UNIT - I

Pharmaceutical analysis :-

Pharmaceutical analysis plays a major role today. Pharmaceutical analysis derives its principle from various branches of science (chemistry, physics, microbiology, nuclear science, electronics etc).

Pharmaceutical analysis is apply mainly two areas / purpose -

i) Qualitative

ii) Quantitative

Pharmaceutical analytical techniques are categorised as -

a) spectral methods - Use light absorption / emission characteristic of drugs,

Ex - UV spectroscopy (absorbance)
IR " (Functional groups)
NHR "

b) Chromatographic method -
Based on affinity / Partition

Ex - Paper chromatography
TLC
HPLC.

c) Electro - analytical technique -
Based on electrochemical properties of the drug.

Ex - Potentiometry
Conductometry
Amperometry

d) Biological and microbiological method -
Based on animal and microorganism.

Ex - Biological assay of some antibiotics and vitamins.

e) Radioactive method - Based on radiation.

Ex - RIA (Radio - Immuno Assay) and other related techniques.

f) Physical method - Based on measurement of physical characteristics.

Ex - Differential thermal analysis (D.T.A)
Differential scanning calorimetry (DSC)
Thermal Mechanical analysis (T.M.A)
Thermal gravometric analysis (T.G.A)

g) Miscelloneous techniques - Based on some titration methods.

Ex - Polarimetric and some other titration.

Spectroscopy :-

Spectroscopy is the measurement and interpretation of electromagnetic radiation (EMR) absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state.

This change may be from ground state to excited state or excited state to ground state.

At ground state, the energy of a molecule is the sum of total rotational, vibrational and electronic energies.

In other words spectroscopy measures the changes in rotational, vibrational and electronic energies.

Electromagnetic Radiation (EMR) :-

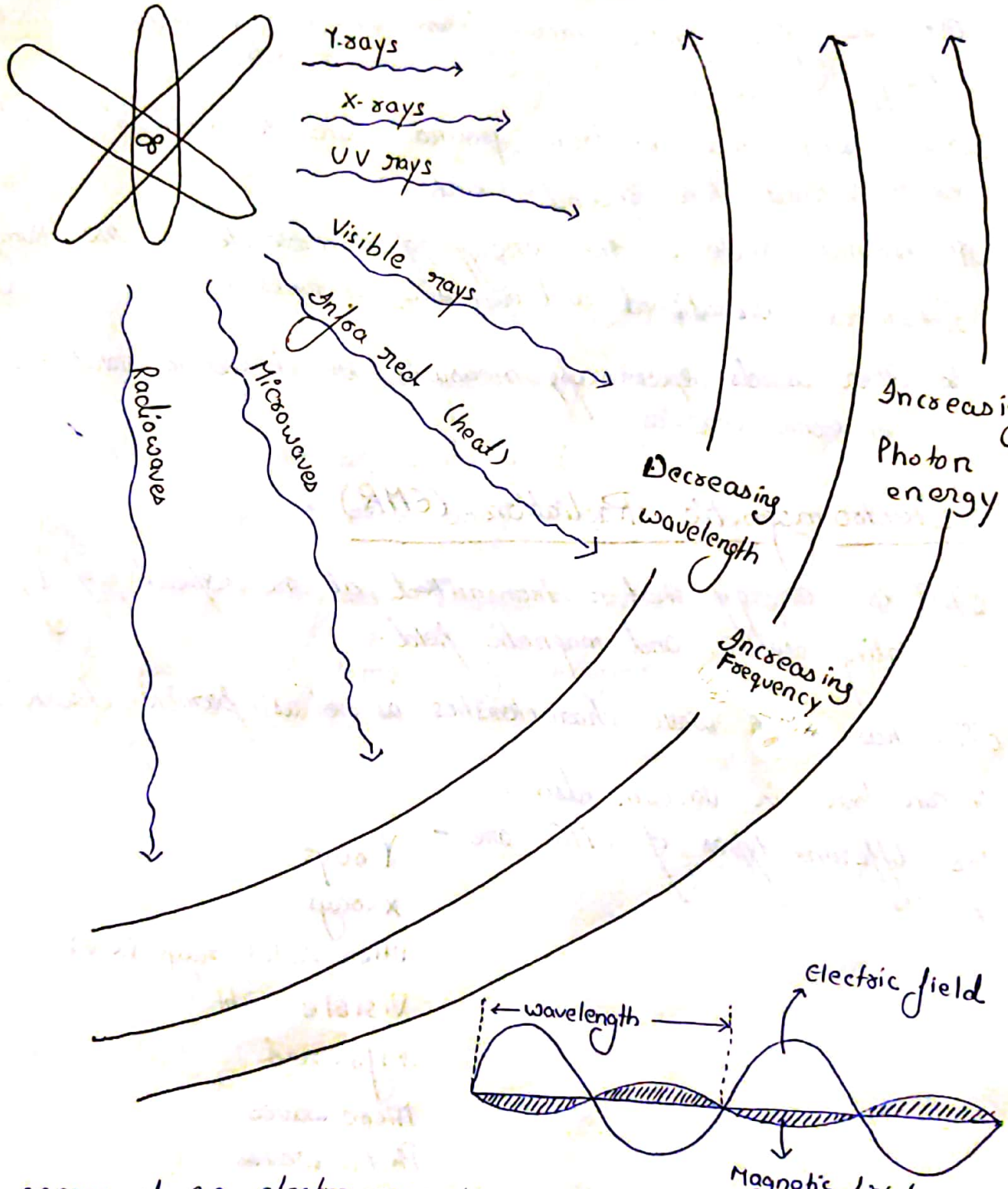
EMR is energy that is transmitted at the speed of light through oscillating electric and magnetic field.

EMR has both wave characteristics as well as particle characteristics.

It can travel in vaccum also.

The different types of EMR are -

- Y-rays
- X-rays
- ultra violet rays (UV)
- Visible light
- Infra red
- Micro waves
- Radio waves.



The energy of an electromagnetic radiation can be given by $E = h\nu$

where E = energy of radiation
h = Plank's constant (6.624×10^{-34} J-sec)
ν = frequency of radiation.

Frequency = c/λ = $\frac{\text{velocity of light in vacuum}}{\text{wave length}}$

$c = 3 \times 10^8 \, ms^{-1}$

Hence $\epsilon = h\nu = hc/\lambda = hc\bar{\nu}$

where $\bar{\nu}$ = wave number = $\frac{1}{\lambda}$

Therefore the energy of radiation depends upon frequency and wave length of the radiation.

Frequency :-

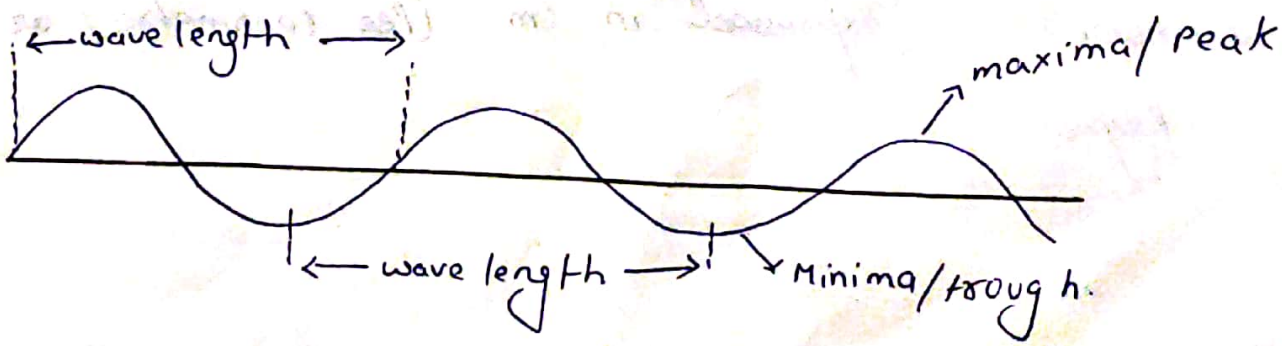
Frequency is the number of complete wave length units passing through a given point in unit time.

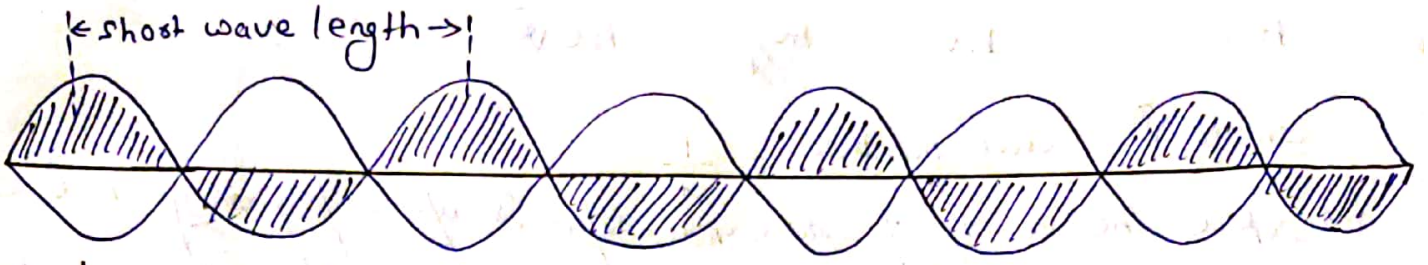
Unit - Hz (Hertz) or cps (cycle per second)

1 Mega Hz = 10^6 Hz
1 kilo Hz = 10^3 Hz
1 Foensel = 10^{12} Hz

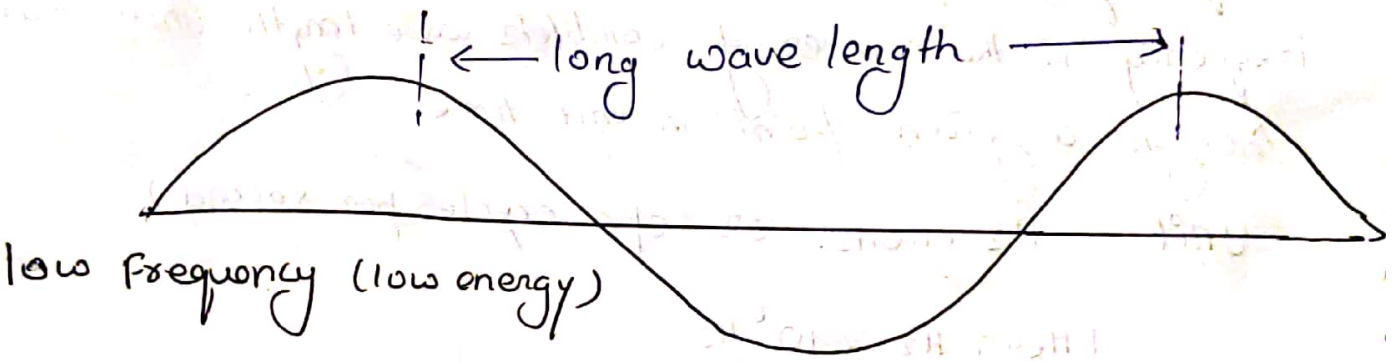
Wavelength :-

Wave length is the distance between two successive maxima or minima or distance between two successive troughs or peaks. Wave length can be measure in metres, cm, mm, km etc.





High frequency (High energy)



low frequency (low energy)

Wave number :-

It is the number of waves per centimetre

$$\bar{\nu} = \frac{1}{\lambda}$$

Wavenumber is expressed in cm^{-1} (Per centimetre) or kayser.

Type of Spectroscopy :-

The electromagnetic spectrum :-

The entire distribution of electromagnetic radiation according to frequency or wavelength

All these waves can undergo interference, be reflected, refracted, diffracted and polarized and travel at the speed of light ($2.998 \times 10^8 m s^{-1}$) in vaccum

Types of spectroscopy :-

1) Whether the study is made at atomic or molecular level :-

a) Atomic spectroscopy - where the changes in energy take place at atomic level.

Ex - Atomic absorption spectroscopy
- Flame photometry → (where either atomic absorption or atomic emission of radiation is being studied)

b) Molecular spectroscopy -
Where the changes in energy take place at molecular level.

Ex - • UV spectroscopy
- Colorimetry
- IR spectroscopy
- Fluorimetry (where the molecular absorption, emission or vibration is being studied).

2) Whether the study is based on absorption or emission of EMR :-

a) Absorption spectroscopy -
Where the absorption of radiation is being studied

Ex - • UV spectroscopy
- IR spectroscopy
- Atomic absorption spectroscopy
- Colorimetry

b) Emission spectroscopy -
Where emission of radiation is being studied

Ex - • Flame Photometry
- Fluorimetry

3) Whether the study is at electronic or magnetic levels :-

a) Electronic spectroscopy
Ex - UV spectroscopy, colorimetry, Fluorimetry - where the study

is done using electromagnetic radiation only (without the influence of magnetic field).

b) Magnetic spectroscopy -

Ex - NMR spectroscopy
ESR spectroscopy

where the study is done using EMR radiation under the influence of magnetic field.

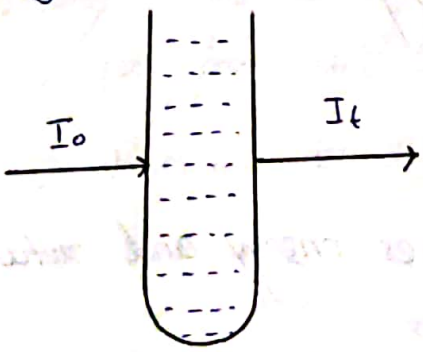
The energy of the molecule can be due to -

Rotational energy : Vibrational energy : Electronic energy = 1 : 100 : 10,000

When in EMR is passed onto a molecule, energy changes take place in a molecule / Atom which can be measure.

Theory of Spectroscopy :-

When EMR travels to a medium containing atom, molecules or ions, anyone of the following may take place.



- Intensity of emergent light (I_t) = Intensity of incident light (I_o)

i.e, $I_t = I_o$

Therefore, no absorption.
No change in energy takes place and no information about the molecule can be derived.

- Reflection, Refraction or scattering, where some studies like nephlometry or turbidimetry are being made.
- Intensity of emergent light < Intensity of incident light, where there is absorption of energy.

Here some information can be derived.

1) Excitation Process :-

Absorption of energy or light followed by transition from ground state to excited state is called as excitation process.

Ground state :-

Is the state of a molecule or an atom which is most stable and has least energy.

Excited state :-

Excited state is a state which is least stable but contains more energy. The life time of excited state is normally 10^{-8} to 10^{-9} seconds.

2) Relaxation Process :-

An atom or molecule looses energy and returns to ground state known as relaxation process

The absorbed energy can be lost by the following ways -

i) Production of Heat (collisional deactivation)

ii) Decomposition into a new species. (Photochemical reaction)

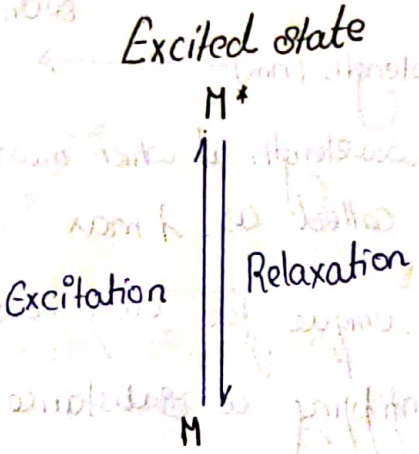
iii) Emission of Radiation of -

a) specific wavelength, characteristics of excited species (as in flame photometry)

b) longer wavelength (as in fluorescence)

c) longer wavelength after a short time lag (as in phosphorescence)

The excitation process, emission process, ground state, excited state and series of events which take place when an EMR is passed on a molecule or atom can be represented by -



Relaxation :-

$M^* \longrightarrow M + 1.\text{Heat}$

2. Emission of specific λ (Flame Photometry)

3. Emission of longer λ (Fluorescence & phosphorescence)

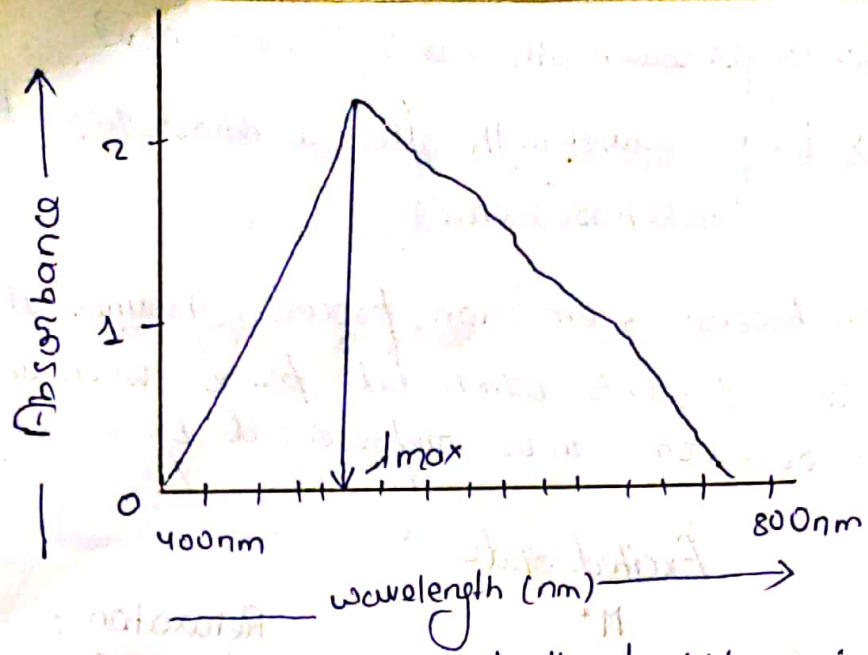
4. Photochemical reaction

Visible Spectroscopy (Colorimetry) :-

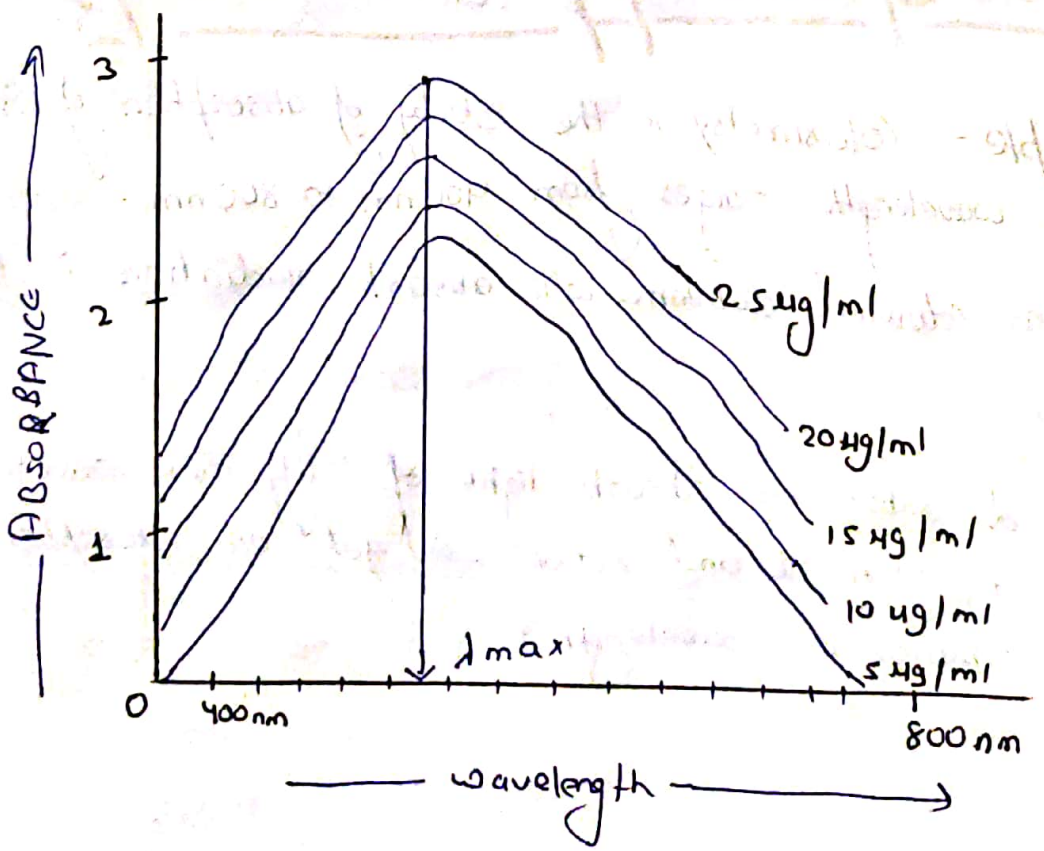
Principle - Colorimetry is the study of absorption of visible radiation whose wavelength ranges from 400 nm to 800 nm.

Any coloured substance will absorb radiation in this wavelength region.

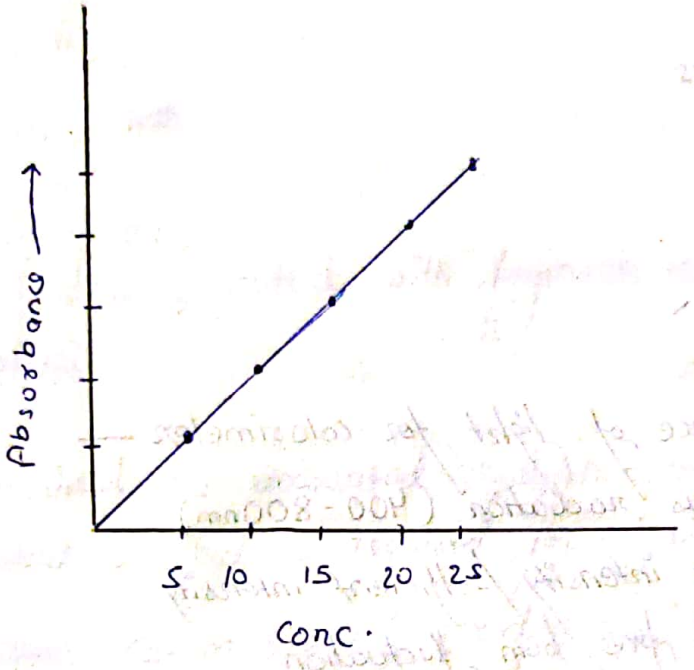
Coloured substances absorb light of different wavelength in different manner and hence we get an absorption curve. (absorbance v/s wavelength)



- In this absorption curve the wavelength at which maximum absorption of radiation is take place is called as λ max
- This λ max is characteristic or unique for every coloured substance and this is useful in identifying a substance.
- λ max is not usually affected by conc. of substance.
- The absorbance of soln increases with conc. of substance



when we plot a graph of concentration v/s absorbance we get a calibration curve or standard curve.



This calibration curve is useful in determining the conc. of amount of a drug substance in the given sample solution or a formulation.

- **Laws governing absorption of radiation :-**

i) Beer's law (related to conc. of absorbing species)

ii) Lambert's (related to thickness / pathlength of absorbing species)

These two laws are applicable under the following condition -

$$I = I_a + I_t$$

I = Intensity of incident light

I_a = Intensity of absorbed light

I_t = Intensity of transmitted light and no reflection or scattering of light takes place.

Instoumentation of Visual Spectroscopy :-

a. Source of light
b. Filters and monochromators
c. Sample cells
d. Detectors

a) Source of light :-

The requirements of source of light for colorimeter —

- It should provide continuous radiation (400 - 800nm)
- It should provide adequate intensity / sufficient intensity
- It should be stable and free from fluctuation
- The sources of light commonly used are —
  - i) Tungsten lamp
  - ii) Carbon arc lamp

i) Tungsten lamp (The lamp consist of a tungstun filament in a vaccum bulb.

ii) Carbon arc lamp :- For a source of very high intensity, carbon arc lamp can be used. It also provides an entire range of visible & visual spectrum.

b) Filters and monochromators :-

- The source of light gives radiations from 400nm - 800nm. This is polychromatic (heterochromatic) in nature (light of several wavelengths)
- In a colorimeter/spectrophotometer we require only monochro-matic light. Hence a filter or monochromator is used which

Converts polychromatic light into monochromatic light.

i) Filters -

They are two types -

1) Absorption filters
2) Interference filters.

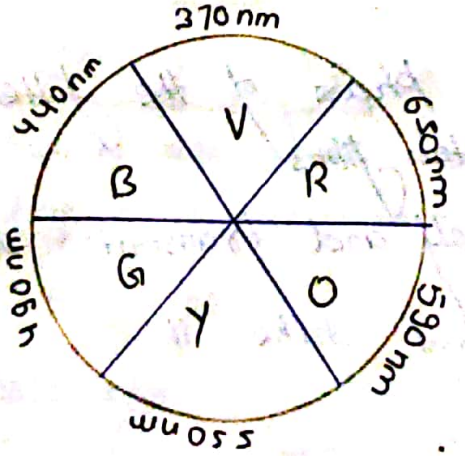
Absorption filter-

Made up of glass, coated with pigments or they are made up of dyed gelatin.

They absorbed the unwanted radiation and transmit the ~~unwan~~ rest of radiation which is required for colorimetry.

These filters can be selected according to the procedure given below -

- Draw a filter V (a circle wave 6 parts)
- Write the colours (VIBGYOR) in clockwise or anticlockwise manner omitting Indigo.
- If the colour of solution is red, use green filter and if the colour of soln is green use red filter. (The colour of filter is opposite to the colour of the soln).
- Similarly we can select the required filter in a colorimeter, based upon the ~~co~~ colour of the soln.



V - Violet
I - Indigo
B - Blue
G - Green
Y - Yellow
O - Orange
R - Red

Absorption filter.

Merits :-

- Simple in construction
- Cheaper
- Selection of filter is easy

Demerits :-

- less accurate since band pass is more (+-30 nm) i.e., if we have to measure at 500nm, radiation ranging from 470nm - 530 nm.
- Intensity of radiation becomes less due to absorption by filters.

2) Interference filter :-

This is also k/a Fabry-Perot filter.

It consist of an coating of transparent dieloctric spacer of low refractive index sandwiched b/w the semi-transparent silver films.

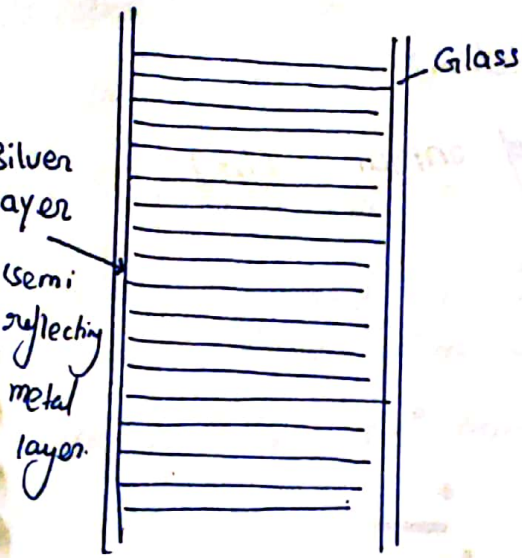
Magnesium fluoride is a common dielectric spacer because because of its hardness.

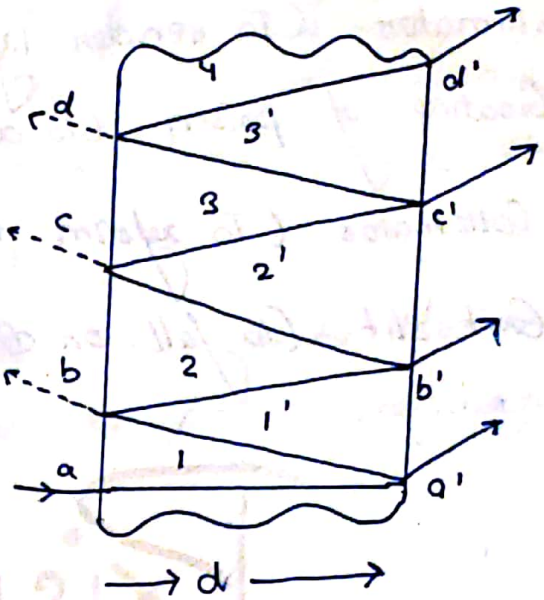
Some other didectric spacer are calcium flouride, silicon monoxide etc.

Working :-

The light inciedent upon the phase of the filter is reflected back and forth b/w the metal films

Each silver film reflects half and transmits the other half of any radiation that strike it.





They can be used with high intensity light source bcz they remove unwanted radiation by transmission and reflection and not by absorption.

Merits -

In expansive

Lower band pass compare to absorption filters and hence more accurate

Demerits -

The band pass is only 10-15 nm and hence higher resolution obtained with monochromators or gratings cannot be achieved.

II. MONOCHROMATERS

Monochromaters are better and more efficient than filter in converting a polychromatic or heterochromatic light into monochromatic light.

A monochromater has the following unit.

- Entrance slit (To get narrow source)

- Collimator (To render light parallel)
- Grating of prism (To disperse radiation)
- Collimator (To reform the image of entrance slit)
- Exit slit (to fall on sample cell).

Prisms :-

- The Prisms disperse the light radiation into individual colours.
- Inexpensive instrument
- Band pass is lower than filters and hence better resolution.
- The resolution depends upon size and refractive index of the prism.
- Material of the prism is normally glass.
- Two types of prisms are :-

i) Refractive type
ii) Reflective type (Littrow type)

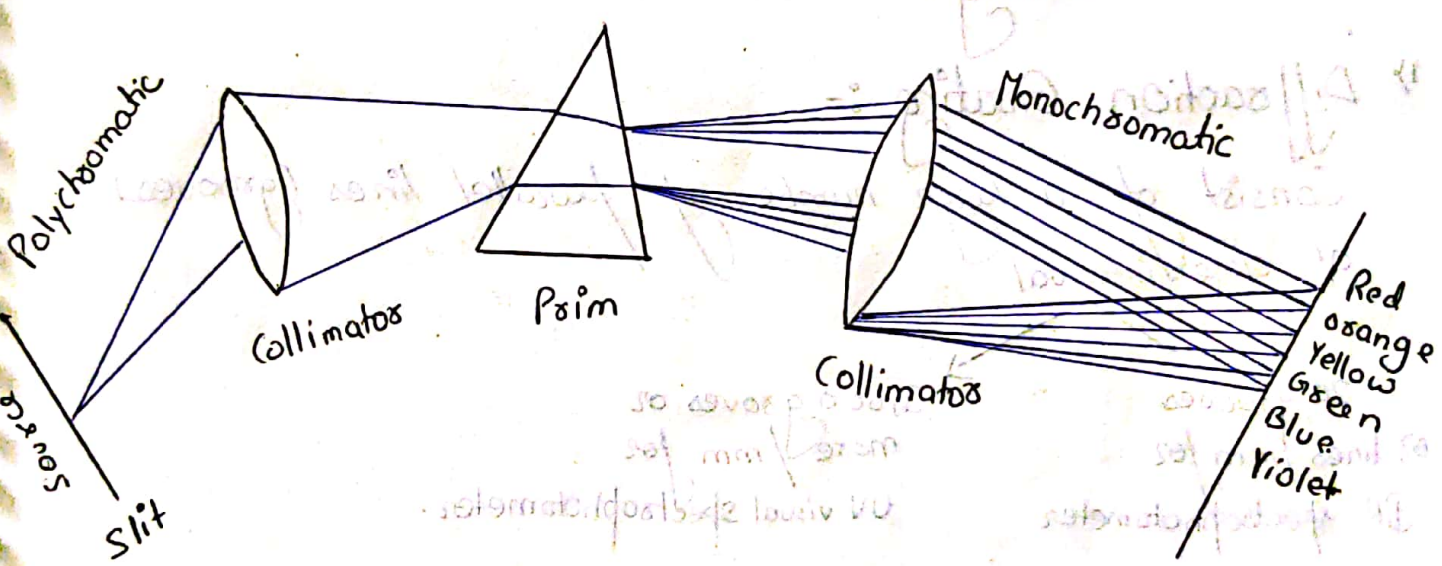
1) Refractive type :-

The source of light, through interance slit falls on a collimator.

The parallel radiation from a collimator are dispersed

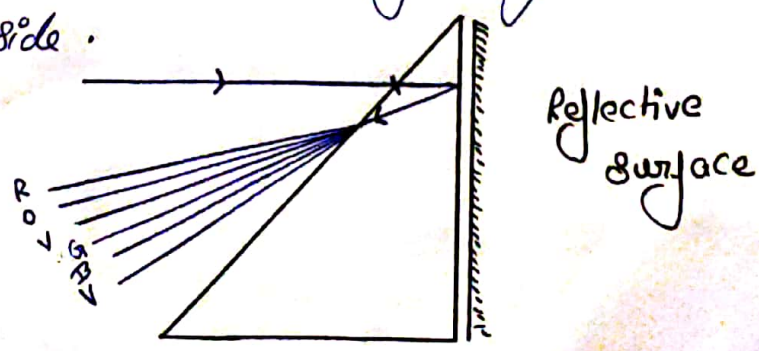
into different colours and wavelength after passing through a prism and by using other collimator, the images of enterance slit are reformed (ones will be either violet, Indigo, Blue, Green, Yellow, Orange or Red)

The required radiation on exit slit can be selected by rotating the prism or by keeping the prism stationary and moving the exit slit.



Reflective type :-

The principle of working is similar to the refractive type except that, a reflective surface is present on one side of the prism. Hence the dispersed radiation gets reflected and can be collected on the same side.



Grating :-

Most efficient for converting polychromatic light into monochromatic light.

± 0.1 nm resolution could be achieved.

Commonly used in spectrophotometer.

They are two types -

i) Diffraction Grating
ii) Transmission Grating.

i) Diffraction Grating :-

Consist of a large number of parallel lines (grooves) at close interval

- 20 grooves or lines/mm for IR spectrophotometer
- 3,600 groves or more/mm for UV visual spectrophotometer.

Gratings are rulings made on some material like glass, Quartz or alkyl halides depending upon the instrument

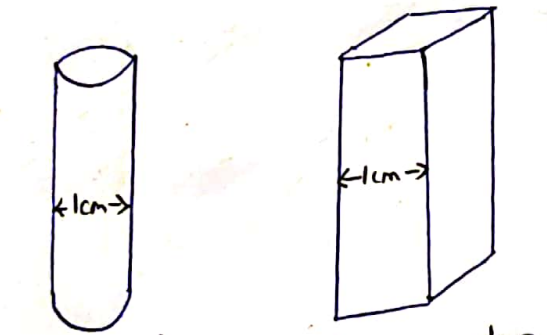
Sample cell :-

Sample cells or cuvettes are used to hold sample solution Their geometry and material varies with the instrument and nature of sample handled

The material of sample cell should not absorbed at the wavelength being observed.

i) Sample volume - small volume cells (0.5ml or less) and large volume cells (5-10ml)

ii) Shape of cell - Cylindrical (like test tube) or rectangular.



Types of Sample cell

iii) Path length (internal distance)- 1cm (normally), upto 10cm (long path length) 1mm or 2mm (short path length) cells are available.

iv) Material - .Fused glass for visible region.
- Polystyrene cells for aqueous solvents but not for organic solvent.
- For UV region must use Quartz cell because glass absorbs UV ~~rog~~ radiation

Glass material is suitable for 380 nm - 2,500 nm.

Detectors :-

Detectors used in UV visual spectrophotometers called as photometric detectors.

Radiation → (Pass through) → Sample cell → Some radiation absorbed and rest transmitted.

Transmitted radiation → Falls on → Detector (light energy)

Detector (light energy) → Read → Record.

Detector (light energy) → converted to → Electric energy or electric signal

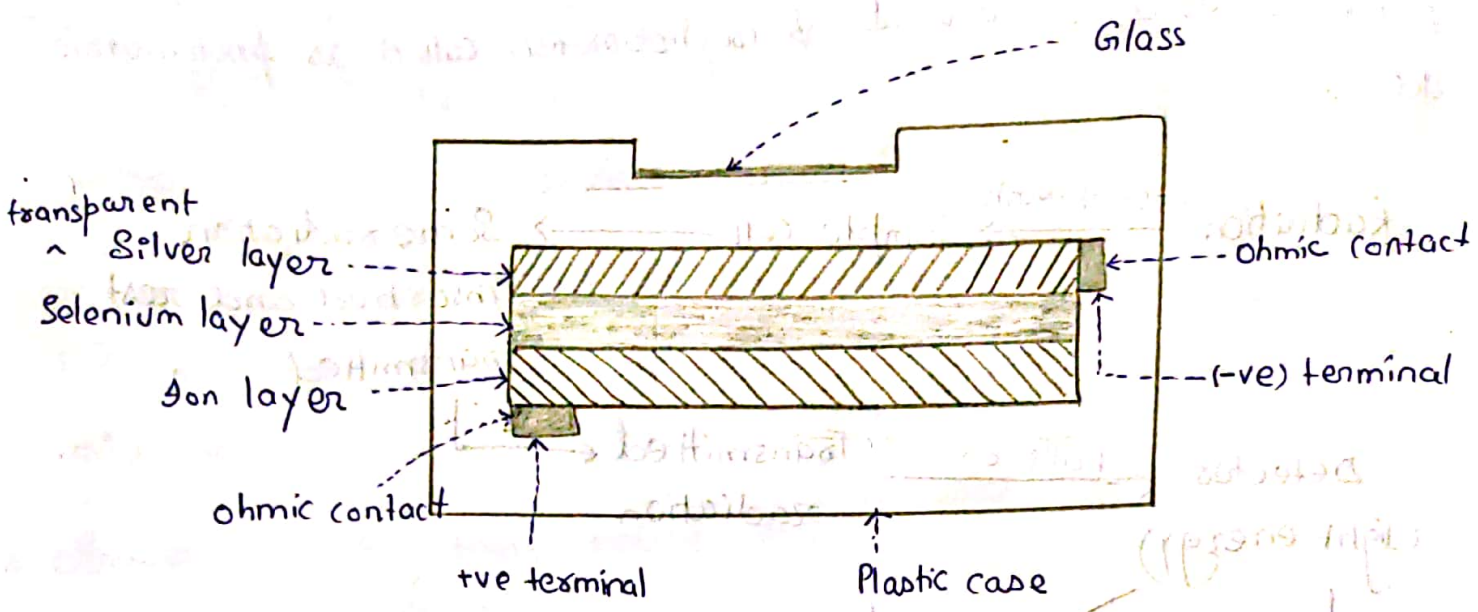
The most commonly used detectors are -

i) Barrier layer cell or photovoltaic cells
ii) Phototubes or photoemissive cells
iii) Photo multiplyer tubes (PMT)

Selecting criteria for suitable detectors :-

- Should be highly sensitive
- Should be cheap and good quality
- Should be durable
- Should give linear response

Barrier layer Cell or photovoltaic Cell :-



- Photovoltaic cell are constructed using a base plate of iron which is coated with a thin layer of semi-conductor material like selenium.
- The selenium is covered with a thin layer of silver, the whole setup is enclosed in a plastic case/housing.
- The top portion of this case is made of transparent material like glass.
- This transparent material allows light photons to pass and fall on the silver layer of the detector.
- The ohmic contacts at iron and silver selenium surface act as electrodes
- The ohmic contact act as junction between two conductors

Working :-

Light (Photon)

↓ Falls on

Detector

↓

Electrons on silver-Selenium are excited

↓

Energy increases

↓

Electrons released from bonds

↓ collected at

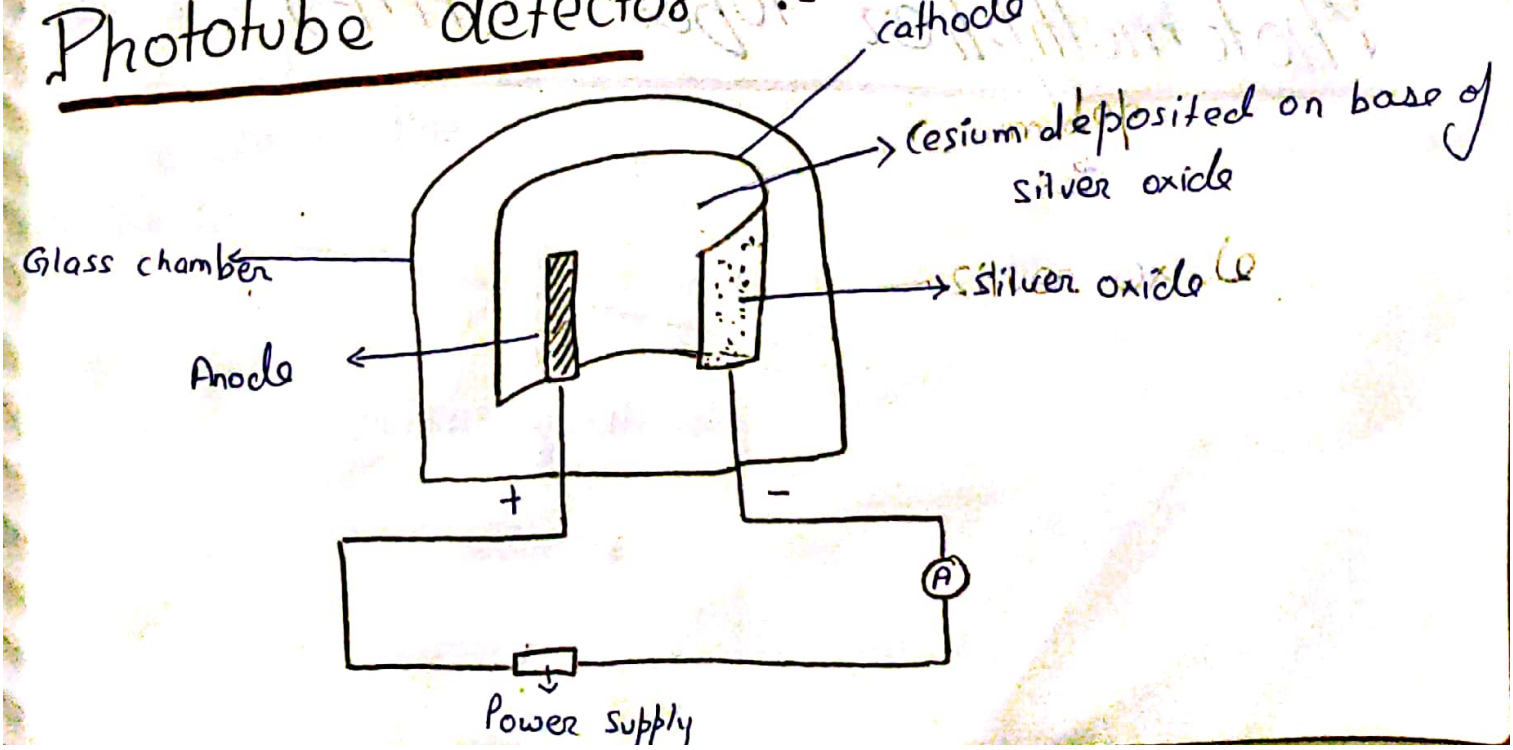
Collector electrode (At silver-selenium surface)

When Galvanometer is connected, the current flows.
As the intensity of light increases, the number of photons increases which releases more electron, that increases the photo current.

Advantage :-

Cheap

Phototube detector :-



Working :-

Composed of evacuated glass tube

↓

Consist of photo cathode (coated with caesium/Potassium/Silver oxide)

+

Anode collector

↓ ← Light radiation

Electron liberated from cathode

↓ towards.

Anode

↓

Current production.

Amount of current $\propto$ Intensity of light radiation.

- Signal from detector can be amplified using an amplifier.
- Better sensitivity than photovoltaic cell.

Photomultiplier tubes (PMP)

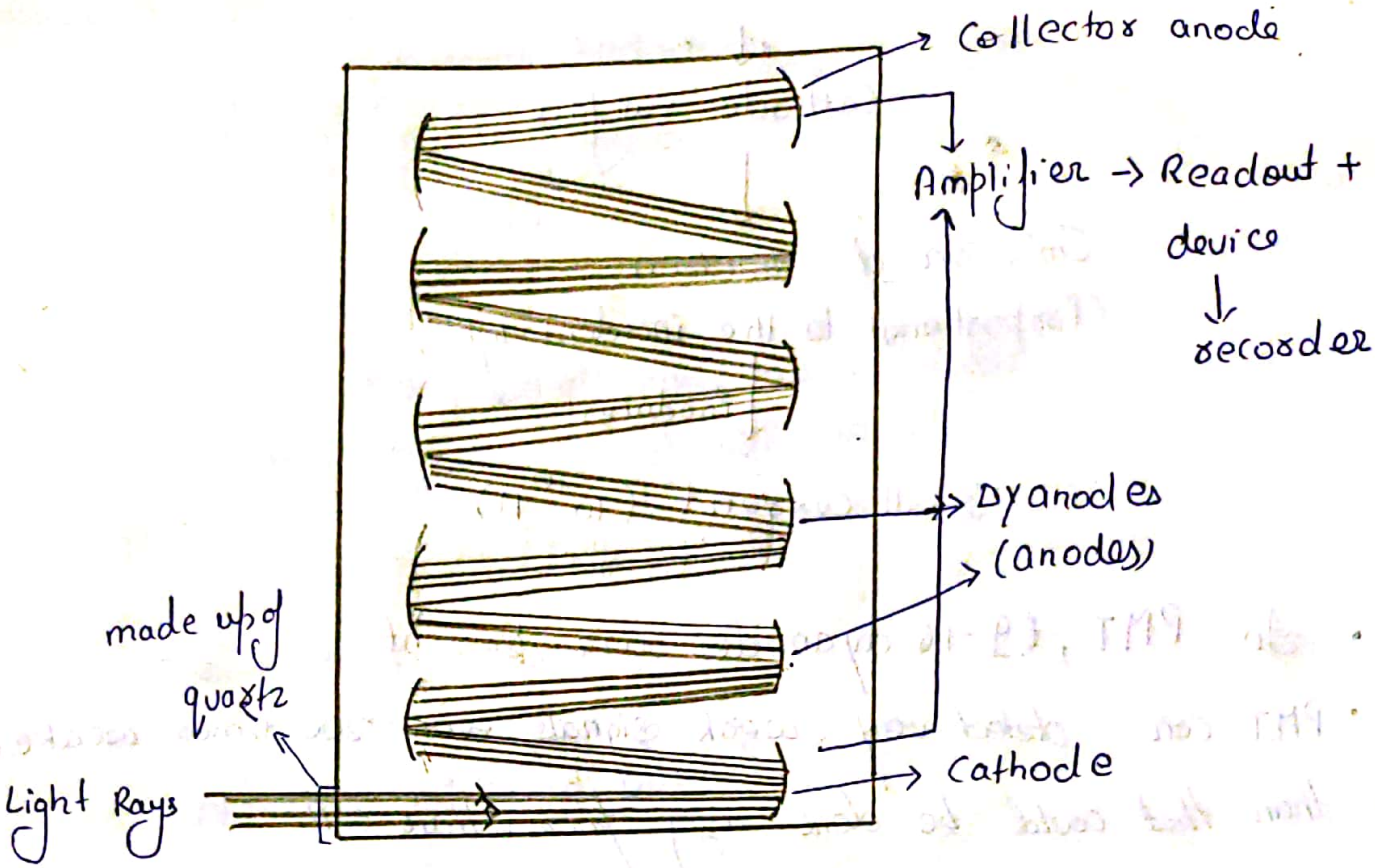


Fig :- Photomultiplier Tube

Photomultiplier tube

consists of

- A cathode
  - Negative electrode
  - Semicylindrical
  - Coated with alkaline earth oxide.
- An anode
  - Positive electrode
  - Coated with alloys, antimony, Gallium, Phosphorus ion and chromi-um ion.

In PMP, quartz window allows

Radiation

Falls on

↓

Cathode surface.

↓

Emission of electron
(Proportional to the incident light)

↓ Produces

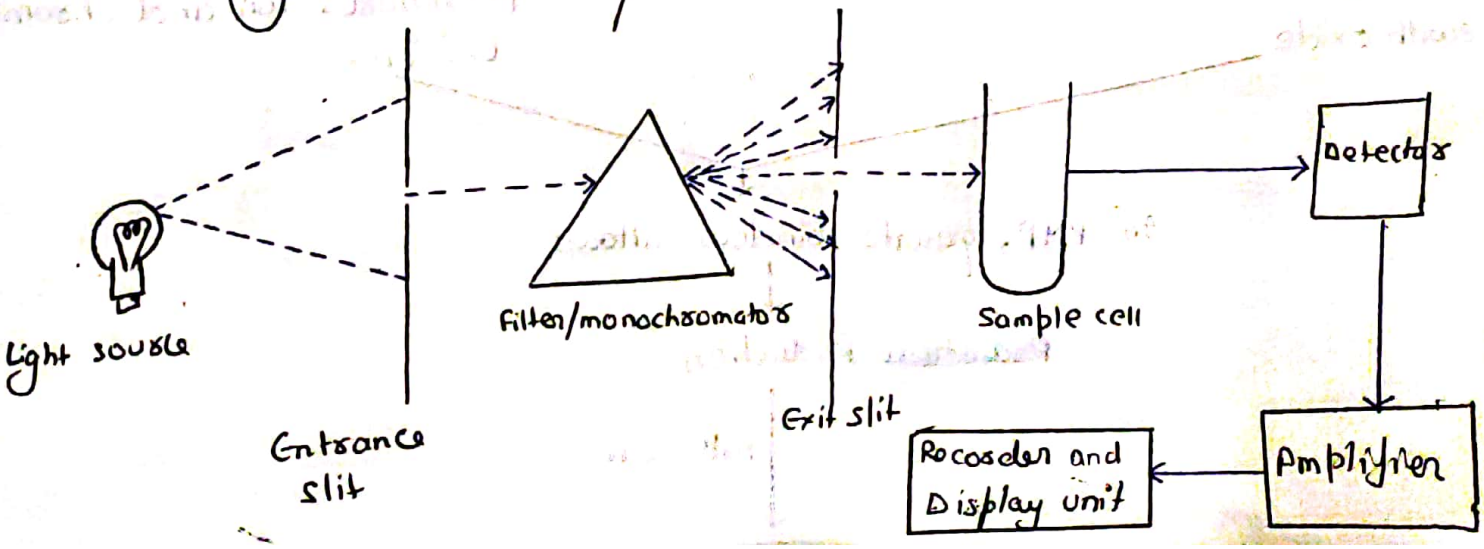
Small current (10^{-10} A)

- In PMT, 9-16 dynodes are present
- PMT can detect very weak signals, even 200 times weaker than that could be done using photo voltaic cell.
- Very high magnification (10^6 times) with the help of dynodes

Types of Colorimeter / Visual spectrophotometer

i) Single beam spectrophotometer
ii) Double beam spectrophotometer

i) Single beam Spectrophotometer :-



Light Radiation

↓ falls on

Slit

↓ then

Pass through filter / Monochromator

(only required wavelength of light pass)

↓ falls on

Sample cell / Reference cell

↓

Absorption of some radiation

↓

Rest is transmitted

↓ which falls on

Detector

(Photovoltaic cell)

↓

Amplifier

↓

Recorder / Display.

Merit -

Simple in construction

In expensive

Easy to operate

Demerits -

- The reading are affected by fluctuations in the intensity of source.
- Rapid scanning to get a spectrum is not possible.
- Recorder cannot be used with single beam time.

11) Double beam spectrophotometer :-

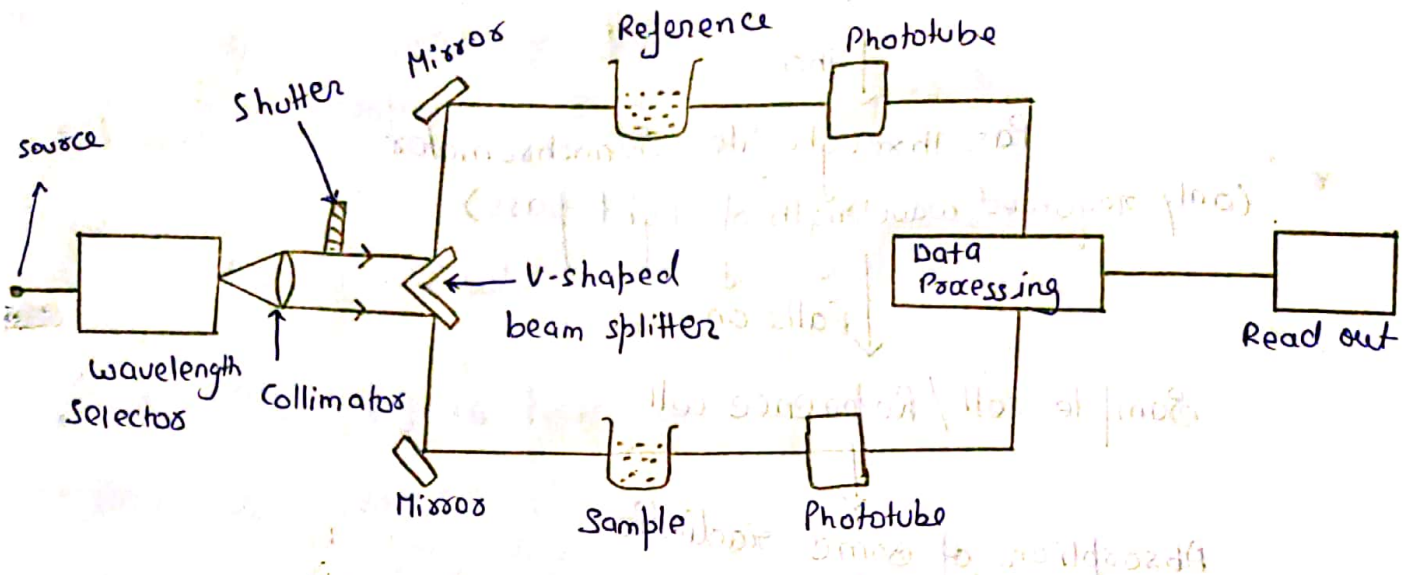


Fig - Double beam spectrophotometer

Light radiation (from tungstun lamp)

↓ Falls on

Slit

↓ by using

Concave mirror

↓ then

Light passes through wavelength selector

↓ then

Light splitted by V-shaped beam splitter

↓ then

Passes through sample / Reference cell

↓

Detetor

↓

Data processing
↓
Read out

Advantage -

High accuracy
Good sensitivity
Wide range
better reliability and repeatibility

Disadvantages -

Intensity of light is reduced to 50%
Complex construction (Instrument)
Skilled person required
Expensive

Criteria for satisfactory visual spectroscopy estimation :-

1) **Stability of colour -**
Color fade of solution occurs due to -

i) Air oxidation
ii) Photochemical decomposition
iii) temperature

2) **Intensity of colour -**
Solution colour should be intense for accurate result in low concentration.

3) **Clearity of Solution -**
Substance should be completely soluble in solvent.

Turbid / Suspension / colloidal sol^n may - absoob the light or scatter the light or both.

4) Reproducibility -

Result should be reproducable

5) Validity of Beer's law -

Colour intensity of the sol^n should be proportional to the conc.

Application of visual spectroscopy

1. Estimation of biochemical compounds in blood, plasma, CSF, urine etc.
   - eg - Glucose, urea, Uric acid, lipid, enzymes, minerals, billirubin etc.
2. Widely used in — hospitals and Laboratories
   Institution
   Food industry
   Paint industry
   Textile industry
3. Used in quantitative estimation of biochemical compounds
   - For the quantitative analysis of drugs by colourimetry, the following characteristics must be known —
     a) light absorbtion characteristics - ie, λ^{max} to be known
     b) validity of beers law - i.e., Conc. range in which

the system obeys linearity

c) Solvate reagent and other conditions

4. **Quality control of purity** - Colourimetry can be used to detect the impurities. Coloured impurities present in a sample give rise to additional peaks or more absorption at particular wavelength.

5. To check water quality (screening chemicals like Fluoride, Cynide Iron, dissolved oxygen, zinc etc)

6. To determine the conc. of plant nutrients (such as phosphorus, nitrates, ammonia etc)

7. Determination of ligand / metal ratio in metallic complexes.

8. **Structure elucidation of organic compounds** - The absorption spectrum of an unknown compound can be compared with known compound so that the most probably structure may be obtained.

U.V spectroscopy

Ultraviolet spectroscopy is concerned with study of absorption of U.V radiation which ranges from 200nm - 400nm.

- compounds which are colourless absorb radiation in U.V region
- Compounds, which are coloured, absorb radiation from 400nm - 800 nm
- In both U.V as well as visible spectroscopy only the balance e^- absorb the energy
- There by the molecule undergoes trangition from ground to excited state.
- This absorption is characteristic and depends on the nature of electron present.
- The intensity of absorption depends upon the conc and path length as given by Beer-Lambert law.
- **Types of electrons :-**

Theis are three possible type of electrons -

1) **σ electroons -**

σ electroons are saturated compounds having single bonds and absorb vaccum U.V rays. (less than 200nm)

11) **π electrons -**

π electrons are unsaturated compounds that possess double and triple bonds in molecules.

eg - $C=C$, $C\equiv C$, $=O$ etc.

iii) n electrons -

These are non bonded electrons which are not involved in the any bond formation.

eg - Sulphur, oxygen, Nitrogen and Halogen.

Principle -

Any molecule has either n, π or σ or a combination of these bonding electrons (σ and π) and non bonding (n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state.

By the characteristic absorption peaks the nature of ~~absorp~~ electron present and hence the molecular structure can be elucidated.

Electronic Transition and excitation process

There are four types of transition for electrons exists in molecule -

i) $n \longrightarrow \pi^*$

ii) $\pi \longrightarrow \pi^*$

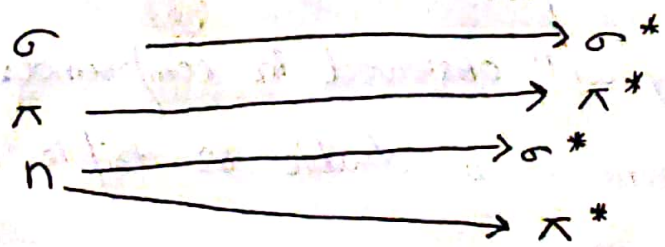
iii) $n \longrightarrow \sigma^*$

iv) $\sigma \longrightarrow \sigma^*$

On UV radiation, electronic transition takes place (because of π electrons) followed by inital vibration and rotation.

The absorbed radiation again comes back to lower energy level because of molecular collision and the energy absorbed is released as heat energy in U.V radiation. This is learned as molecular phenomena

Bonding orbital (BO) Antibonding orbital (A.BO)



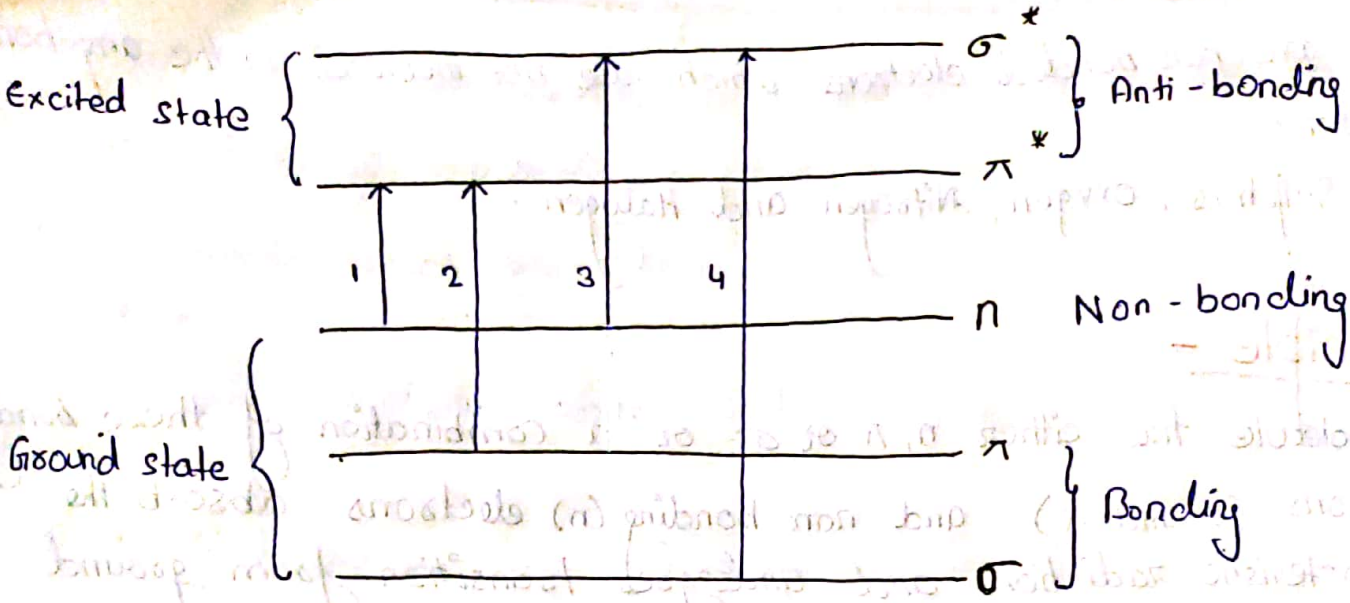


Fig :- Energy level of electronic transition

The higher energy orbital is called as Anti-bonding orbital, because of extra energy, they cannot form bond.

Ground state energy orbital is called as bonding orbital.

The energy required for excitation for different transition are —

$n \longrightarrow \pi^* < \pi \longrightarrow \pi^* < n \longrightarrow \sigma^* < \sigma \longrightarrow \sigma^*$

more than 200nm | less than 200nm

$n \longrightarrow \pi^*$ requires lowest energy

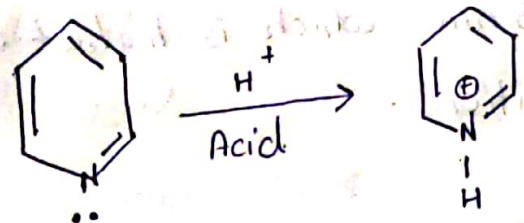
$\sigma \longrightarrow \sigma^*$ requires highest energy for excitation in U.V region

Types of electronic transitions :-

1) $n \longrightarrow \pi^*$:-

These transition requires lower energy and longer wavelength. This can be very well observed in compounds where n electron is present in a double or triple bonded compound.

eg - Aldehydes, ketones, Nitro compounds.



Peak present

Peak disappears.

Peaks due to this transition are called as "R Bands"

2) $\pi \longrightarrow \pi^*$ transition -

This type of transition gives rise to B, E and K Bands.

Types	Due to
1) B bands (Benzenoid bands)	Aromatic and Hetero aromatic system
2) E bands (Ethylenic bands)	Aromatic systems
3) K bands ($\pi \longrightarrow \pi^*$)	Conjugated system

These type of transition requires more than 200 nm (wavelength).

3) $n \longrightarrow \sigma^*$ transition -

Requires approximate 175 nm - 250 nm.

As the peaks are at the lower end of UV spectrum, it can be called as end absorption.

This transition occurs in saturated compounds, with hetero atoms like S, O, N or halogen

It requires lesser energy when compare to $\sigma \longrightarrow \sigma^*$ transition.

eg - ~~Alkaloids~~ Alcohols, Aldehydes, ketones etc.

Water - 167 nm

Methylene chloride - 169 nm

Methanol - 203 nm

Ethanol - 204 nm

Chloroform - 237 nm

$\sigma \longrightarrow \sigma^*$ transition :-

Requires energy of 125 - 135 nm which is highest energy requires in all other transitions.

- Vaccum UV region

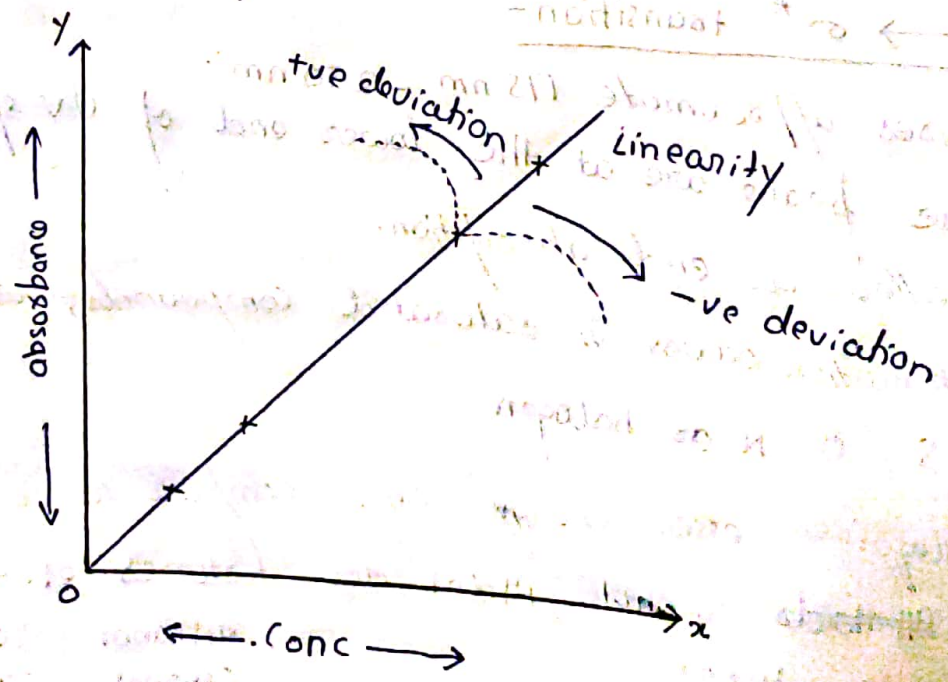
eg- Hydrocarbons (methane, Propane etc)

- **Deviation from Beer's-Lambert's law :-**

A system is said to obey Beer's law when a plot of Concentration Vs absorbance gives a straight line (linear graph).

If the deviation is bend towards x-axis, i.e., with the increase in conc. there is a decrease in absorbance called as negative deviation.

If the deviation is higher towards y-axis, i.e., with the increase in conc., there is a proportional increase in absorbance called as positive deviation.



Reasons for deviation →

Instrumental Error

Chemical error

Other phenomenal effects

Solvent error

Physical factors.

Instrumental error :-

Error due to light source

Improper slit width

Type of radiation defect due to monochromator malfunction.

Detector malfunctioning due to st. radiation falling on detector.

Chemical error -

a) Association of molecules -

Methylene blue exists as monomer at a λ-max of 660 nm (10^{-5} molar conc.) Methylene blue exist as dimer at a λ-max of 600 nm (10^{-4} molar conc.)

b) dissociation of Molecules -

Potassium dichromate in high conc. exists as orange sol^n. (λmax - 450 nm). But on dilution, dichromate ions are dissociated into chromate ions which is yellow coloured (λ-max - 410nm)

Hence when 450 nm is used for absorbance measurement, deviation from Beer's law is seen

$$Cr_2O_7^{-2} + H_2O \xrightarrow{\text{Dilution}} 2H^+ + 2CrO_4^-$$

(orange) (yellow)

λmax - 450nm λmax - 410 nm

c) Incomplete Reaction
d) Ionisation of molecule
e) pH change in Soln
f) Polymerisation of the molecule
g) Solvation of the molecule
h) Adsorption of the molecule.

Other Phenomenal effect

Photo-degradation of the molecule.

Solvent Error

Chloroform and CCl_4 absorb strongly at 250nm. Hence can be used for measurements at wavelength.
Wavelengths above 280 nm.

Physical factors

Wavelength selection (as absorption changes with wavelength)

IR Spectroscopy

Infra red spectroscopy or vibrational spectroscopy is concerned with the study of absorption of infra red radiation, which results in vibrational transition.

IR spectra is mainly used in structure elucidation to determine the functional group.

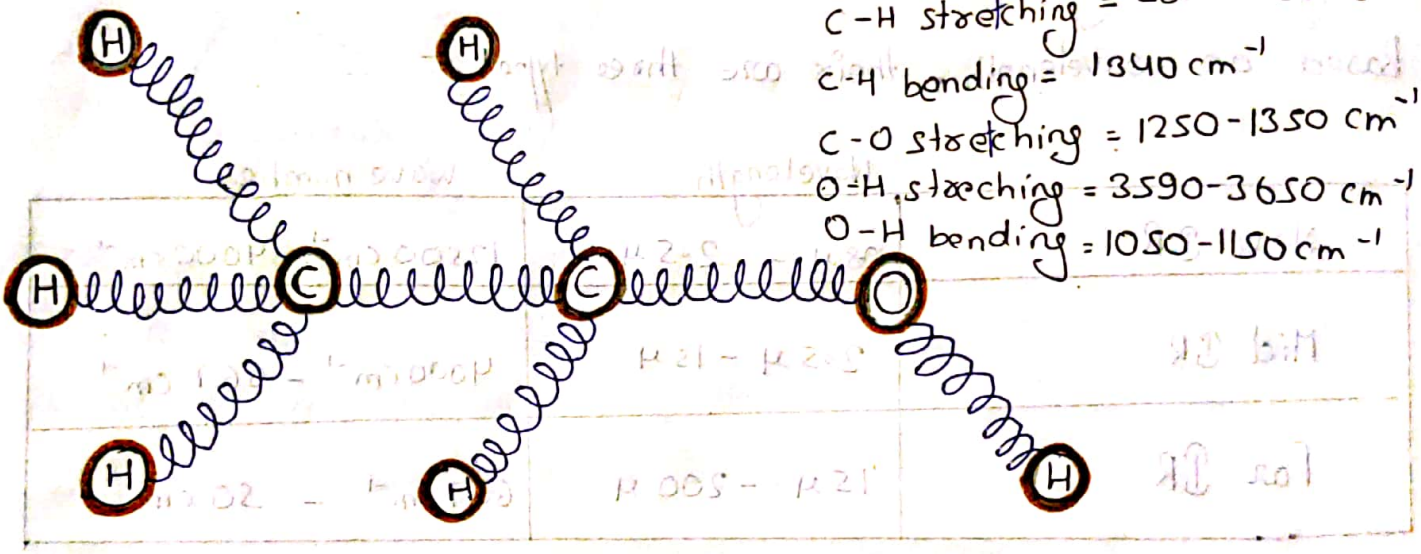
Based on wavelength, their are three types :-

	Wavelength	Wave number
Near IR	0.8 μ – 2.5 μ	12500 cm^{-1} – 4000 cm^{-1}
Mid IR	2.5 μ – 15 μ	4000 cm^{-1} – 667 cm^{-1}
Far IR	15 μ – 200 μ	667 cm^{-1} – 50 cm^{-1}

Principle –

Atom / Groups of atoms (on any molecule) → connected by → Bonds [* similar to spring, * Flexible in nature] → b/c of → Continuous motion of the molecule → they → Maintain some vibration & Frequencies → which is characteristic to every position of molecule → k/a Natural frequency of vibration → when

Applied IR frequency = Natural frequency → Absorption of IR radiation takes place → then → Peak is observed.



C-H stretching = 2850-2960 cm^{-1}
C-H bending = 1340 cm^{-1}
C-O stretching = 1250-1350 cm^{-1}
O-H stretching = 3590-3650 cm^{-1}
O-H bending = 1050-1150 cm^{-1}

- Every bond or portion of molecule or functional groups requires different frequency for absorption.

Hence characteristic peak is observed for every functional groups or part of the molecule.

* In pharmaceutical analysis we use infrared radiation (mid IR).

In IR spectra we use wave numbers instead of wavelengths because wave numbers are larger values and easy to handle than wavelengths which will show only small differences b/w functional groups.

Wave number :-

Number of waves present per cm, which can be calculated from the wavelength —

$$\frac{1}{\text{wavelength in } \mu} \times 10^4 = \text{wave number per cm or } cm^{-1}$$

Types of vibration :-

Molecular vibrations or fundamental vibrations are classified as -

Stretching vibration.

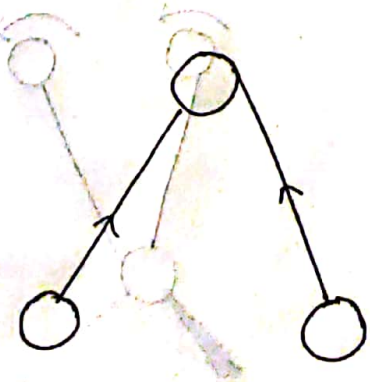
Bending vibration.

1) Stretching vibration -

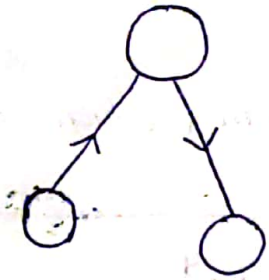
The distance between the atom increases or decreases but atoms remain in same bond axis.

There are two subtypes

i) Symmetrical stretching - In which two hydrogen atoms move either towards or away from the central carbon atom, results in changing of interatomic distance. or no change in valency angle



ii) Asymmetrical stretching - One hydrogen atom approaches the carbon atom while the other moves away from carbon atom.



2) Bending Vibration :-

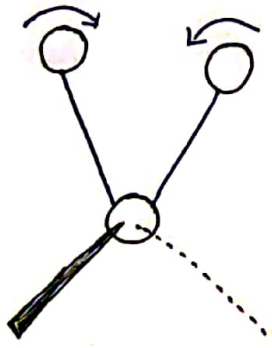
When a three atom system forms part of larger molecules it involves vibrations which essentially involve oscillation of the atom or group or whole and is perpendicular to the chemical bond

Types of bending Vibration :-

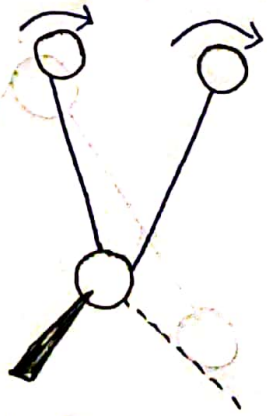
1) Inplane bending -

a) Scissoring or symmetrical bending - Two atoms attached to central atom either move towards or away from each other.

b) Rocking - The structural unit swings back and forth in the plane of the molecule.



Scissoring



Rocking

ii) Out of plane bending -

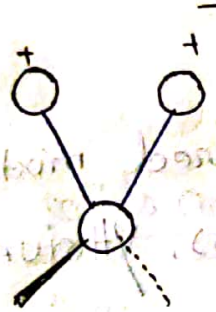
a) Wagging - The structural unit swings back and forth out of the plane of the molecule

b) twisting - The structural unit rotates about the bond that joins it to the rest of the molecule.

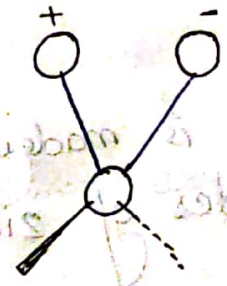
Or

In which one atom is above the plane and other is below the plane

out of plane bonding -



Wagging



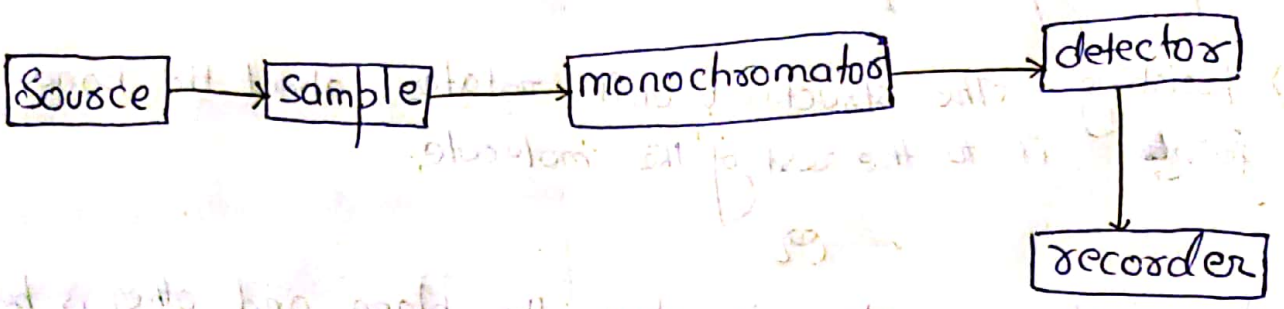
Twisting

+ → above the plane

- → below the plane

The bending vibration requires less energy and longer wavelength

Instrumentation :-



1) Source :-

Light sources are -

i) Nernst Glower - This is made up of sintered mixture of oxides of zirconium (Zr), yttrium and Erbium (Er)

ii) Globar Source - This is made up of silicon carbide rod heated to 1,500 Kelvin by passes of electric current to give hot globar and emits radiation.

This source is very useful for mid IR region.

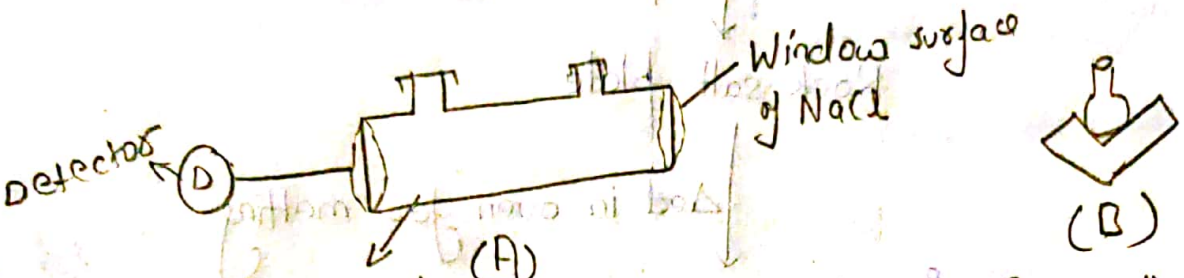
iii) Laser diode -

This is achieved by very high electric potential across a p-n junction in a gallium arsenide. Most diode operate at red and near IR wavelength.

iv) Various Ceramic clay materials -

2) Monochromatic Sample :-

For gases - A special type of gas cell with IR transparent window of NaCl is used.



(B)

Gas cell placed on V shaped metal block in IR spectrometer

For solids and liquids - Theis are four methods -

i. Residue
ii. Melt
iii. Mull
iv. Disc

1) Residue -

Concentrated soln of sample
↓ dissolved in volatile solvent (Ether, chloroform, ethanol)
Fed dropwise
↓ on
Rock Salt plate
↓ solvent allowed to evaporate
Leaving behind a film of solid

ii) Melt

Few mg of waxy samples or low melting sample

↓ deposited on

Rock salt plate

↓ Δed in oven for melting

Second rock salt plate is put over the first

↓

Thin film of sample in liquid state

iii) Mull :-

5mg Sample

↓

Grounded in Mortar and Pestal

↓

Add 1 drop of mulling agent (Nujol)
(Refrasified paraffin oil)

↓

Make paste

↓

Transfer the paste on [Potassium bromide or Sodium chloride] disc

↓

Press the sample disc with another disc

↓

Place these disc into plate holder of spectrometer

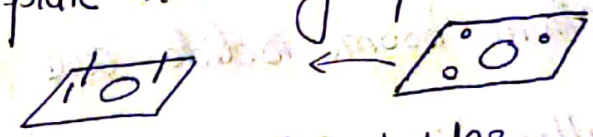


Fig :- Plate holder

iv) **Disc** :-

2mg Sample

↓

Mixed and grounded

↓

Add 200mg of pure and dry alkali halide (KCl, KBr)

↓

Mixed well

↓

Load into a die

↓ High pressure, Vacuum

Formation of a solid and robust glass like disc

(Sample is uniformly distributed throughout the supporting alkali)

Monochromator :-

Filters madeup of Lithium fluoride or prisms made of KBr and Caesium Iodide, Sodium chloride or Thallium boomo iodide are used.

Diffraction Gratings made up of alkali halides are also used.

Grating rotates slowly.

This rotation sends individual frequencies to the detector.

Detector :-

- At the wavelength, where the sample has absorbed, the detector will recieve a weak beam from the sample while the reference beam will retain full intensity.
- This lead to a pulsating or alternating current to flow from detector to amplifier.
- On the other hand, at the frequencies where the sample doesn't absorb, both the beams will have equal intensities and the current flowing from the detector to the amplifier will be direct and not alternating.
- Theis are different types of detectors —
  - Thermocouple, Golay cell, Bolometers, Thermistors, Pyroelectric detectors.

Recorder / Plotter :-

They are used to record the IR spectrum, on white paper or transparent sheets

Application :-