UNIT - Ist

UV VISIBLE SPECTROSCOPY

- Analysis →
  It is the quantitative & qualitative determination of any sample

- Spectroscopy →
  Spectrum + scopy
  EMR ← Light
  to determine

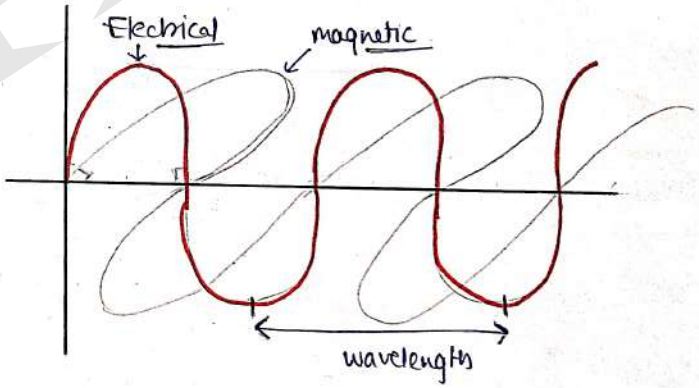
- It is the branch of science which is used for quantitative analysis of matter/sample by using light (Electro-magnetic radiations)..

(eg.) amount of substance in sample etc..

- EMR → Electromagnetic Radiations ~
  Electric + magnetic

- Light flow in the form of wave which contain photon — packets of energy

- It contains electric & magnetic radiations perpendicular to each others..



- Wavelength (λ) → Distance b/w the two consecutive waves (crest/trough). → nm..

- frequency (υ) → No. of waves (cycle) in one sec. → hertz (Hz)..

frequency, υ = c / λ
c — velocity of light
λ — lambda

• Gamma Rays	< 0.001 nm
• X-rays	< 0.01 - 10 nm
⊙ U.V	200 - 400 nm
⊙ Visible light	400 - 800 nm
• Infra Red	0.8 - 200 μm
• Microwaves	0.01 - 1 m
• Radio waves	1 - 10^7 m

ENERGY ↓

• **UV Visible Spectroscopy →**

Used for quantitative analysis of matter / sample by using (UV) (Ultra violet) and (Visible) light...

• the overall range of wavelength of UV & visible is 200 - 800 nm.

• **Principle →**

- EMR contain energy (photons)

$$E = h\nu$$ ∴ h = Plank's constant 6.626×10^{-27} erg. sec

$$\nu = \frac{c}{\lambda}$$

$$E = h \cdot \frac{c}{\lambda}$$

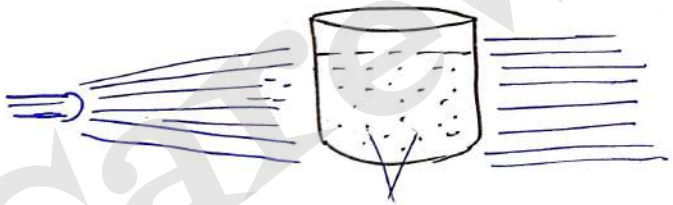
• $E \propto \frac{1}{\lambda}$, Frequency ↑ = Wavelength ↓

Wavelength ↓ = Energy ↑

When light (EMR) is passes through a sample

Principle →

- EMR light contain packets of energy (photons)..
- Now, when this light passes through the sample,
- A particles/molecules of sample absorb this light..



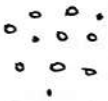
particles absorb this light → this light contain energy

So, particles get excited —

Absorbance / Absorption

Stop light

emission



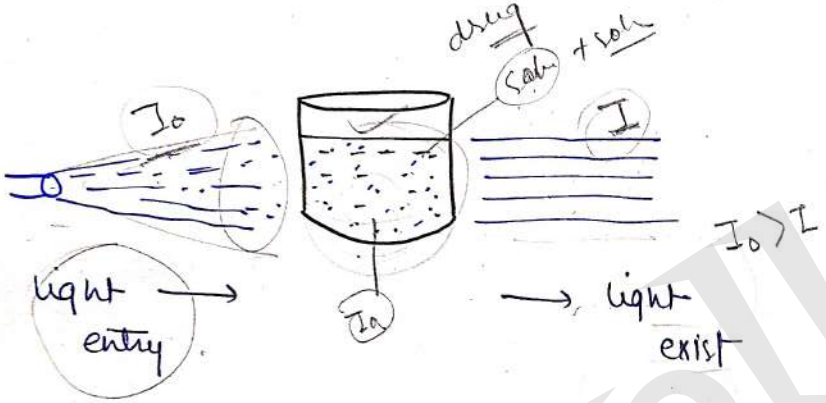
Absorption spectroscopy (UV-Visible, IR)

Absorption

EMR → Sample → transmission

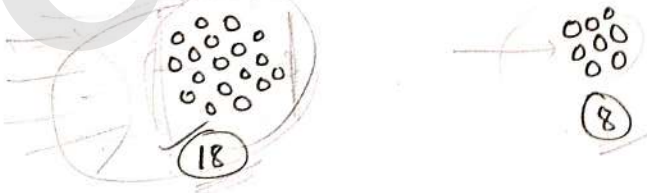
Sample → Emission → Emission spectroscopy (flame photometry)

- Now, UV-Visible spectroscopy's principle depends upon the absorption of light.



because particle absorb some light.

eg. — who absorb more light — ??



- So, if we determine the intensity of light, then we calculate the concentration of particles —

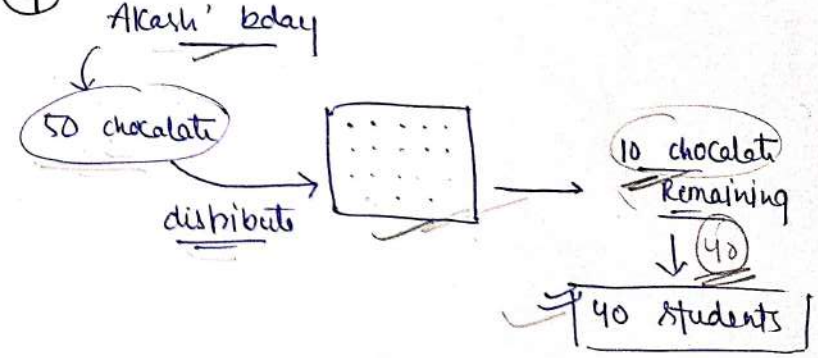
> Absorption ∝ concentration of particles

Absorption↑ = concentration↑

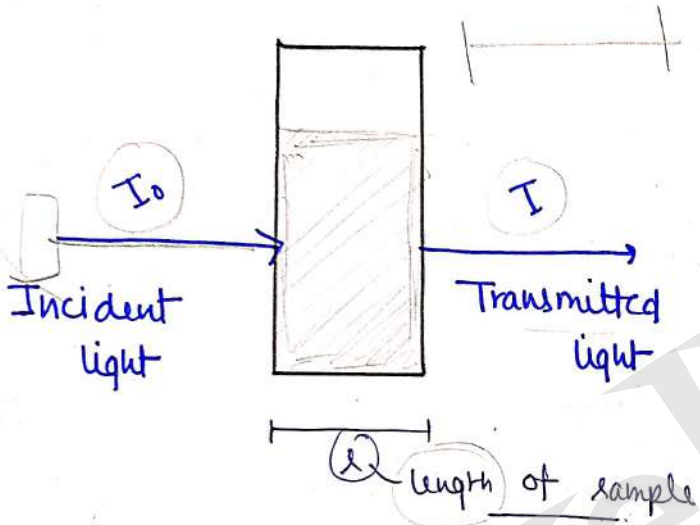
So, Now, we have to calculate the absorption.

How — ??

eg.



- principle based on **Beer-Lambert law**.



Acc to this,

Light Absorption is directly proportional to path length and the concentration of the solution.

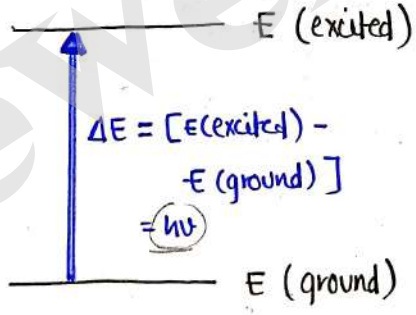
$$A = \varepsilon l c$$

ELECTRONIC TRANSITIONS

The absorption of Electromagnetic radiation causes electrons to be excited, which results in promotion from a bonding / non-bonding orbitals to an anti-bonding orbitals i.e. $n-\pi^*$, $\pi-\pi^*$

OR

- Energy absorbed in the UV-Vis region produce change in electronic energy of molecules (ground state (E_0) to excited state (E_1)) resulting from transition of electron in the molecule.



- three types of electrons involving in organic molecules :—

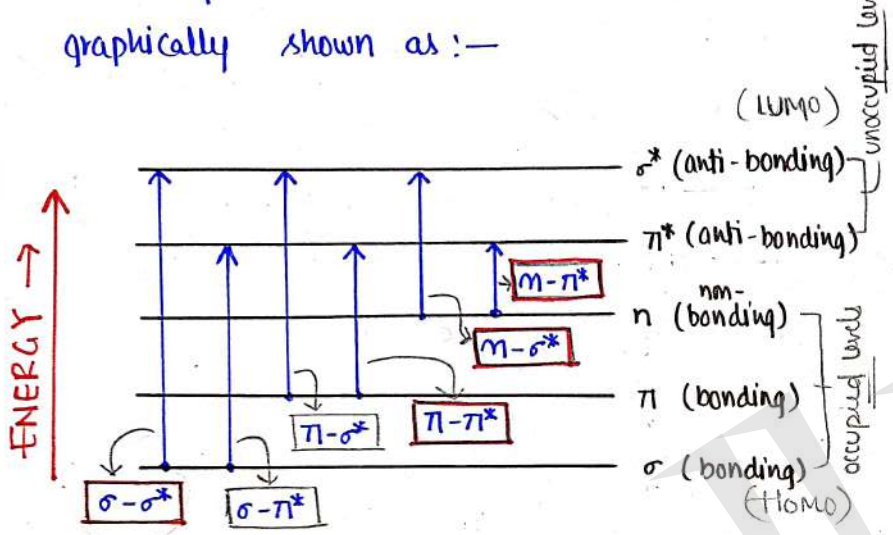
i) σ - electrons :→ these electron are involved in saturated σ-bonds, these are the lowest-energy occupying molecular orbital i.e. σ-orbitals / σ-bonds

ii) π - electrons → these electron are involved in unsaturated hydrocarbons. these π-orbitals / π-bonds lie at higher energy levels.

iii) n - electrons → these electrons are involved in lone pair or non-bonding (n) orbitals
non-bonding
Trap
- they also lie at higher energy level

eg. Nitrogen, Oxygen & Halogens etc.

* The possible electronic transitions can graphically shown as :—



- The possible electronic transitions are :-

i) $\sigma - \sigma^*$ transition
ii) $\pi - \pi^*$ transition
iii) $n - \sigma^*$ transition
iv) $n - \pi^*$ transition
v) $\sigma - \pi^*$ transition
vi) $\pi - \sigma^*$ transition

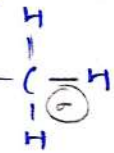
} theoretical possible (show absorption in region above 200 nm)

- The most probable transition is from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).

i) $\sigma \rightarrow \sigma^*$ transition :- A transition of an electron from a sigma bonding orbitals to the higher energy anti-bonding sigma orbitals ($\sigma - \sigma^*$)

- high energy required [$\because \frac{1}{\lambda} = E$]
- very short wavelengths

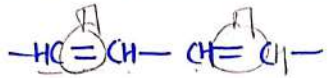
(eg). Methane (CH_4)



show wavelength of 125 nm

ii) $\pi - \pi^*$ transition → π electron in a bonding orbital is excited to corresponding anti-bonding π^* orbital.

- Available in compounds with unsaturated centres.

(eg). Alkenes,  alkynes, carbonyl, etc..

- Alkenes generally absorb in the region 170 to 205 nm.

iii) $n-\sigma^*$ transition → It involves saturated compounds with atoms containing lone pair of electrons O, N, S ..

- (eg) NH_3 
- wavelength → 150 - 250 nm.

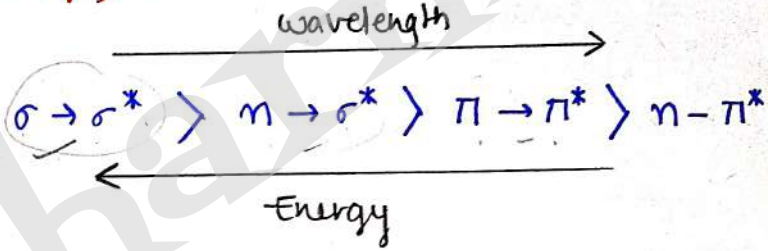
iv) $n-\pi^*$ transition → It involves an electron of atoms containing lone pair is excited to π^* anti-bonding orbitals

- required least energy & longer wavelength

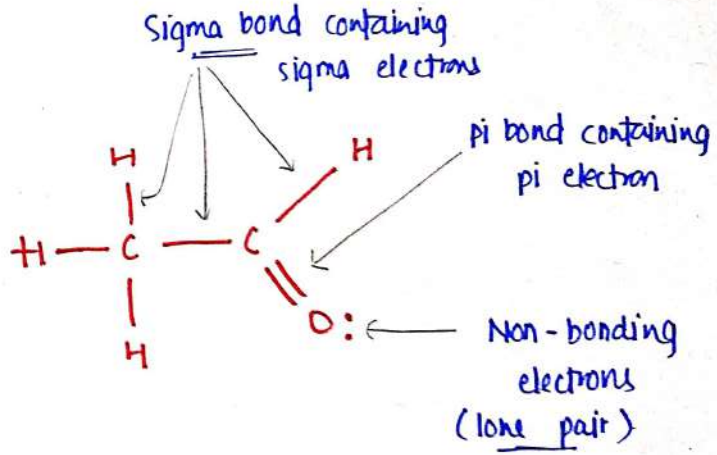
(eg).  ,  etc.

- wavelength → 280 - 300 nm

Energy :-



(eg.)



- Three types of electrons

CHROMOPHORES

Chromophores are the molecules or parts of molecules that absorb light in the UV-Visible region and responsible for imparting colour to the compounds.

(reflect a certain color)

eq. Nitro-compound (–NO) are generally yellow in color.

Colorless benzene + (–NO) ⟶ Pale Yellow color

+hydroxyl group (Auxachrome) → dark yellow.

- Some of the important chromophores are ethylenic, Acetylenic, Carbonyls, esters, Nitrile group etc.

(π-π*: ethylenic, Acetylenic; π-π*, n-π*: Carbonyls)

Types

Independent chromophores

- A single chromophore imparts color to the compound.

eq. azo group (–N=N–), Nitro group (–NO)

Dependent chromophores

- More than one chromophore is required to produce color in compound.

eq. C=O groups, C=C groups etc.

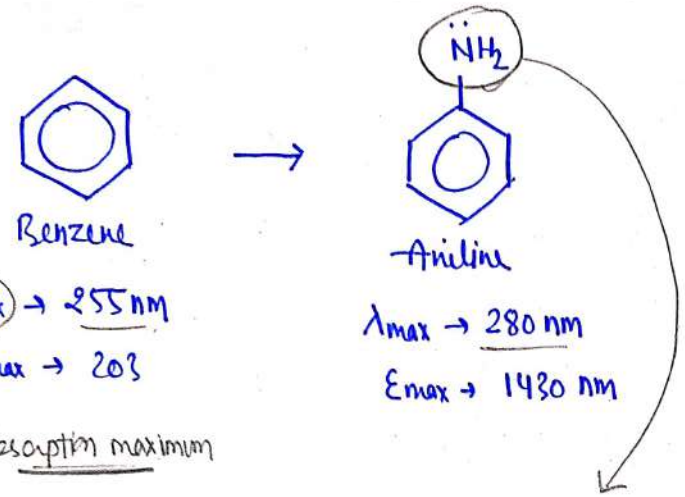
Acetone: one ketonic group → colorless; 2 ketonic group → Yellow; 3 ketonic group → Orange

Auxachromes

It is a group of atoms that get attached to the chromophore and increase the colorfullness of the chromophore.

- Also called as Color enhancing groups.

Eg.

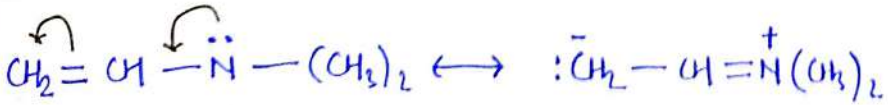


- $-OH$, $-OR$, $-NH_2$, $-NHR$, $-NR_2$, $-SH$ etc.

⊙ Also auxochromic groups increases the wavelength of molecules.

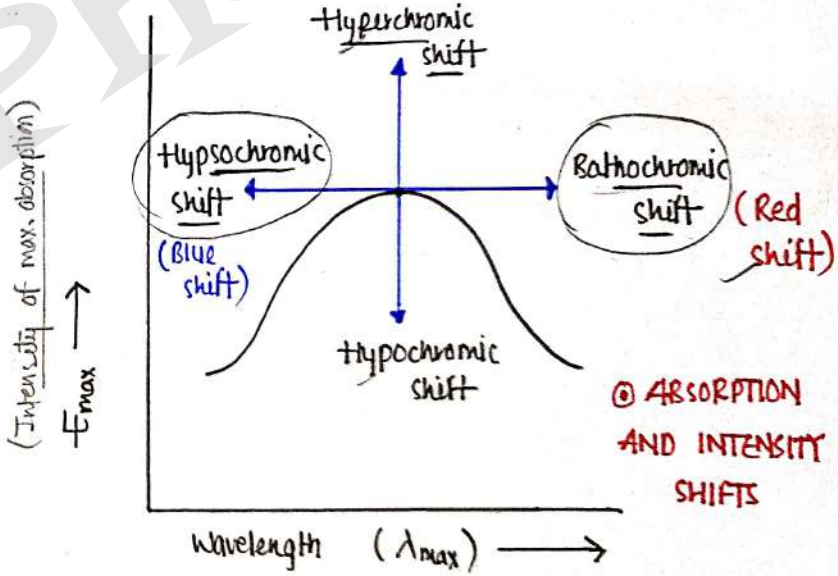
Mechanism →

they contain non-bonding electrons, which extended the conjugation of the chromophores by sharing the non-bonding electrons.



SPECTRAL SHIFTS

The position of absorption maximum and absorption intensity can be modified in different ways by some structural changes or solvent changes :-



i) Bathochromic shift (Red shift) → It is an effect in which the absorption maximum (λ_{max}) is shifted towards longer wavelength due to presence of an auxochrome or by the

change of solvent.

(eg.) The $n-\pi^*$ transition for carbonyl compounds experiences bathochromic shift (red shifts) when the solvent polarity is decreased.

ii) Hypsochromic Shift → (Blue shift)

It is an effect in which the absorption maximum is shifted towards shorter wavelength.

iii) Hyperchromic Shift →

It is an effect in which the intensity of absorption maximum increases ie. the value of ε_{max}

eg. Auxochrome increases the intensity of Absorptin.

iv) Hypochromic Shift →

It is an effect in which the intensity of absorption maximum decreases i.e. the value of ε_{max} decreases.

It may be caused by the introduction of group which distorts the geometry of molecules.

(eg.) Biphenyl - ε_{max} 19000 whereas 2-Methyl biphenyl at ε_{max} 10250

SOLVENT EFFECT ON ABSORPTION SPECTRA

The choice of solvent used in UV spectroscopy is most important because solvent can change the absorption max.

- A most suitable solvent is one that does not itself absorbs radiation in the region under investigation (UV-Vis radiation).

(#) Polarity ↓ → Absorption maximum (λ_{max}) wavelength ↑

Solvent polarity

- Most commonly used solvent is 95% ethanol.

Solvents	λ of Absorption
Ethanol	210 nm
Hexane	210
Methanol	210
Cyclohexane	210
Water	205
Benzene	280
Chloroform	245

Water > Methanol > Ethanol > Benzene > Hexane

← Polarity

- The position as well as the intensity of absorption maximum get shifted by changing the solvent polarity.

- (eg.) The α,β- unsaturated carbonyl compounds shows two different shifts.

i) $n-\pi^*$ → Absorption band moves to shorter wavelength by increasing the solvent polarity ←

- λ_{max} of acetone is 279 nm in hexane (non-polar) as compared to 264 nm in water (polar)

ii) $\pi-\pi^*$ → Absorption band moves to longer wavelength by increasing the solvent polarity →

iii) $n-\sigma^*$ → same as $n-\pi^*$ (shorter wavelength) ←

BEER & LAMBERT'S LAW

- Beer's law → Absorbance is directly proportional to the conc^n of sol^n

$$A \propto c$$

- Lambert's law → Absorbance is directly proportional to the length of sol^n

$$A \propto l$$

On Combining,

$$A \propto cl$$

$$A = \varepsilon cl$$

where, ε = molar absorption coefficients

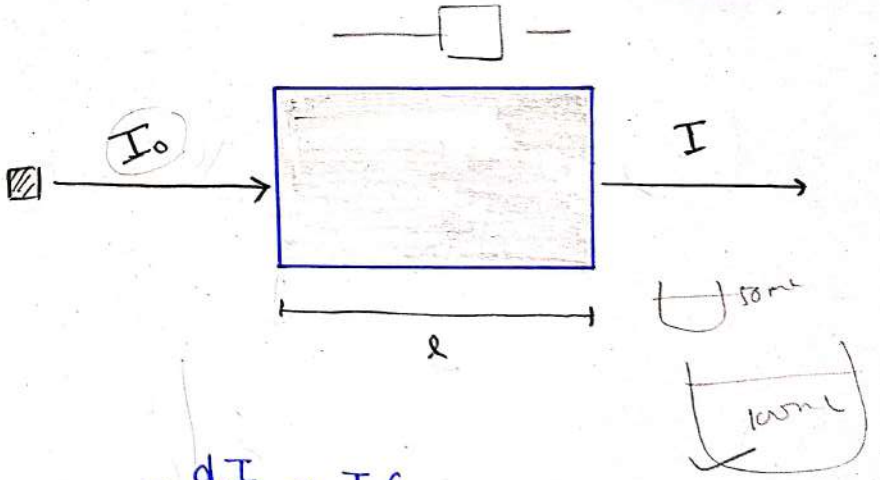
c = conc^n of sol^n (mol/litre)

l = length of light pass through sample

✻ Derivation →

Acc to Beer-lambert's law

When a monochromatic light is passed through a solution containing the absorbing sustance, the decrease in the intensity of light with path length is proportional to the concentration of solution and the intensity of light.



$$-\frac{dI}{dl} \propto I \cdot C$$

$$-\frac{dI}{dl} = KIC$$,

where,

I = intensity of light
c = conc^n (mol/litre)
l = thickness
K = constant.

Interchange,

$$-\frac{dI}{I} = KC.dl$$

Integrating both sides,

$$-\int_{I=I_0}^{I=I} \frac{dI}{I} = KC \int_{l=0}^{l=l} dl$$

$$-\ln \frac{I}{I_0} = KCl \quad \left[\ln \frac{I_0}{I} = KCl\right]$$

$\ln(x) = \log_e(x)$

$$2.303 \times \ln \frac{I_0}{I} = 2.303 \times K C l$$

$$\log_{10} \frac{I_0}{I} = \varepsilon C l \quad \text{(new constant)} \quad \text{——— (1)}$$

$A > 0$
$T < 100\%$

$T = \frac{I}{I_0}$

Transmittance → Amount of light emitted after absorption.

Now,

$$A = \log_{10}\left(\frac{1}{T}\right) \quad \text{——— (2)}$$

Rough condition

$A \propto \frac{1}{T}$

put the value of (T) into eq. 2

$$A = \log_{10}\left(\frac{1}{I/I_0}\right)$$

$$A = \log_{10}\left(\frac{I_0}{I}\right) \quad \text{——— (3)}$$

Acc to eq. (1) & (3)

$$A = \varepsilon . Cl$$

ε → Molar absorptivity (Molar extinction coefficien

A → Absorption

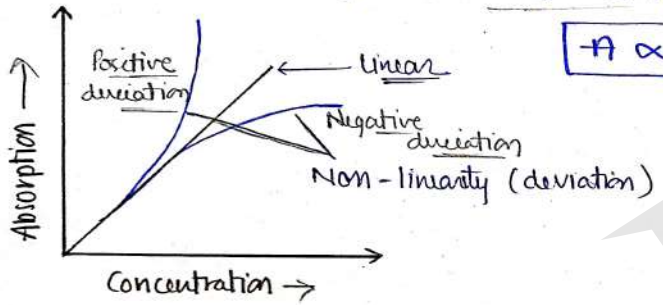
Cl → Concentration & path length

Deviation →

Acc to law,

Absorption is directly proportional to conc^n of solution.

$A \propto C$



Types :—

i) Real deviation / True deviation

ii) Chemical deviation

iii) Instrumental deviation / spectral deviation.

i) Real deviation → It is due to higher concentration of molecules in solution.

Also due to refractive index of absorbing medium.

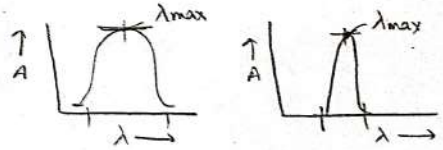
ii) Chemical deviation →

It is due to chemical changes such as association, dissociation, pH changes etc. in absorbing medium.

Eg. phenol → 270 nm, phenoxide anion → 287 nm

iii) Instrumental deviation →

It is due to polychromatic radiation, it leads to negative deviations. Monochromators are used to prevent this.



INSTRUMENTATION

in UV - Visible Spectroscopy

- Source of Radiation, wavelength selectors, Sample cells, detectors - photo tube, Photomultiplier tube, Photo voltaic cells, Silicon photodiode.

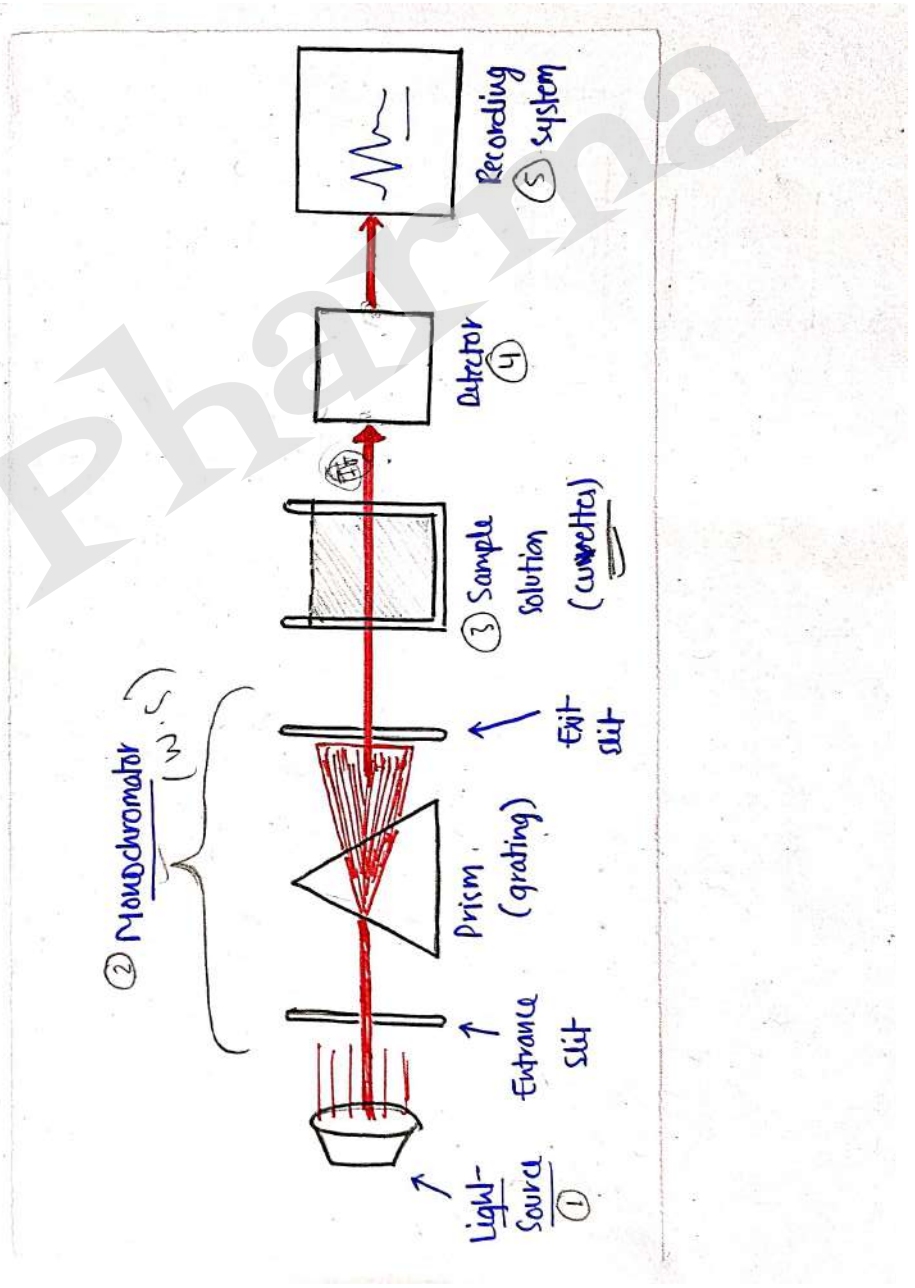
Spectrophotometer :→

A spectrophotometer is made up of two instruments :—

UV Spectrophotometer

Spectrophotometer

- Spectrometer → produce Light of any wavelength
- photometer → used to measure the intensity of Light.



· The essential parts of a spectrophotometer are :-

i) Source of Radiation
ii) Wavelength selector (Monochromator)
iii) Sample cells or Cuvettes
iv) Detector
v) Recording System

i) Source of Radiation →

The best source light is the one which is more stable, more intense and which gives the range of spectrum from 180 - 760 nm.

⊙ Hydrogen discharge lamp → In these lamps, hydrogen gas is stored under high pressure. When an electric discharge is passed through the lamp, excited hydrogen molecules will be produced which emit UV radiations.

⊙ Deuterium lamp → It is similar to hydrogen discharge lamp, but filled with deuterium (D_2) in place of hydrogen.
It offers 3-5 times more intensity than hydrogen lamp.

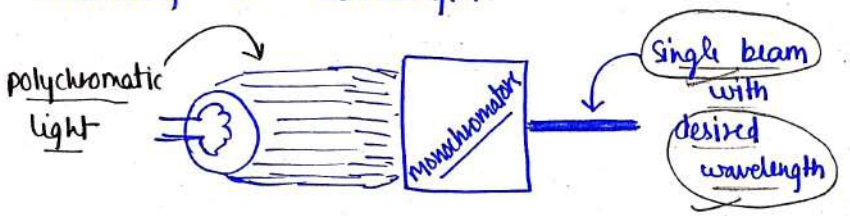
⊙ Xenon discharge lamp → In this lamp, xenon at 10-30 atm pressure is filled ~~with~~ it and it has two tungsten electrodes.
- Greater intensity than hydrogen lamp.

⊙ Mercury Arc →
This contain mercury vapours but not continuous so not widely used.

ii) Wavelength selector →
It consists of monochromat and slits..

⊙ Monochromators → It is used to disperse the radiation

according to wavelength.

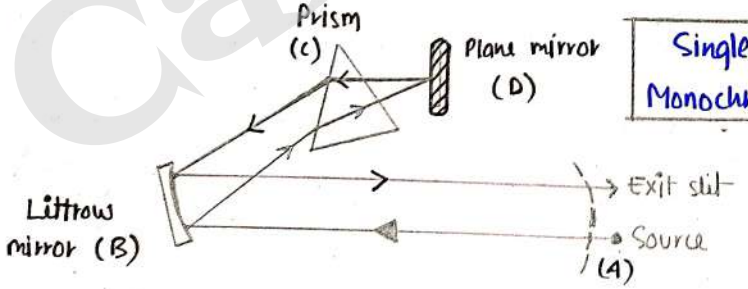


- The essential elements of a monochromator are an entrance slit, a dispersing element, and exit slit.
- The dispersing element may be a prism or grating.

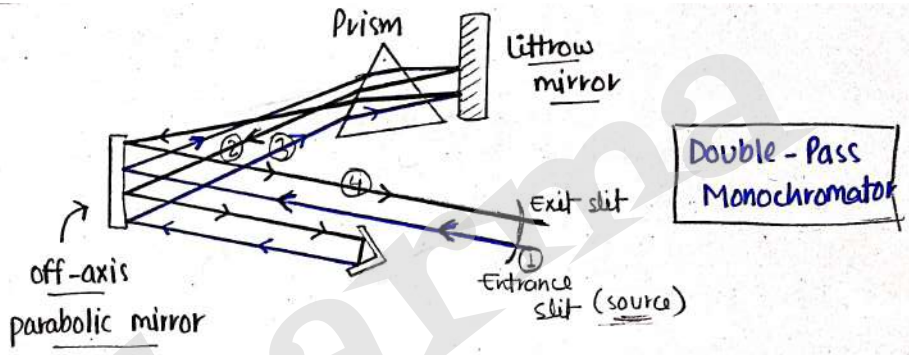
generally made of glass, quartz or fused silica.

widely used in UV spectrophotometers.

⊙ Prism Monochromator → two types →



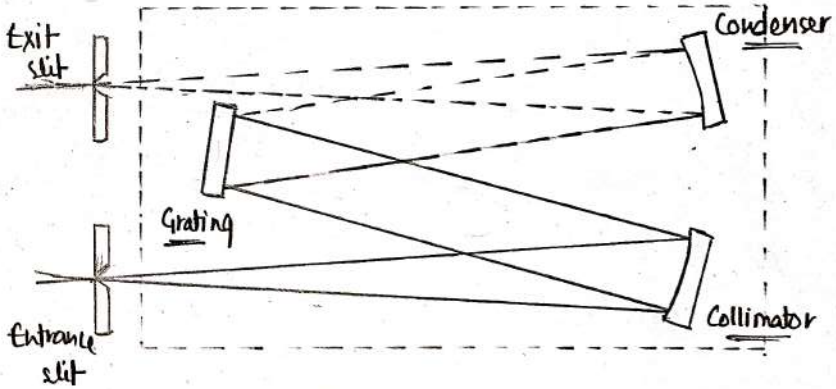
Single - Pass Monochromator



Double - Pass Monochromator

⊙ Grating monochromator →

It provide an alternative means of producing monochromatic light.



It consists of series of parallel lines (grooves) which reflected through highly polished surface of glass, quartz or alkyl halides (grating)..

⊙ Slits →

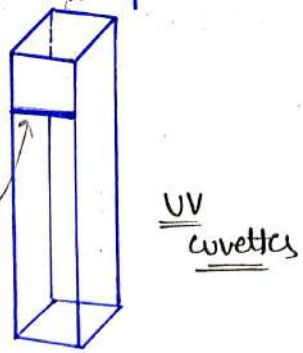
Entrance slit for entry of light and exit slit passed the single radiation of desired wavelength.

** Wavelength selector are the device which get the light from source of radiation and passes the light with desired wavelength which is required for detection to the sample (cuvette).

(iii) Sample Cells / cuvettes →

These are sample containers, which holds the liquid samples.

- they are transparent & usually made up with quartz or fused silica also silicate glass..



UV cuvettes

iv) Detectors →

Detectors used in UV-Visible spectrophotometer can be called as photometric detectors.

- These are those devices which converts light source into electrical signal which finaly displayed by recording system.
- Most commonly used detectors are :—

i) Photo tubes / photo - ~~emmi~~ emissive cells

ii) Photomultiplier Tubes (PMT)

iii) Photovaltaic cells or Barrier - layer cells

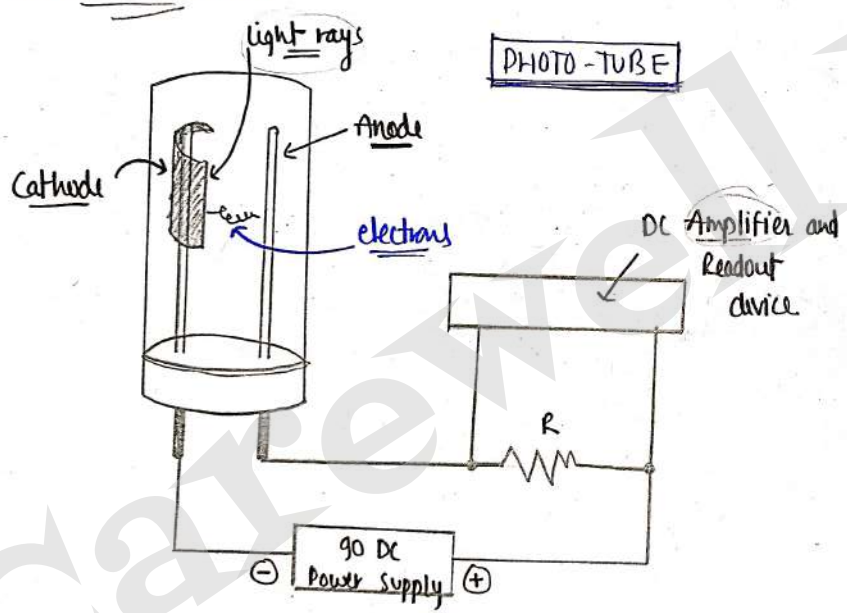
•) Phototubes or Photo - emissive cells →

- It is composed of an evacuated glass tube, which consist of a photocathode and a collector anode.

- The photocathode is coated with element of

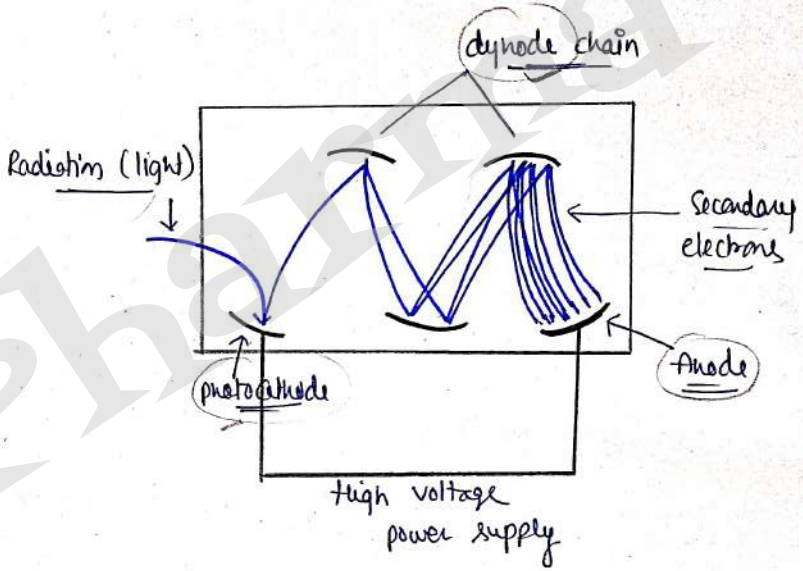
high atomic volume like Caesium, potassium or silver oxide, which can librate (emitted) electron when light radiation falls on it.

- This flow of electrons toward anode produce a current proportional to the intensity of light radiation.



- Phototubes have better sensitivity when compared to photovoltaic cells and hence are widely used.

6) Photomultiplier tubes (PMT) →



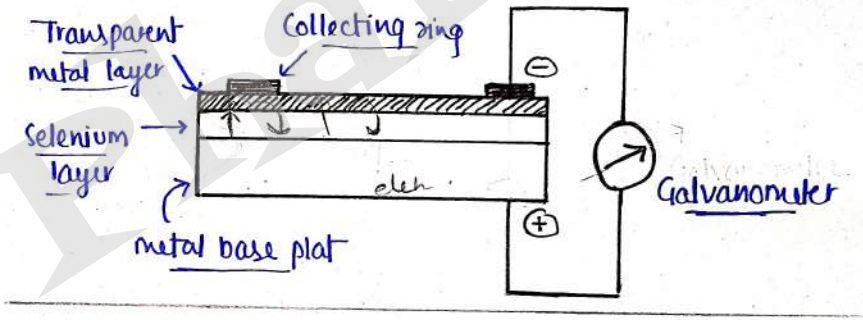
- The principle employed in this detector is multiplication of photoelectrons by secondary emission of electrons.
- It consist of a photocathode and a series of anode (dynodes).
- Each dynode is maintained at 75-100V higher than the proceeding one & electron emission is multiplied by a factor

of 4 to 5 due to secondary emission of electrons, hence an overall factor of 10^6 is achieved.

- PMT can detect very weak signals.
- Now, finally the large number of electrons arrive at collector (anode).
- The number of electron falling on the collector measures the intensity of light incident on the cathode surface.
- The response time of PMT is 10^{-9}s.

• Photovoltaic cells (Barrier-layer cells)

- Also known as Photronic cell and operates without the use of a battery
- It consists of a metal base plat (iron/aluminium) that act as an electrode, on this thin layer of semiconductor metal (like selenium) is deposited.
- then this covered by a very thin layer of silver or gold that act as second collector electrode



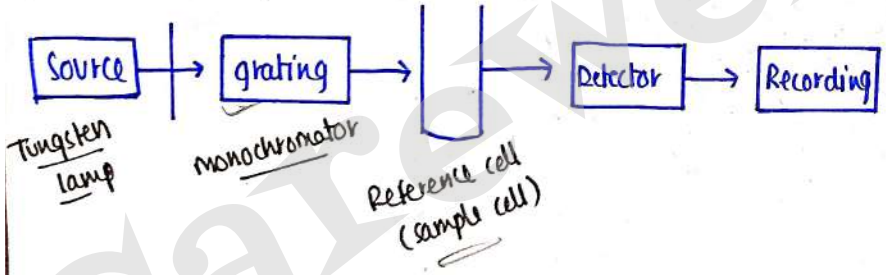
- When light radiation falls on selenium layer, electron are generated at the selenium - silver interface.
- These electrons are collected by the silver, this creates a electric voltage difference b/w the two electrode (silver & base cell) and causes the flow of current.
- Now, this flow is directly proportional to the intensity of the incident radiation beam.

v) Recording systems →

The signal from the detector is finally received by the recording system, it can be done by a recorder pen.

UV - Visible Spectrophotometer

1) Single beam UV spectrophotometer :—



- Step involved :—

i) UV radiation is given off by the source

ii) A convex lens gathers the beam of radiation and focuses it on the slit.

iii) The inlet slit permits light from the sources to pass, but block-out stray radiation

iv) The light then reaches the monochromator, which split it up according to wavelength.

v) The exist slit is positioned to allow light of the required wavelength to pass through.

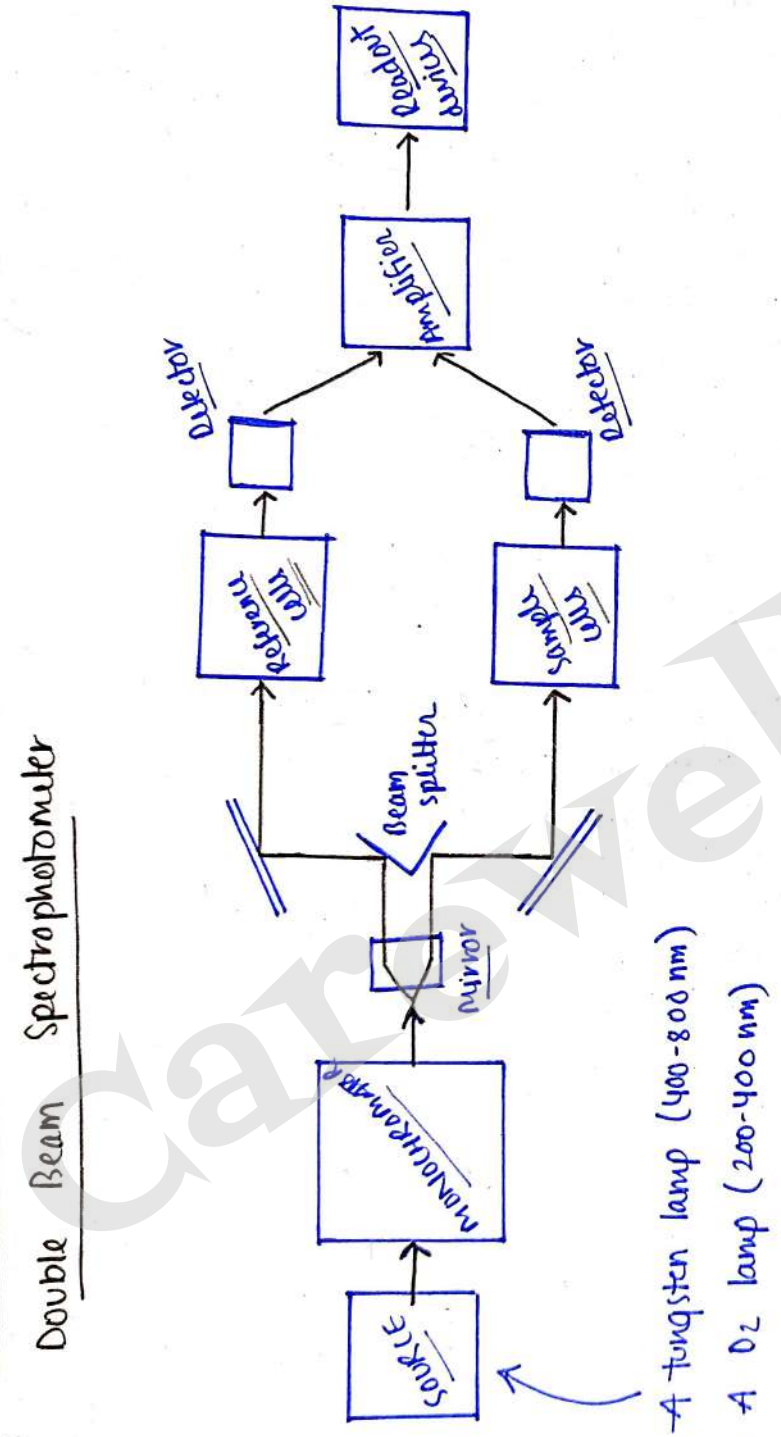
vi) Radiation at all other wavelength is blocked out

vii) The selection radiation passess through the sample cell to the detector, which measures the intensity of the radiation reaching it.

viii) By comparing the intensity of radiation before and after it passes through the sample, it is possible to measure how much radiation is absorbed by the sample at the particular wavelength used.

ix) The output of the detector is usually recorded on graph paper.

Double Beam Spectrophotometer



• Step involved :—

i) The radiation from the selected source passes through a fixed slit to the surface of reflection grafting (monochromator), and from the diffracted radiation, the desired wavelength is selected.

ii) The selected beam of light falls on V-shaped mirror, called a beam splitter that splits the radiations into two beam; one of which passes through the reference cell containing pure solvent and other simultaneously passes through the sample solution cell.

iii) The transmitted light from the two cells go to the photoelectric detector alternatively, and the difference in the absorbance by the solvent and the sample solution is measured electronically with very high accuracy.

- Applications of UV Visible Spectroscopy

UV-Vis spectroscopy has been mainly applied for :—

i) Detection of functional groups - It is applied to detect the presence or absence of a chromphore.

ii) Extent of conjugation - conjugation can shifts the absorption to longer wavelength. (↑absorption & ↑wavelength)

iii) Identification of a unknown compound - By comparing the spectrum of an unknown compound with that of a known compound.

iv) Detection of impurities - Additional peak can be due to impurities in sample and can be compared with that of standard materials.

v) Difference in conjugated & non-conjugated compounds - By comparing their wavelength.

※ Quantitative analysis :—

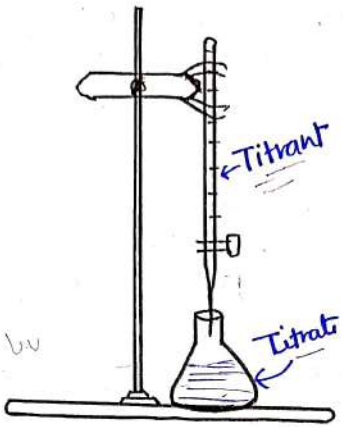
i) Spectrophotometric titrations
ii) Single components analysis
iii) Multi component analysis

i) Spectrophotometric titration →

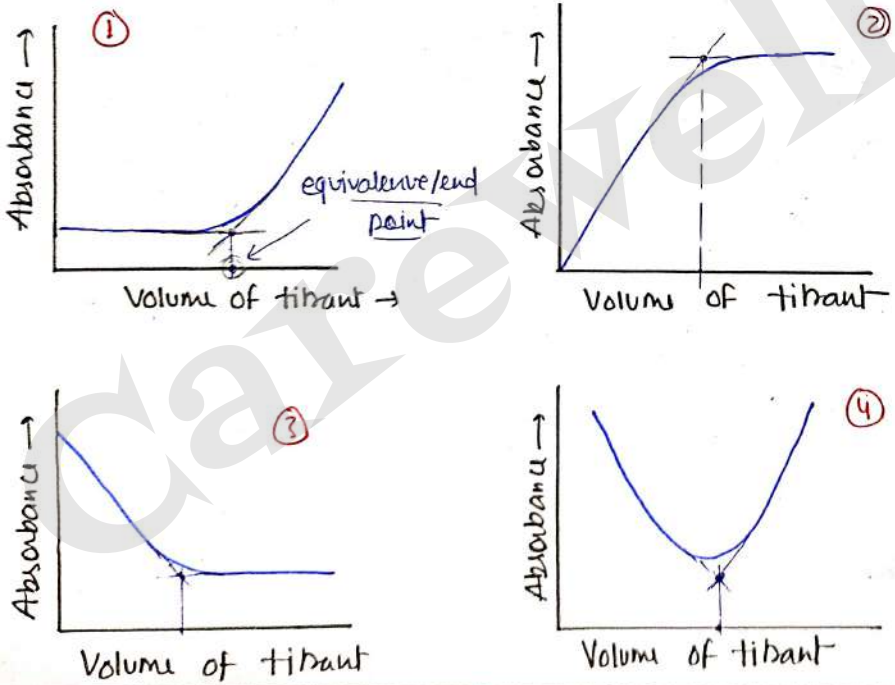
It is the process of determining the quantity of a sample by adding measured increments (increasement of a titrant until the end point...

- The endpoint is where the graph is discontinuous.

- The titrations are based on Beer's law ($A \propto C$)
- Titration curve is plot of absorbance Vs volume of titrant



Titration Curves



ii) Single Component analysis →

If sample is consists of only drug that absorbs radiation and excipient does not absorb radiation at λ_{max} of drug.

- Methods use - Standard absorptivity value, By using $A = abc$, By using Beer's curve. etc.

iii) Multicomponent analysis → (A ∝ C)

If same sample has more than one drug and all of them absorb at same wavelength then this analysis is required

OR

If sample carry excipients, impurities product which absorb at λ_{max} of drug then multicomponent analysis is required.

- Methods use -
  - Simultaneous equation method
  - Difference spectrophotometry.
  - Absorptivity factor method
  - Dual wavelength method

etc..

XX — Completed — XX

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