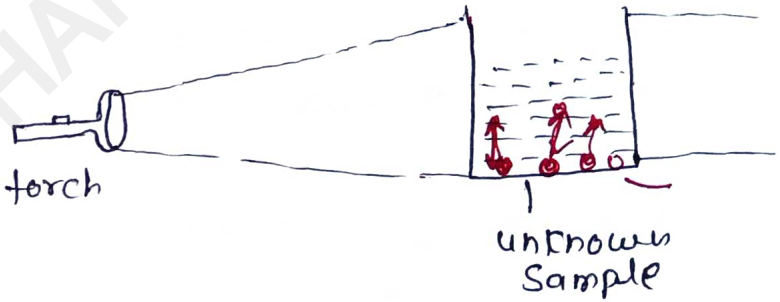
- Absorption Spectrum,
- Emition Spectrum,

Absorption Spectrum

↓

when molicules are goes from ground stage to Excited Stage.



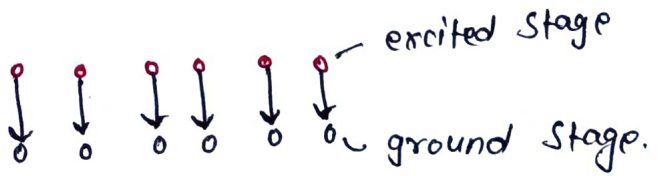


Emition Spectrum

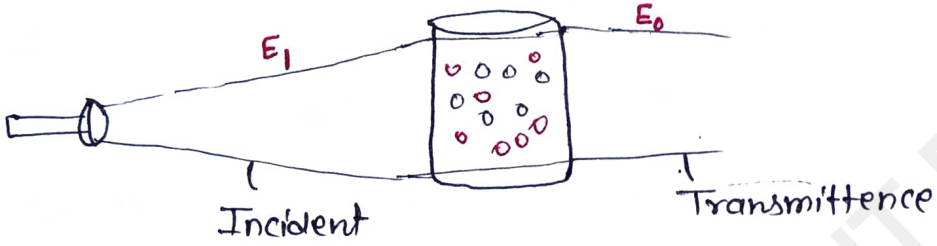
↓

when molicules are loose their energy and comes from Excited stage to ground stage.

→



- UV Spectroscopy is based on Absorption Spectrum.
- Emition spectrum is based on IR Spectroscopy



- If concentration is high then absorption is also high.
- If conc^n. is low then absorption is also low.

Absorption ∝ Concentration

$E_1 - E_0$ = Absorption

Theory ↓

→ The principle of UV-Spectroscopy is based on the absorption of UV light or visible light by chemical compounds, which results in the prodn of distinct spectra.

→ when the matter absorbes light, it undergoes excitation and de-excitation, resulting in the production of a spectrum

→ when matter absorbes UV radiation, the

electron present in it undergo excitation.

→ This causes them to jump from a ground state to excited state.

→ it is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.

Unit-1 - UV visible Spectroscopy

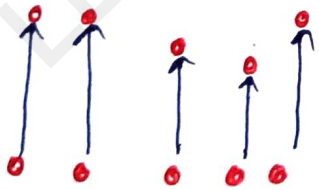
Lecture-3 - **Electronic Transition** Imp

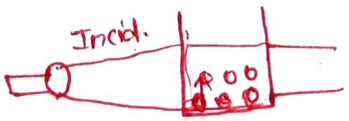
→ Electronic Transition

- Electronic Transition in molecules depend upon promotion of electrons from ground state to the higher or excited state.

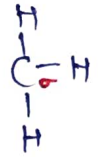
electrons
↓
σ - alpha
π - pie
n - lone pair

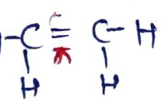
Higher energy state.
σ* - alpha antibonding
π* - pie antibonding
n* - Non bonding.

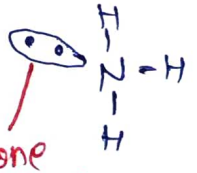


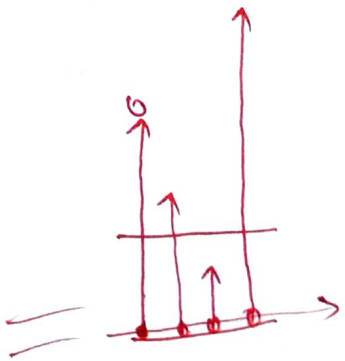


Examples

σ bond - CH_3 → 

π bond - $CH_2=CH_2$ → 

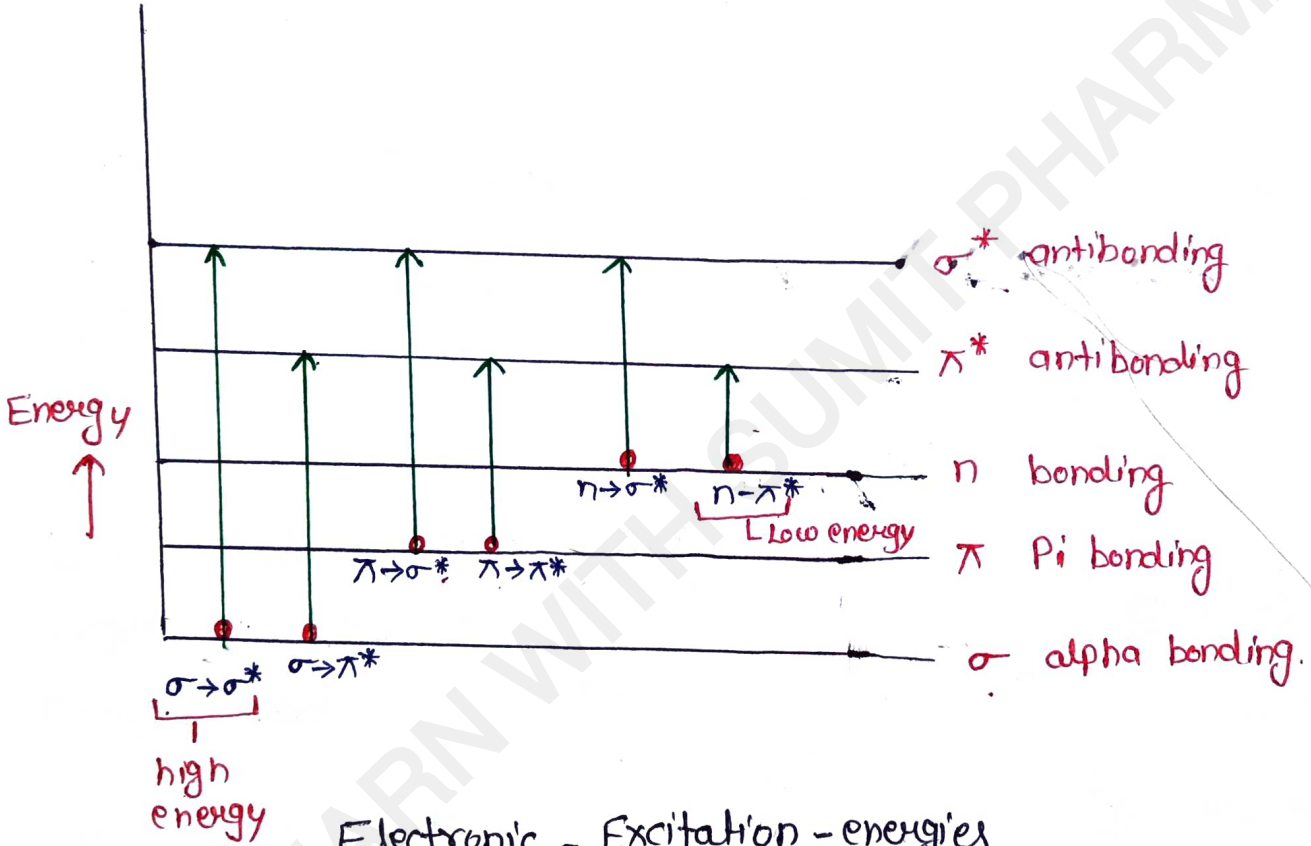
n - NH_3 → 



Types of e Transition →

4 types →

① $\sigma \rightarrow \sigma^*$ transition
② $\pi \rightarrow \pi^*$ transition
③ $n \rightarrow \sigma^*$ transition
④ $n \rightarrow \pi^*$ transition.



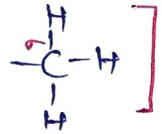
Electronic - Excitation - energies

$[n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \sigma^* < \sigma \rightarrow \sigma^*]$

① $\sigma \rightarrow \sigma^*$

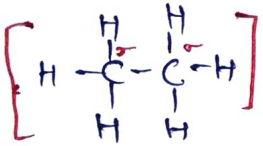
- High energy absorb molecules
- Low wavelength

freq. ∝

ex → CH_4 → 

C_2H_6

↓



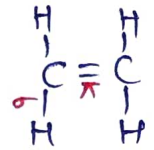
- Those compound which have single bond (σ) having high energy absorbing capacity.

wavelength = 125 nm

(wavelength of UV = 200-400 nm)

② $\sigma \rightarrow \pi^*$

ex → $CH_2 = CH_2$



- Compounds having 1 π bond and 1 σ bond.

wavelength = 180 nm

③ $\pi \rightarrow \sigma^*$

ex → $CH_2 = CH_2$

wavelength = 170 nm.

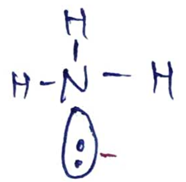
④ $\pi - \pi^*$

ex → ① $H-C \equiv C-H$ ③ $C \equiv C-C \equiv C$ (di-ene)

② 

wavelength = 205 nm.

(5) $n \rightarrow \sigma^*$

ex →  amonia

wavelength = 250 nm

(6) $n \rightarrow \pi^*$

ex 

wavelength = 300 nm

UNIT-1 UV-Visible Spectroscopy

Lecture-4 Chromophore & Auxochrome

① Chromophore

Definition →

chromophores are those molecules which shows the colour after absorption of light are known as chromophores.

ex →

① The compounds which have = bond, ≡ bond and multiple bonds. [C=C, C=O, C≡N] they absorb light upto 200 nm.

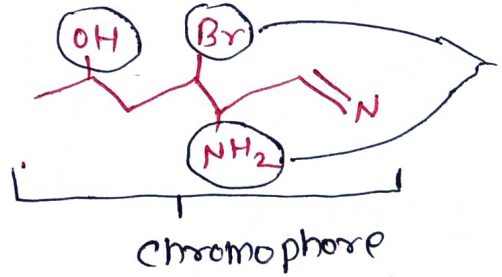
② non-conjugated di-enes, absorbs light less than 200 nm [C=C-C-C=C]

③ Conjugated di-enes absorb the light upto 200 nm. [C=C-C=C-C]

② Auxochrome

- Auxochromes are those functional group or elements which produce the colour inside the chromophore are known as Auxochromes.

ex →



→  → 255 nm

→  → 270nm

→  → 280 nm

VISITOR

Unit-1 UV-visible Spectroscopy ✓

Lecture-5 **Absorption and Intensity Shift**

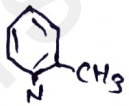
Definitions

① **Hyperchromic Shift**

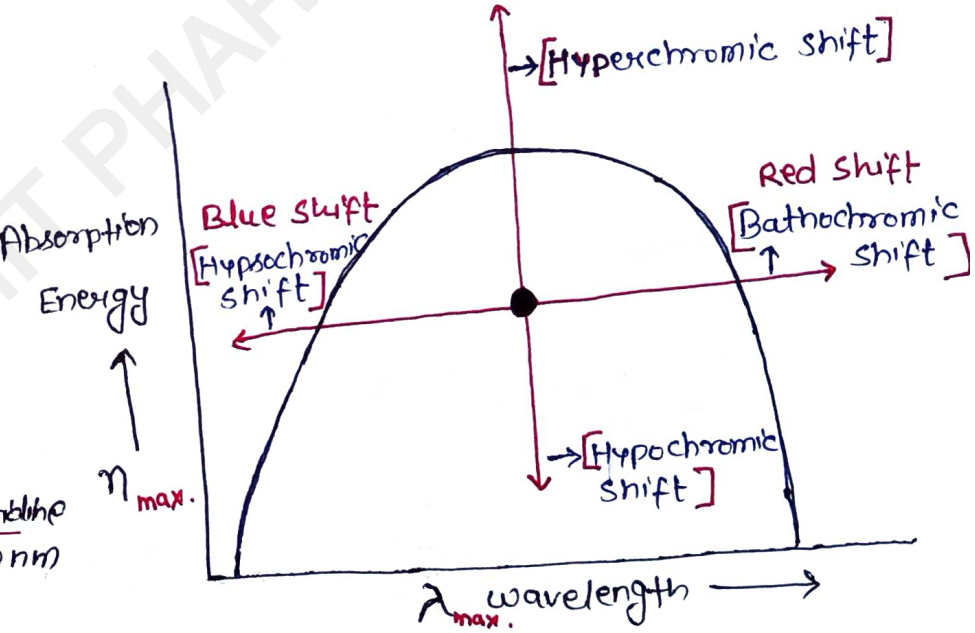
- when intensity of absorption is increased ↑ from low to high is called Hyperchromic shift.

ex →

Pyridine
$\lambda_{max.}$ = 257 nm



2-methyl Pyridine
$\lambda_{max.}$ = 260 nm

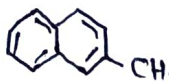


② **Hypochromic Shift**

- when intensity of absorption is decreased ↓ is called Hypochromic shift.

ex →

Naphthlene
ε = 19000



2-methyl naphthlene
ε = 10250

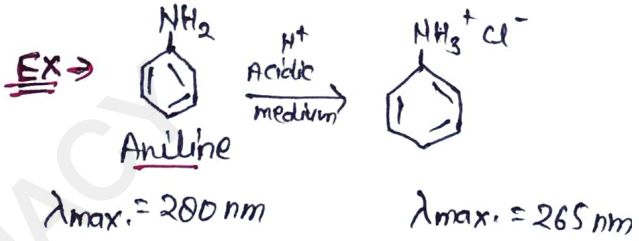
③ **Bathochromic Shift**

- when the wavelength of absorption is increased ↑ is called Bathochromic Shift.

ex → An Auxochrome group like -OH, $-OCH_3$ causes absorption of compound at longer wavelength.

(4) Hypsochromic Shift

- when the wavelength of absorption is decreased ↓ is called Hypsochromic Shift.

Ex → 

- Bathochromic Shift is also known as Red Shift ✓
- Hypsochromic Shift is also known as Blue Shift ✓

INSTRUMENTAL METHOD OF ANAYSIS

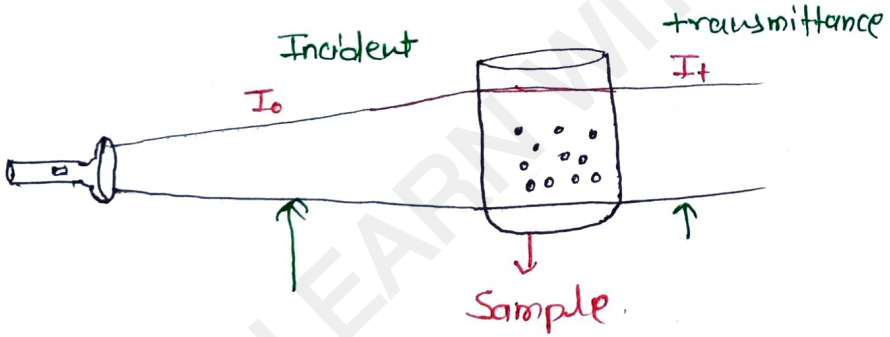
B-Pharm 7th sem

UNIT - I

Lecture - 2

Topic → Principle of UV spectrophotometer or Beer's Lambert Law

- The principle of UV-Spectrophotometer is based on the absorption Spectrum and it is based on the principle of Beer's Lamberts Law.



Absorption = $I_0 - I_T$

Beer's Lamberts Law

- Beer and Lambert both are diff. Scientists and laws are also different but we will study both laws together so this is called Beer's Lamberts law.

First we will discuss both laws seperately →

① Beer's Law

- According to Beer's law the absorption of any monochromatic light is

directly proportional to the concentration of the sample.

$A \propto C$ —— (1)

Absorption ∝ Conc^n

(2) Lambert Law

- According to Lambert, the decrease in intensity of light is directly proportional to the path length travelled by the light

$A \propto L$ —— (2)

Absorption ∝ Path Length

→ The absorption of light is directly proportional to the path length travelled by light.

Beer's Lamberts law

Beer's Law – $A \propto C$ – (1)

Lambert Law – $A \propto L$ – (2)

combine eq. (1) and (2) –

$A \propto C \cdot L$

$A = \varepsilon CL$

where, C = Conc^n

L = path Length

ε = absorption coefficient.

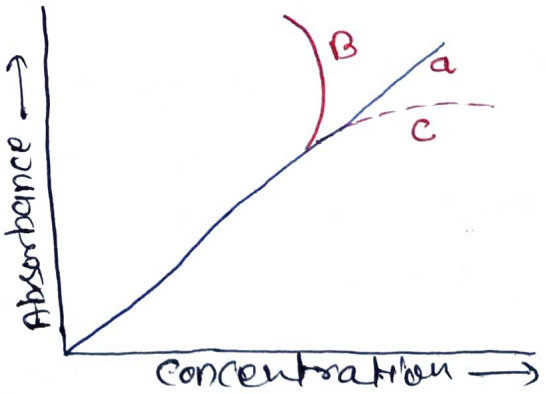
Some error occurs

Deviation of Beer Lambert Law

- Deviation from the law may be positive or negative according to at whether the resulting curve is concave upwards.
- The deviation from the Beer's law may be due to interaction of the solute molecules with each other or with solvent or may be due to instrumental factors.
  - Some of deviation are-

① Real deviation

- At high conc^{n}, the substance absorbed more readily than the equation requires.
- The real deviation describes the absorption behavior of dilute solution only.
- It is regards as limiting law.



- At high conc^{n} the average distance b/w the spicies responsible for absorption.
- This interaction is capable of interacting their ability to absorb a given wavelength of radiation.
- As degree of interaction depends upon the conc^{n}. , the occurrence of this phenomenon causes deviation from the linear relationship b/w absorbance conc^{n}.

② Chemical deviation

- The absorbance may changes with conc^{n} because of effects such as hydrolysis association, polymerization, ionization hydrogen bonding etc.
- Deviation from beer's law and frequently encountered as a consequence of association, dissociation or reaction of the absorbing spicies with the solvent.

ⓐ Dissociation of molecule :- The dissociation of polymeric acohol - increases as the solution is diluted. The

monomer indicate negative absorption or deviation deviation, while polymer shows positive deviation from the beer's law.

(b) Association of molecule :- Conjugated particles will cause turbidity i.e scattering of the beam of radiation scattering out of the beam will give an apparent increases of absorption scattering absorption.

(c) Incomplete reaction of Substance :- It forms inter mediate substance causes diff. colours and change the absorption max. of substance.

(d) Change in pH :- As pH of solvent system is directly proportional to the absorbance at high pH (or for basic) sub. Absorbance alter towards shorter wavelength and vice versa.

(e) Presence of impurities :- The presence of impurities that fluoresces or absorb at the required wavelength may also cause deviation from the Beer's Law.

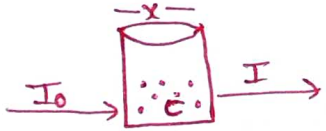
BEER - LAMBERT LAW (Long 5/7 marks) (detail explained)

① BEER's Law

- Beers law states that "the intensity of a beam of monochromatic light decreases, exponentially, with increase in the concentration of absorbing species arithmetically.

Accordingly,

$$-\frac{dI}{dx} \propto K'Ic$$



where, C = Conc^n of solution in moles litre^{-1}
K' = molar absorption coefficient.
x = thickness of medium.

Suppose I_0 is the intensity of the radiation before entering the absorbing solution ($x = 0$)

I is the intensity of radiation passing through thickness x.

$$-\int_{I_0}^{I} \frac{dI}{I} = \int_{x=0}^{x=x} k'C\,dx$$

$$\frac{I}{I_0} = e^{-k'Cx}$$

or

$$\boxed{I = I_0\, 10^{-KCx}}$$

where $K = \frac{k'}{2.303}$ known as molar extinction coefficient of absorbing solution.

Lambert's Law

when a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of light.

$$\boxed{-\frac{dI}{dt} \propto I} \quad \text{or} \quad \boxed{-\frac{dI}{dt} = KI}$$

where, I = Intensity of incident light of wavelength λ

t = thickness of medium.

K = proportionality factor.

So by putting $I = I_0$ when $t = 0$

$$\log_n \frac{I_0}{I_t} = kt$$

$$I_t = I_0 e^{-Kt} \quad \text{— (2)}$$

where, I_0 = intensity of incident light
I_t = intensity of transmitted light

On enhancing eq-(2) to natural log-

$$[I_t = I_0 10^{-Kt}]$$

where, $K = K/2.3026$

(absorption coefficient)

"defined as reciprocal of the thickness which is required to reduce the light to 1/10 of its intensity."

$$\left[\frac{I_t}{I_0} = 0.1\right]$$

- The ratio of $\log I_0/I$ is termed as absorbance A.

$$A = \log \frac{I_0}{I_t}$$

and ratio I_0/I_t is termed as transmittance T.

BEER-LAMBERT LAW

on combining both laws-

$$\log \frac{I_0}{I} = KK'cx$$

$$\left[\log \frac{I_0}{I} = \varepsilon cx = A\right]$$

where, I_0 = Intensity of Incident light

I = Intensity of transmitted light.

c = Concentration of solution in moles/litre

x = Path length of the sample (usually 1 cm)

ε = molar extinction coefficient.

A = Absorbance.

$$A = \varepsilon c x = \log \frac{I_0}{I}$$

$$= \log 1/T$$

$$= \boxed{-\log T}$$

Solvent effect on absorption spectra

- A most suitable solvent is one that does not itself absorb in the region under investigation, so a dilute solution of sample is prepared for spectral analysis.
- Most commonly used solvent is 95% ethanol.
- Other solvents which are transparent above 210 mμ are n-hexone, methyl alcohol, cyclohexane, acetonitrile etc.

Solvents with upper wavelength limit

Solvent	λ of absorption (mμ)
• Ethanol, Hexane, methanol, cyclohexane, Diethylether	210
• water	205
• Benzene	280
• Chloroform	245
• Tetrahydro furan	220
• Carbon Tetrachloride	265

- Hexane and other hydrocarbons can be used because less polar and least interaction with molecule under investigation.
- For UV spectroscopy: ethanol, water and cyclohexane are best.

- The position as well as intensity of absorption get shifted as we change the polarity of solvent.
- α, β unsaturated carbonyl compounds shows two diff. shifts.

(i) $n \rightarrow \pi^*$ transition (less intense)

In such case → absorption band moves to shorter wavelength by increasing polarity of the solvent.

- In this transition ground state is more polar than excited state.
- For example - λmax. of acetone is at 270 mμ in hexane and in water λ max. 264 mμ.

(ii) $\pi \rightarrow \pi^*$ transition (intense)

In such case → absorption band moves to longer wavelength by increasing polarity of solvent.

- For example - value of absorption max. in ethanol will be

greater than observed in hexane.

(iii) $n \rightarrow \sigma^*$ transitions

Very sensitive to hydrogen bonding (alcohol and amines form hydrogen bonding with solvent molecule) occurs due to presence of non-bonding electrons on hetro atoms.

So transition requires greater energy.

So we say that :-

(a) when a group (carbonyl) is more polar in ground state than excited state then stability increasing polarity of the solvent stabilises the non-bonding electrons in ground state due to hydrogen bonding.

So absorption shifted to lower wavelength.

(b) when a group is more polar in excited state then absorption gets shifted to longer wavelength with increase in polarity of solvent which helps in

Stabilising the non-bonding electrons in the excited state.

So we can say that -

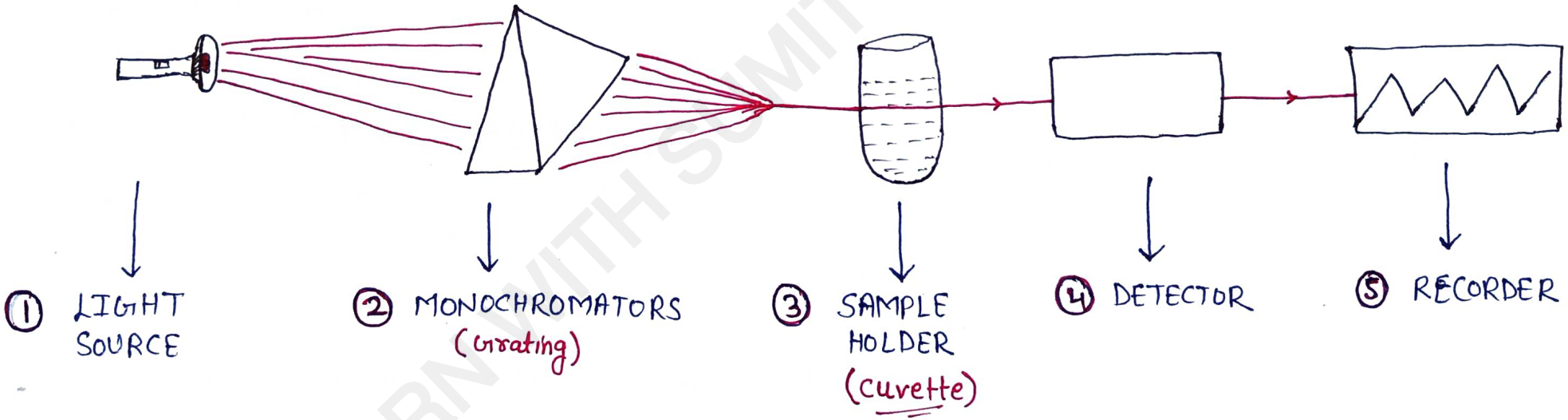
Increase in polarity of the solvent generally shift $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ bands to shorter wavelength and $\pi \rightarrow \pi^*$ bands to longer wavelength.

Unit-1 UV- visible Spectroscopy.

Lecture-6 **Instrumentation of UV Spectroscopy** ✓

Instrumentation of UV

The various components of a UV-Spectrometer are :-



① Source of Light

- ⓐ Hydrogen Discharge Lamp.
- ⓑ Xenon discharge Lamp.
- ⓒ mercury arc lamp.

② monochromators

- ⓐ Gratings

③ Sample holders / cuvettes

For Sample filling.

④ Detectors

- ⓐ Barrier layer cell / photovoltaic cell
- ⓑ Photo tubes / photo emissive tube.
- ⓒ Photomultiplier tube.

⑤ Recorder

- ⓐ Single beam UV Spectrophotometer.
- ⓑ Double beam UV Spectrophotometer.

① Source of Light

ⓐ Hydrogen discharge Lamp

- In Hydrogen discharge Lamp pair of electrodes is enclosed in a glass tube (provided with Silica or quartz window for UV radiation to pass through) filled with hydrogen gas.
- They are stable in nature.
- It gives radiation from 120-350 nm.

ⓑ Xenon Discharge Lamp

- it possesses two tungsten electrodes separated by some distance.
- These are enclosed in a glass tube (for visible) with quartz or fused silica and xenon gas at 10-30 atm. pressure is filled under pressure.
- This is a good source of continous plus additional intense radiation. Its intensity is higher than Hydrogen Discharge lamp.

© Mercury arc lamp

- In mercury arc lamp, mercury vapor is stored under high pressure and excitation of mercury atoms is done by electric discharge.

Demerit

- Not suitable for continuous spectral studies (because it doesn't give continuous radiations).

(2) Monochromators

@ Grating

- Grating are most effective one in converting a polychromatic light to monochromatic light.
- As a resolution of +/- 0.1nm could be achieved by using gratings., they are commonly used in spectrophotometers.

- Gratings are of two types -

① Diffraction grating ② Transmission Grating.

1. Diffraction grating

- Most refined dispersion of light is obtained by means of diffraction gratings.
- These consist of large number of parallel lines (grooves) about 15000/30000/inch is ruled on highly polished surface of glass, quartz or alkyl halides.

2. Transmission grating

- It is similar to diffraction grating but refraction takes place instead of reflection.
- Refraction produces reinforcement.
- This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

③ Sample Holders / cuvettes

- The cells or cuvettes are used for handling liquid samples.
- The cell may either be rectangular or cylindrical in nature.
- For study in UV region: the cells are prepared for quartz or fused silica whereas colour corrected fused glass is used for visible region.
- The surfaces of absorption cells must be kept scrupulously clean. No fingerprints or blotches should be present on cells.
- Cleaning is carried out washing with distilled water or with dilute alcohol, acetone.

④ Detectors

- Device which converts light energy into electrical signals, that are displayed on readout devices.
- The transmitted radiations falls on the detector which determines

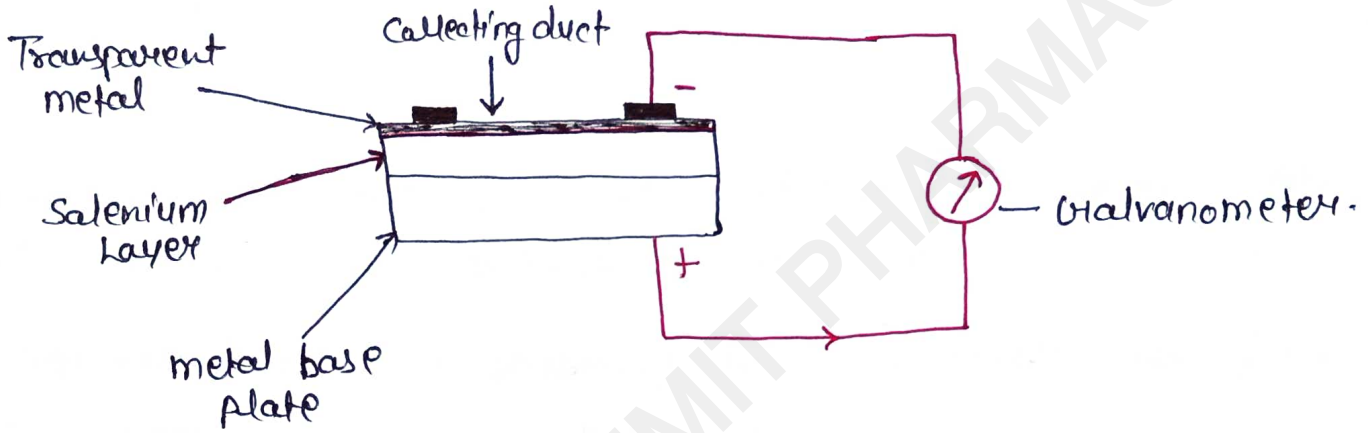
the intensity of radiation absorbed by sample.

Following types of detectors -

1. Barrier layer cell/photovoltaic cell

- The detector has a thin metallic layer coated with silver or gold and acts as an electrode.
- It also has a metal base plate which acts as another electrode.
- These two layer are separated by a semiconductor layer of selenium.
- when light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer.
- This creates a potential diff. b/w two electrodes and causes the flow of current.
- when it is connected to galvanometer, a flow of current observed which is proportional to the intensity and

wavelength of light falling on it.



2. Photo tubes / Photoemissive Tubes

- Consists of a evacuated glass tube with a photo cathode and a collector anode.
- The surface of photoelectrode is coated with a layer of elements like cesium, silver oxide or mixture of them.
- When radient energy falls on photo sensitive cathode, electrons are emitted which are attracted to anode causing current to flow.

- more sensitive compared to barrier layer cell and therefore, widely used.

3. Photo multiplier tubes

- The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.
- In a vaccum tube, a primary photo-cathode is fixed which receives radiation from the sample.
- Some 8-10 dynodes are fixed each with increasing potential of 75-100V higher than preceding one.
- Near the last dynode is fixed an anode or electron collector electrode.
- photo-multiplier is extremely sensitive to light and is best suited where weaker or low radiation is received.

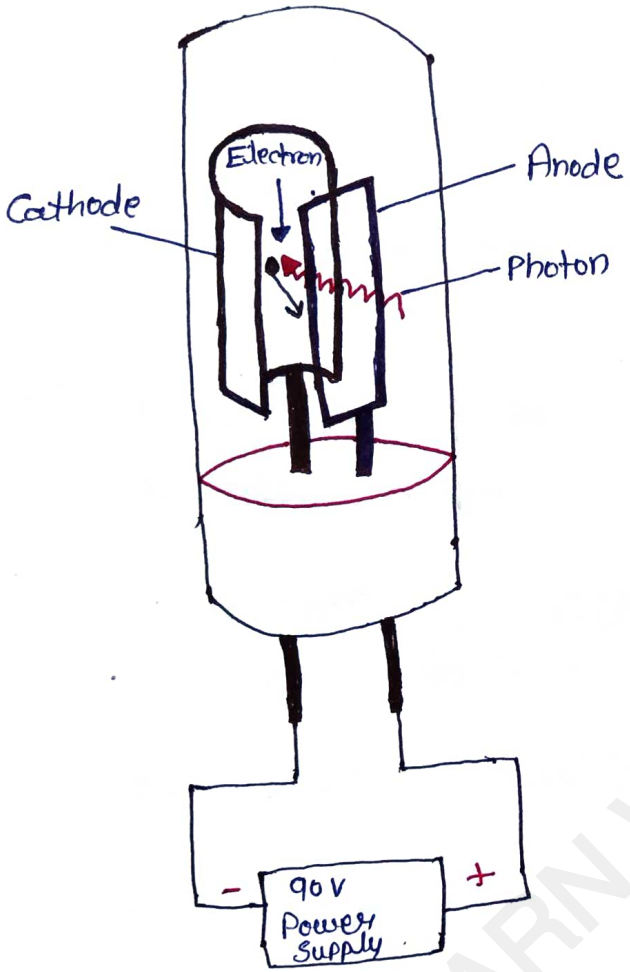
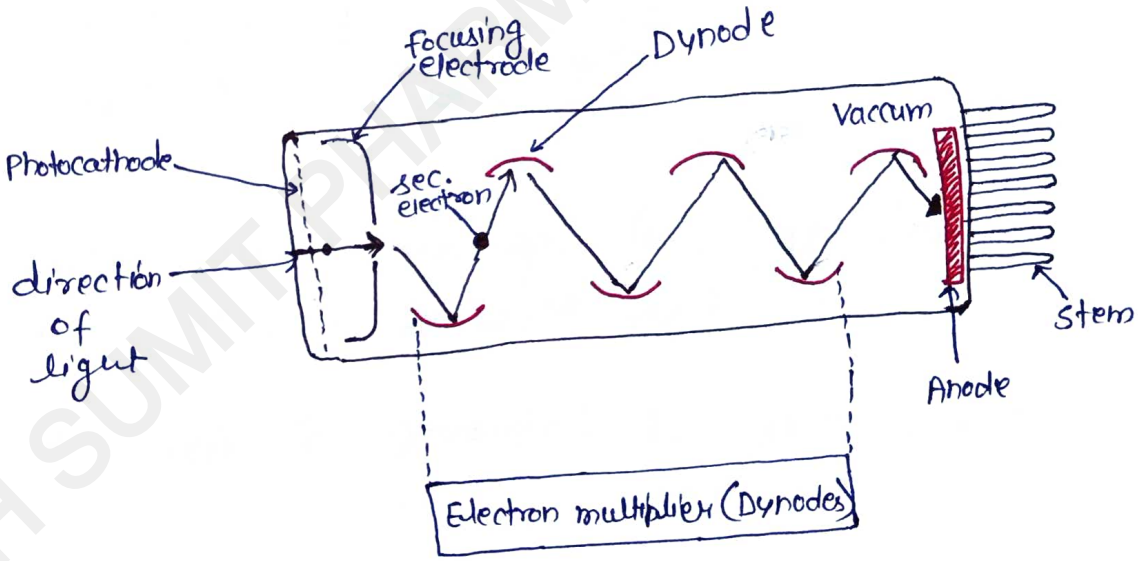


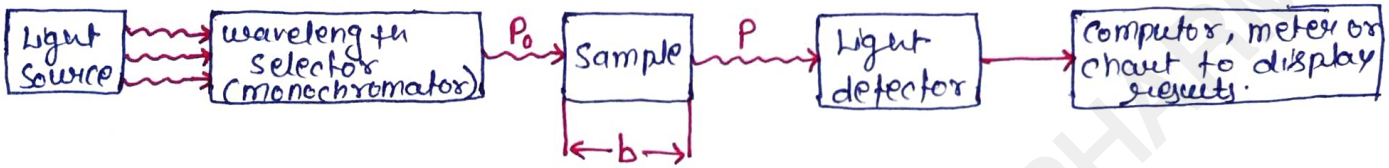
Photo tube / photo emmisive tube



Photomultiplier tubes

INSTRUMENTS

① Single beam Spectrophotometer



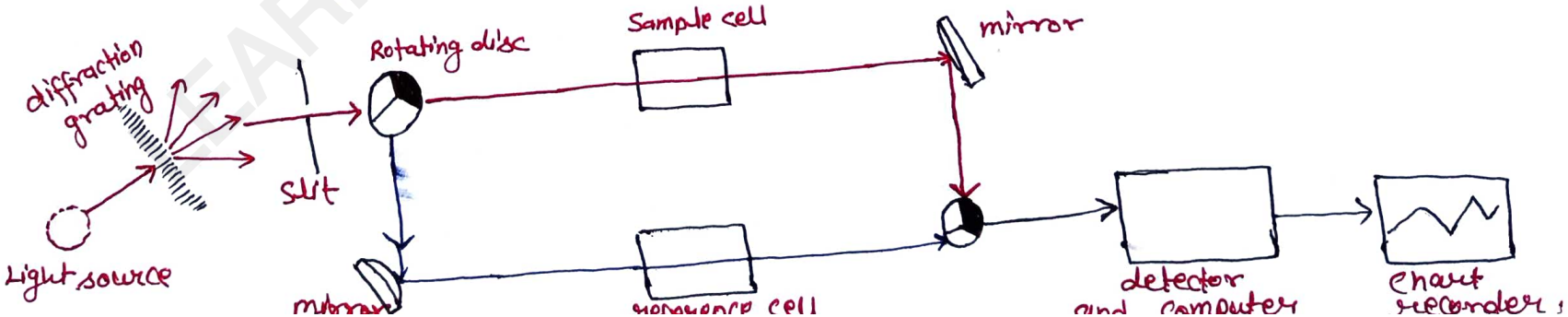
- Light from the source is carried through lens and/or through aperture to pass through a suitable filter.
- The type of filter to be used is governed by the colour of the solution.
- The sample solution to be analysed is placed in cuvettes.
- After passing through the solution, the light strikes the surface of detector (barrier-layer cell or phototube) and produces electrical current.
- The output of current is measured by the deflection of needle of light-spot galvanometer or micro ammeter.

- This meter is calibrated in terms of transmittance as well as optical density. The readings of solution of both standard and unknown are recorded in optical density units after adjusting instruments to a reagent blank.

(2) Double Beam UV-Spectrophotometer

- Double Beam instrument is the one in which two beams are formed in the space by a U-shaped mirror called as beam splitter or beam chopper.

- Chopper is a device consisting of a circular disc. one third of disc is opaque and one third is transparent, remaining one third is mirrored. It splits the monochromatic beam of light into two beams of equal intensities.



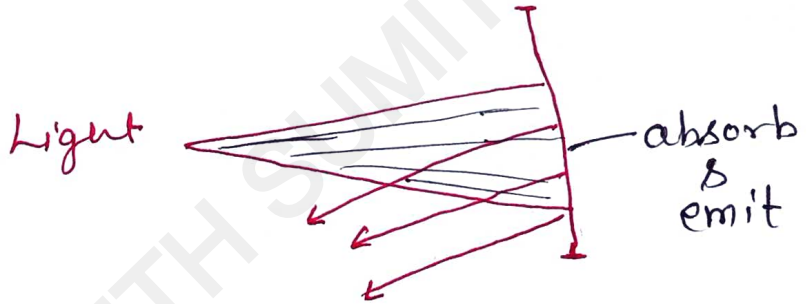
Applications of UV spectroscopy

- it is used in determination of molecular weight of a molecule.
- It is used in determination of impurities present in the sample.
- The unknown conc^{n}. of a solution can be determined using this spectroscopy.
- It is used in characterisation of aromatic compound and in detection of conjugation.

unit-1 chapter-2 **FLUORIMETRY**

→ Fluorescence

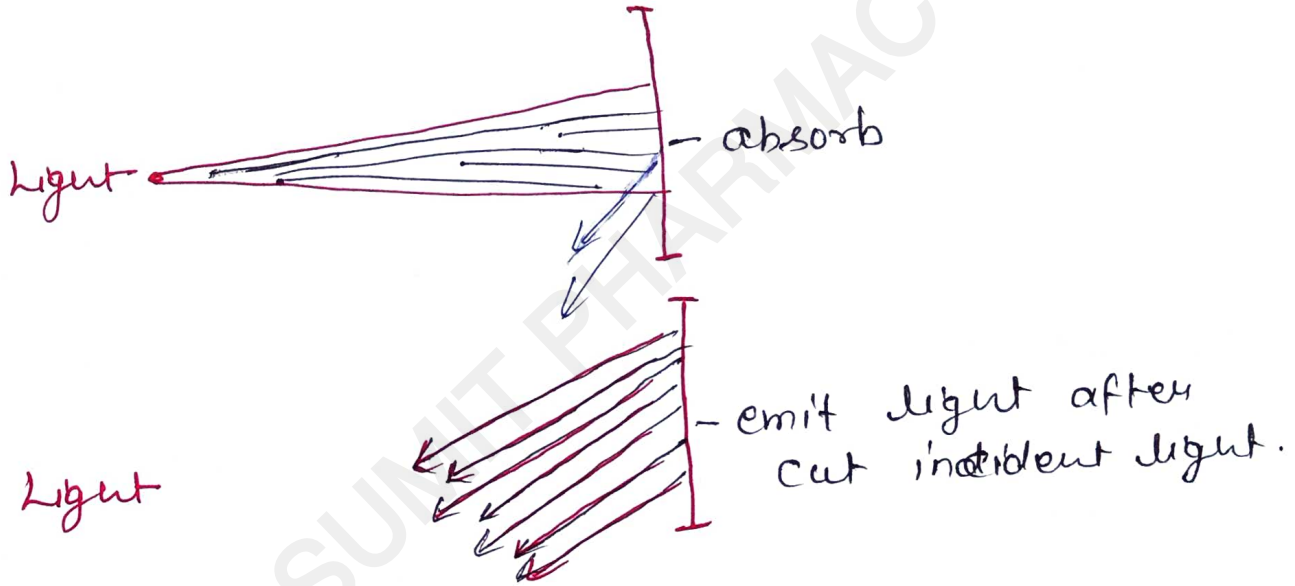
- When a beam of light is incident on certain substances, they emit visible light or radiations.
- This phenomenon is known as fluoroscence and the substances showing this phenomenon are known as fluorescent substances.



→ phosphorescence

- when a beam of light is incident on certain subs., they emit light continuosly even after the incident light is cut off.
- This type of delayed flowrescence is called phosphorescence.

and the substances are called phosphorescent substances.



→ **Flowrimetry Analysis**

- The process or phenomina in which we find out the concentration of any unknown sample with the help of flowriscence and phosphoriscence phenomina, this is called flowrimetry Analysis.

⇒ Principle of flowrimetry Analysis

To understand principle involved in flowrimetry we have to know following basic electronic states as mentioned below -

① Singlet ground state

- A state in which all the electrons in a molecule are paired (↑ or ↓)

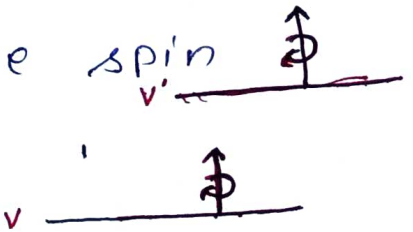
② Doublet state

- A state in which an unpaired electron is present.

e.g. Free radical (↑ or ↓)

③ Triplet state

- A state in which unpaired electrons of same spin are present, (unpaired and same spin) (↑↑)



④ Singlet excited state

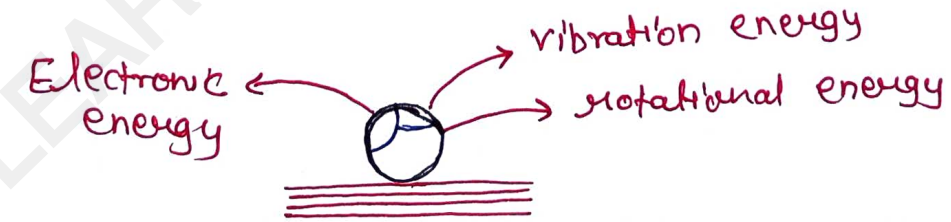
- A state in which electrons are unpaired but of opposite

Spin like (unpaired and opposite spin) (↑↓)

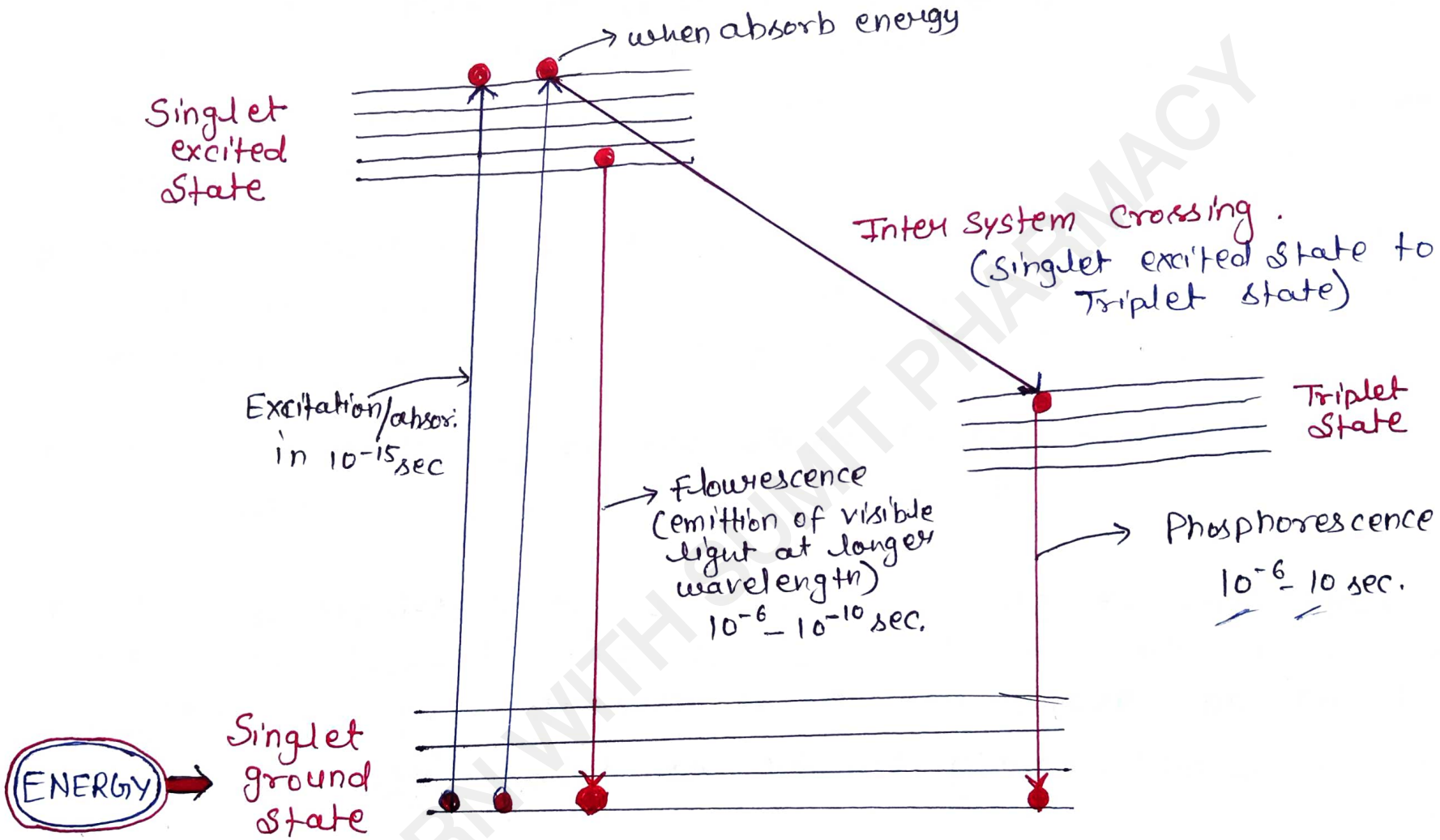
⇒ Absorption of UV/visible radiation causes transition from singlet ground state to singlet excited state. As this excited state is not stable, it emits the excess energy and return to ground state.

⇒ Flourescence is the phenomina of emission of radiation when there is transition from singlet excited state to singlet ground state.

Excitation wavelength → wavelength of absorbed radiation.
Emmission wavelength → wavelength of emitted radiation.



Molecule at ground state having three types of energy.



Theory →

↦ when molicules are irradiated by light of the suitable wavelength, it will absorbed and the molecules more

from ground state to first exited singlet electronic state, as a result of absorption.

→ From the excited singlet state, the following phenomenon takes place -

- Due to unstability of excited singlet state, the excited molecules will return to the ground state by collisional deactivation without emitting any radiation.
- The molecules in excited singlet state may emit radiations as UV or visible light photon (Flourescence).
- The molicules with relatively stable excited state may undergo transition to a metastable triplet state and after sometime return to ground state by emmittion of an ultraviolet or visible photon. (Phosphorescence).

⇒ The process of crossing from a singlet state to a triplet state is called as Intersystem crossing.

Unit-1 Chapter-2 Flourimetry

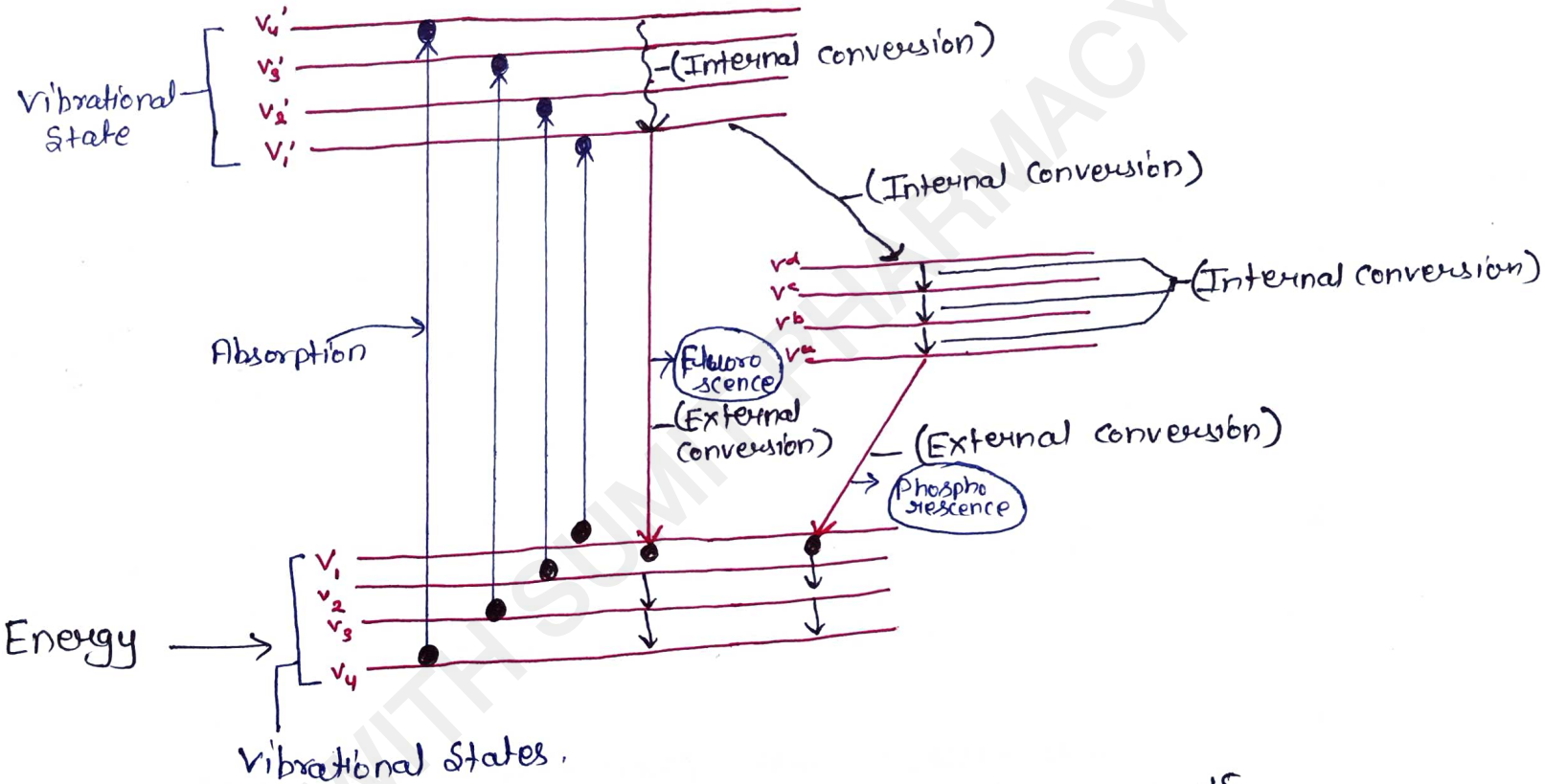
Lecture-2 Internal and External Conversion.

① Internal Conversion

- Internal conversions is a transition of $\bar{e}$ from higher to a lower electronic state in a molecule or atom.
- It is also called "Radiationless de-excitation" because no photons are emitted.
- It is non radiative process that allows the molecules to return to the ground state by Vibrational, relaxation or other relaxation process.

② External Conversion

- It is non-Radiative process that allows the molecule to return the ground state as a result-of collision with solvent molecules.



Time taken to comp. following operations

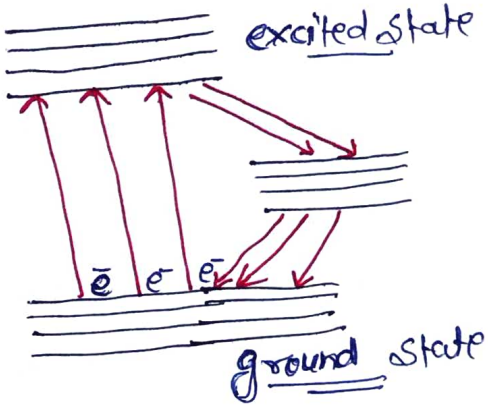
- Absorption – 10^{-15} sec
- fluorescence – 10^{-10} – 10^{-7} sec
- Phosphorescence – 10^{-6} – 20 sec
- Internal conv. – 10^{-11} – 10^{-9} sec.
- Vibrational Relax. – 10^{-12} – 10^{-10} sec
- Intersystem crossing – 10^{-10} – 10^{-8} sec.

Unit-1 Chapter-2 - Fluorimetry

Lecture-3 - molecular structure & fluorescence

① Absorbency - e^- pair

- The compounds should have lone pair e^- or free e^-, they shows the absorbency and flowrescence.

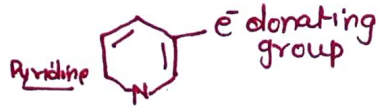


② =(double bond) Resonance

- The compounds which have π bonds in alternate manner are shows the resonance.
- They also shows the absorbency and fluorescence.

③ Hetrocyclic - e^- donating group

- Some Hetrocyclic compounds also showing Fluorescence but on one condition they have a e^- donating group.



ex- $-NH_2$, OH, OCH_3

④ Electron withdrawing group

- If we attach a e^- withdrawing group with that compound which shows flouresence, then due to e^- withdrawing group the fluorescence property will be decreases ↓.

e^- withdrawing groups → COOH, NO_2, N≡N, Cl, Br etc.

⑤ Polycyclic Compounds

- polycyclic compounds like Vit. K, nucleotides, Vit. A are also shows the property of fluoroseence because they contain some free e^-.

⑥ metal chelate

- Some molicules are making chelate by coordinating with metals, due to this the molicules became rugid and due to rugidity they shows the property of fluorescence.

Chelate → rugid → free e^-

⑦ Chromophores position → Chromophores are those compounds which shows the colour after absorption of light.

- If we attach chromophores with some compounds then

they also shows property of fluorescence.

(8) Ionisation

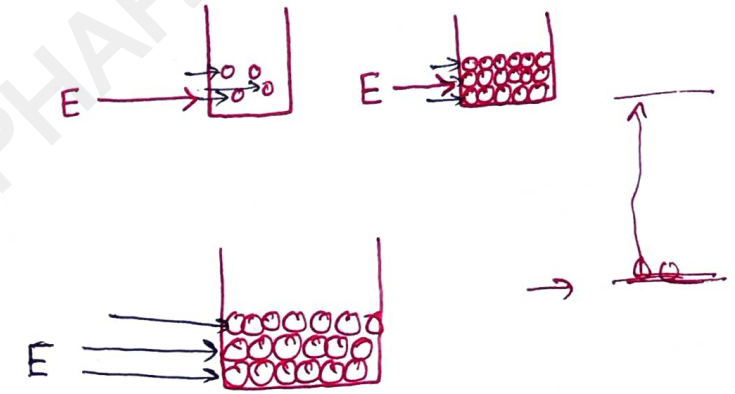
- Due to ionisation, the compounds shows the property of fluorescence.
- because ionisation increases the resonance which leads to increase in free e^-

Unit-1 Chapter-2 FLUOROMETRY

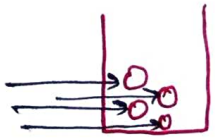
Lecture-4 Factors Affecting Fluorescence

① Concentration – Conc^{n} $\propto \frac{1}{\text{Fluorescence}}$

- If the conc^{n} of particles increased in the solution then all particles can not absorb the radiation only few particles absorbs the radiation and they shows the fluorescence and other particles are remain in the ground state.



- If the conc^{n} of particles decreased in the solution so they can easily vibrate and go to the excited state from ground state because of sufficient space.



② Oxygen [Oxygen $\propto \frac{1}{F}$]

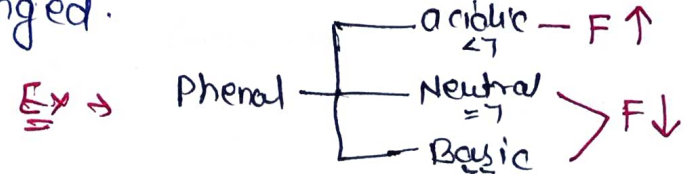
- When molecules are present in exposure of oxygen, then the molecules are oxidised and due to oxidation, the $\bar{e}$ density is decreased, so their fluorescence intensity is decreased.

③ Photo decomposition [P.D $\propto \frac{1}{F}$]

- For the fluorescence a particular intensity and wavelength light is required.
- If the intensity and wavelength is much high, it causes decomposition of molecules which is called photo decomposition and it decreases fluorescense.

④ PH

- It is to be expected that alteration of pH of a solution have a significant effect on fluorescence if the absorption curve of the solute is changed.



⑤ Temperature & viscosity

$\left[\text{Temp} \propto \frac{1}{F}\right]$ $\left[\text{viscosity} \propto \frac{1}{F}\right]$

- If we increase the temp. of solution the molecules are decomposes. and fluorescence will decreased.
- If we increase the viscosity of the solvent, then molecules are trapped inside and can't vibrate., because of this fluorescence will be decreased.

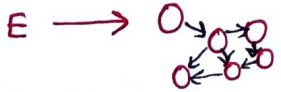
⑥ Impurities

- If any type of impurity are present in the Solution then the property of fluorescence will be decreased because some of the radiation are absorbed by the impurity.

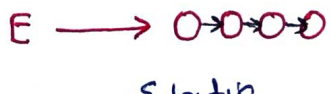
ex → Iodine Impurity.

⑦ Chemical Quenching

- Collision
- Static



Collision Quenching.



Static Quenching

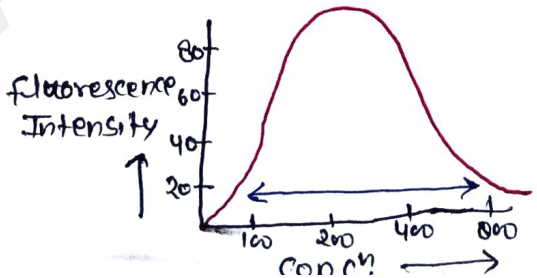
FLUORESCENCE QUENCHING

- Decrease in fluorescence intensity is known as fluorescence Quenching.
- It may occur due to change in concentration, PH, Temp., viscosity etc.

Types of Quenching
- Self Quenching / concentration Quench.
- Chemical Quenching
- Static Quenching
- Collision Quenching.

① Self Quenching / conc^n Quenching

- it occurs when the conc^n of fluoresencing molecules increase in a simple solution. The fluorescence intensity is reduced in highly conc^n solution.

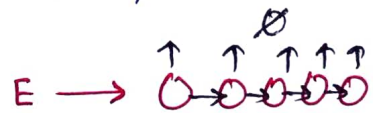


② Chemical Quenching

- Fluorescence intensity decreased due to the factors like change in pH, presence of oxygen, halides & heavy metals.
- Aniline at pH 5–13 gives fluorescence but at <5 & >13 it does not exhibit fluorescence.
- Halides like chloride, Bromide, Iodide & e^- withdrawing groups like NO_2, COOH etc. leads to Quenching.

③ Static Quenching

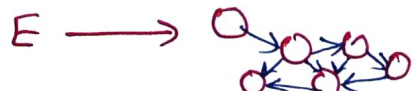
- It occurs due to complex formation quenching of fluorescence.



e.g → caffine shows Quenching & reduce fluorescence of Riboflavin.

④ Collision Quenching

- It occurs due to increase in Collision.



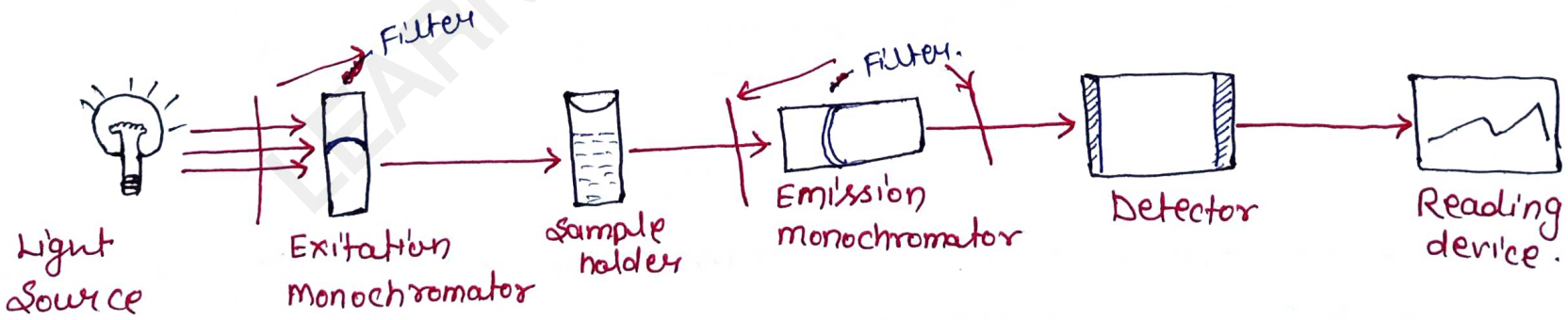
Unit-1 Chapter-2 FLUORIMETRY

Lecture-5 Instrumentation of fluorimetry

Instrumentation of fluorimetry

It contains 4 main components -

1. Source of Light (Lamp)
2. Filters and monochromators,
3. Sample cells,
4. Detectors (photomultiplier tubes)



① Source of Light (lamp)

ⓐ mercury arc lamp

mercury vapours at high pressure gives intense lines on continuous background above 350 nm. at low pressure mercury vapour gives an additional line at 254 nm.

ⓑ Xenon arc lamp

Produce intense radiation on passing current through spectrum is continuous over the range b/w 250-600 nm peak intensity about 470 nm.

ⓒ Tungsten Lamp

It is low intensity lamp produce radiation of visible region (doesn't offer UV radiation)

② Filters and monochromators

ⓐ Filters These are optical filters work on the principle of absorption of unwanted light and transmitting

the required wavelength of light.

(i) Primary filters

Absorbs visible radiation and transmit UV radiation.

(ii) Secondary filters

Absorbs UV radiation and transmit visible radiation.

(b) Monochromators

- They convert polychromatic light into monochromatic light.
- They isolate a specific or particular range of wavelength of radiation source.

(i) Excitation monochromators

- Provides suitable radiation for excitation of the molecule.

(ii) Emission monochromators

- Isolate only radiation emitted by the fluorescent molecules.

(C) Sample cells

- These are used for holding liquid samples made up of quartz or glass.
- Cylindrical or rectangular in shape 10mm or 1cm length.

(D) Detectors

- Converts light signals into electric signals.

3 types
- Photovaltic cell
- Photo tube
- photomultiplier Tubes (Best for fluorimetry) ✓

∵ [All types of detectors are discussed in spectrophotometer already.] ✓

APPLICATIONS OF FLUORIMETRY

① used in determination of Uranium (used extensively in the field of nuclear research).

② In general Inorganic ions do not show fluorescence. but some of them form fluorescent chelate with non-fluorescent organic molecules.

example

ⓐ used in determination of Ruthenium ion with 5-methyl 1-1, 10 Phenanthroline forms the complex ion which fluoresces strongly at pH 6.

ⓑ used in determination of aluminium (III) in alloys.
Alluminium (III) form complex with dye pantochrome at pH 4.8 which fluorensces strongly.

ⓒ Used in the estimation of traces of boron in steel by complex formation with benzoin.

ⓓ Cadmium can be estimated by precipitating with 2-(2-hydroxyphenyl) - benzoxazole in presence of tartrate.
complex on dissolving in glacial acetic acid give a bright blue fluorescence in UV light.

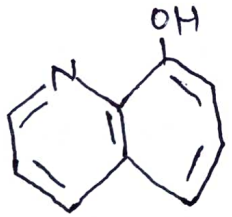
(e) Calcium can be estimated by fluorimetry with calcein solution.

(3) Fluorescent Indicators

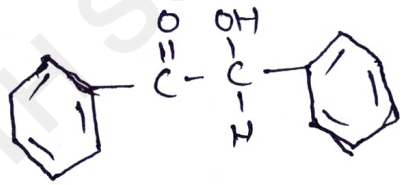
Intensity and colour of fluorescence of many substances depends on pH solution, so can be used in acid base titration (fluorosent indicators)

(4) Fluorometric Reagent ✓

Fluorometric reagent for cation analysis have aromatic structure with 2 or more donor functional groups that form chelate with metal ions.



(8-Hydroxy quinaline)



(Benzoin)

(5) In Determination of Vitamin B_1 and B_2.

(6) Organic analysis - it is used to carry out qualitative as well as quantitative analysis of many aromatic compounds in cigarette smoke, air, pollutant, concentrates, automobile exhausts.