Chapter:

Molecular Spectroscopy

UV-Visible Spectroscopy

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Chemistry with MJS

Chemistry Preparation by MJS

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(SPECTROSCOPY)

Interaction of E-M radiations with matter.

(1) Importence / Scope :-

* Electronic transitions takes placed due to absorbence of electromagnetic radiation.

(i) All types of organic molecules which have synthesized, they are identified in spectroscopy.

→ Identification may be of atoms or molecules.
→ Structure elucidiation (NMR) / MS
→ Identification of functional groups. (IR)
→ Identification of Configurational isomers.
→ Colored / colorless (UV- Visible)

* Spectroscopy may be

(1) Atomic Spectroscopy
(i) AFS
(ii) AAS
(iii) AES
(iv) Coupling Technique. (GC-MS)

(2) Molecular Spectroscopy:-

(i) UV-Visible

(ii) Infra-Red

(iii) Nuclear magnetic Resonance (NMR)

(iv) Mass Spectroscopy. (MS)

"Best Technique for Structure elucidation is NMR." "

* Colored Compound = Visible light absorbed.
* Colorless Compounds = UV.

(Spectroscopy)

" Interaction of electromagnetic radiation with the matter (Analyte/Sample/Compl molecules etc) "

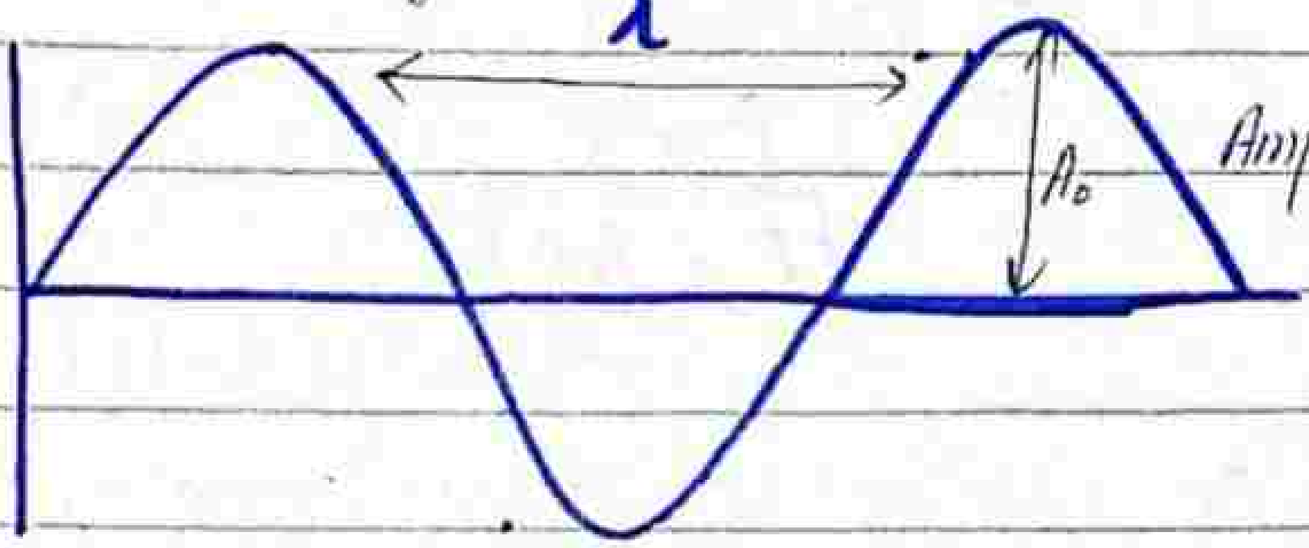
* Electromagnetic Radiations :-

Radiation which oscillate matter due to electric and magnetic charges.

Form of E produced as a result of electromagnetic process and is known as **radiant Energy**.

→ They do not require any medium to propagate. (Vacuum) ✓

They are mutually ⊥ and are co-planar.



★ **Frequency:-** (f)

→ No. of wave pas from one point in one second is called frequency.

→ f

→ Unit = Hertz. or Cycle/s (cs^{-1})

★ **Wave Number:-** ($\bar{\nu}$)

→ Wave passing in one cm distan in one second is wave numbe

→ cm^{-1} (Reciprocal cm)

★ **Amplitude:-** (Å) m

Max distance / Displacement of a wave is called amplitude.

* From average position of EM

★ **Wave length:-** The distance between two crest or troughs of the wave.

$E \propto f, \bar{v}$

$E \propto \frac{1}{\lambda}$

* Energy :-

→ Joules.

→ $E \propto$ frequency

$$E \propto f$$

$$\boxed{E = hf}$$

* Or Energy is inversally proportional to wavelength.

$$E = \frac{hc}{\lambda}$$

* Wave no. is inverse of wavelength.

$$E \propto \bar{v}$$

$$f \propto \bar{v}$$

$$\lambda \propto \frac{1}{\bar{v}}$$

$$\boxed{E = hc\bar{v}}$$

* h = plank's constant = 6.63×10^{-34} Js

* C = 3×10^{10} cm/s or 3×10^{8} m/s.

* Numerical :-

An electromag. Radiation propagates with wavelength $\lambda = 600$ Å. What will be its energy.

$$E = \frac{hc}{\lambda}$$

$$E = \frac{6.63 \times 10^{-34} \times 3 \times 10^{8}}{600 \times 10^{-10}}$$

$$E = 3.315 \times 10^{-19}\ J$$

(Conversions)

dm

cm 10x

mm

μm.

nm

pm

cm

fm

am

1 of m = dm

$10 \times 10^{-15}\ m =$

$10^{-14}\ m = 10^{-8}$ dm

2.5×10^{-2} cm → 40 cm^{-1}

40 cm^{-1}

4×10^{2} nm ⟶ cm^{-1}

$4 \times 10^{2} \times 10^{-9}\ m$

$4 \times 10^{-7}\ m = 4 \times 10^{-5}$ cm

4×10^{-5} cm = 25000 cm^{-1}

small → large divide.

$\frac{4 \times 10^{2}}{10^{7}}$: $\frac{4 \times 10^{9}}{1 \times 10^{10}}$

$\frac{4 \times 10^{3}}{10^{+7}}$: 4×10^{-5}

100 cm → mm

10^{-2} 10^{-3}

100 cm → 10 X 100 mm

1000 mm.

4×10^{2} nm ——— mm.

n

E	10^{18}	G	10^{9}	H
P	10^{15}	M	10^{6}	
T	10^{12}	Kg	10^{3}	

(1) 5×10^{-3} nm = ——— m

(2) 0.3×10^{-1} cm = ——— μm

(3) 2×10^{-10} m = ——— A°

(4) 3×10^{-11} cm^{-1} = ——— μm

(Conversions)

→ When we have to convert **Smaller unit** into **Bigger unit** We divide it with the gap

* **Example :-**

4×10^{2} nm into ——— cm

$\frac{4 \times 10^{2}}{10^{7}}$ cm = 4×10^{-5} cm

(1) 5×10^{-3} nm into m

$$\frac{5 \times 10^{-3}}{10^{9}} = 5 \times 10^{-12} \text{ m}$$

$5 \times 10^{-3} \times 10^{-9}$ m $= 5 \times 10^{-12}$ m

(2) 0.3×10^{-1} cm into μm

(1) $= 0.3 \times 10^{-1} \times 10^{-2} \times (10^{-6}) \times 10^{6}$

$= 0.3 \times 10^{3}$ μm

(2) $0.3 \times 10^{-1} \times 10^{4}$ $\quad 10^{0} = 1$

0.3×10^{3}

(3) 2×10^{-10} m into Å

$= 2 \times 10^{-10} \times 10^{-10} \times 10^{10}$ Å m

$= 2$ Å

(4) 3×10^{-11} cm^{-1} into μm

$= \frac{1}{3 \times 10^{-11}}$ cm

$= 0.33 \times 10^{11} \times 10^{-2} \times 10^{-6} \times 10^{6}$

0.33×10^{15} μm

LEC #2

31-May-22

* ElectroMagneticRadiations :-

→ IR, UV-Visible, Microwaves, Radiowaves.
X-Rays, γ-rays, cosmic rays.

→ Velocity is same for each ($3 \times 10^8 ms^{-1}$) but wavelength and frequency varies.

Cosmic Rays · γ-rays · X-rays · UV-Visible · IR · Microwaves · Radiowaves.

$\lambda \uparrow$

$E \downarrow$

$E \propto \frac{1}{\lambda}$

NMR = Radio waves

UV-Visible = Ultraviolet

Range :- λ

UV = 10 – 400nm

Visible = 380 — 780 / 400 — 800nm

IR = 10 – 14000 cm^{-1} (Wave no.)

Rdiowavel = 600 MHz — 900 MHz

10 – 400 Far IR | 400 – 4000 cm^{-1} Mid | 4000 – 14000 cm^{-1} Near-IR |

(2) 10 - 400 nm → cm

10 nm → cm

$10 \times 10^{-9} \times 10^{-2} \times 10^{2}$ m

= $\boxed{10 \times 10^{-7}\ cm}$

(2) 400 nm → cm

= $400 \times 10^{-9} \times 10^{-2} \times 10^{2}$

= $\boxed{400 \times 10^{-7}\ cm}$

(3) = $10 \times 10^{-7} \times 10^{-2} \times 10^{-6} \times 10^{6}$ m

= $\boxed{10 \times 10^{-3}\ \mu m}$ = 0.01 μm

(4) = $400 \times 10^{-7} \times 10^{-2} \times 10^{-6} \times 10^{6}$

= $\boxed{400 \times 10^{-3}\ \mu m}$ = 0.4 μm

→ **UV- Visible** light / Spectroscopy carries out electronic transitions.

→ While **I-R** radiations only vibrates (bond)

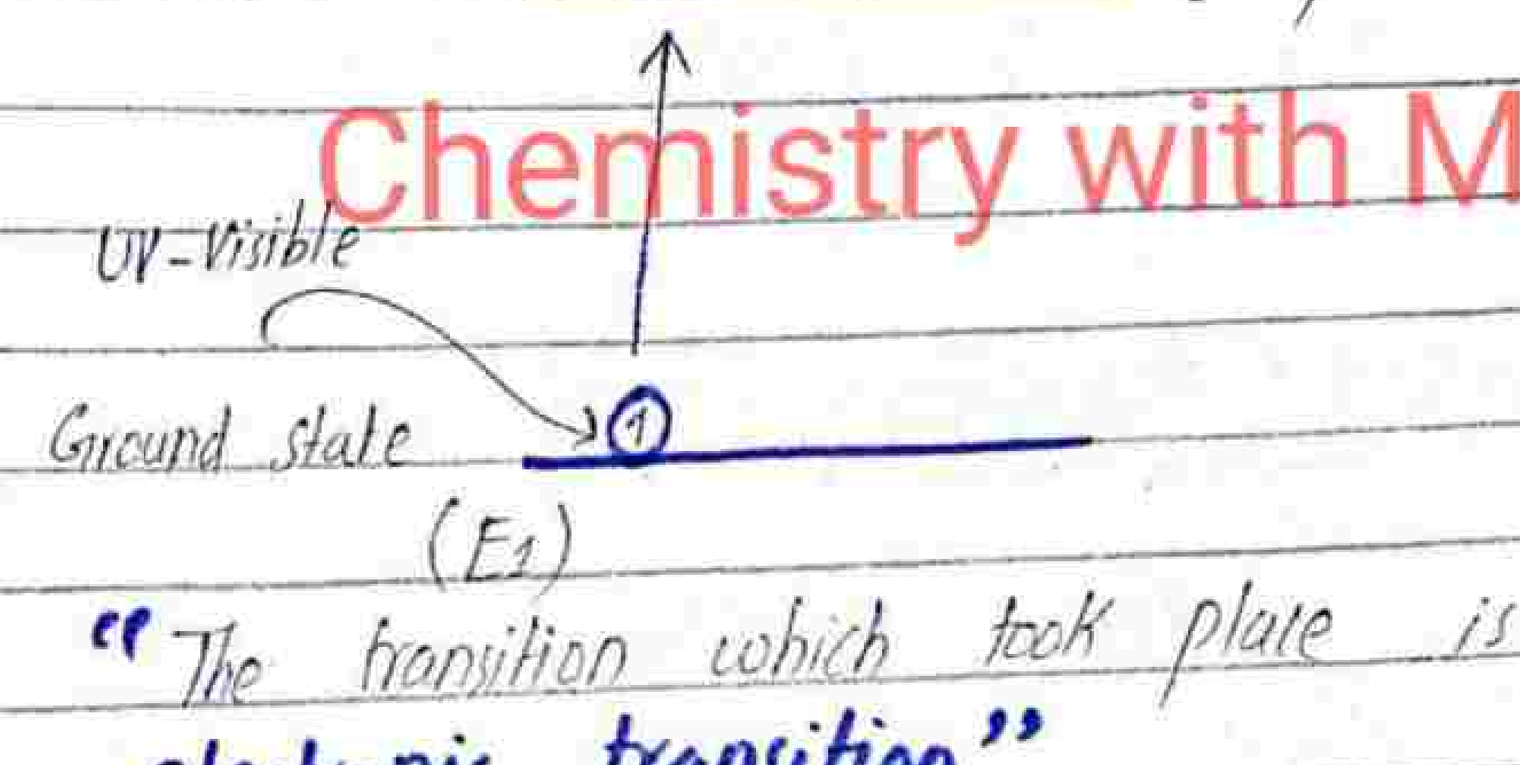
→ **Micro-** waves carry out only rotational transitions.

(When we deal with electronic transitions, all other transitions are involved (Vibrational + Rotational also).

"UV-Visible Spectroscopy"

* Interaction of Ultraviolet radiations with the matter Visible

Principle: Electronic Spectroscopy



"The transition which took place is electronic transition"

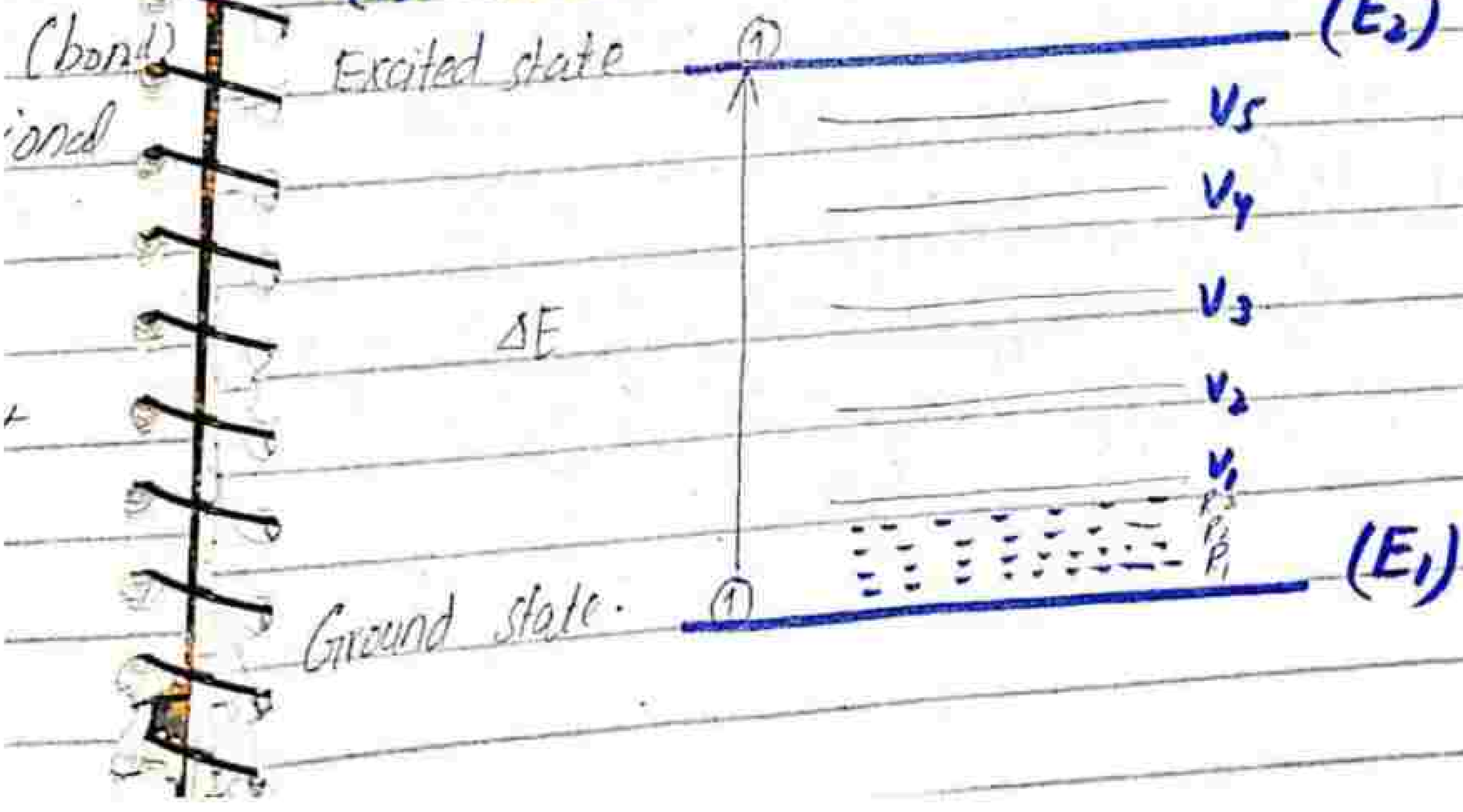
* Transitions / Energy levels.

These are mainly three.

(1) Electronic transitions :-

(2) Vibrational transitions

(3) Rotational transitions.



* In UV = [200-400nm] region is best / Significant.

- It is also called **Electronic Spectro-scopy.**
- All three types of transitions are happening in UV- Visible
- In case of IR (Vib + Rot) no Electronic
- In microwaves, only **rotational** transitions.

UV - Visible

$\lambda \downarrow$ (10 - 400 nm) $\quad$ $\lambda \uparrow$ (400 - 800 nm)

$E \uparrow$ $\quad$ $E \downarrow$

* Regions (10 - 400nm) UV

Ultraviolet light is used.

→ **10-200nm** Far-UV (C) } Vacuum UV
O_2, N_2 are absorbed.

→ **200-300 nm** Mid-UV (B) } Significant

→ **300-400 nm** Near UV (A)

* Far-UV

→ As O_2 & N_2 are absorbed, so evacuation have to be done. "Evacuated Instrument" is required which is expensive.

→ High λ_{max}

→ Low E required

* Visible Region :- (400 - 800)

(380 - 780)

→ Visible light for colored compounds

→ $\lambda\uparrow$ $E\downarrow$

→ There is some conjugation highly (shifting of π e⁻). As π-bond is weaker than σ bond. So, less energy is required to shift π-electrons. ↑ λ_{max}

(More conjugation = Less Energy) high.

→ As a result wavelength will be

Conjugation ↑ ΔE ↓ λ ↑

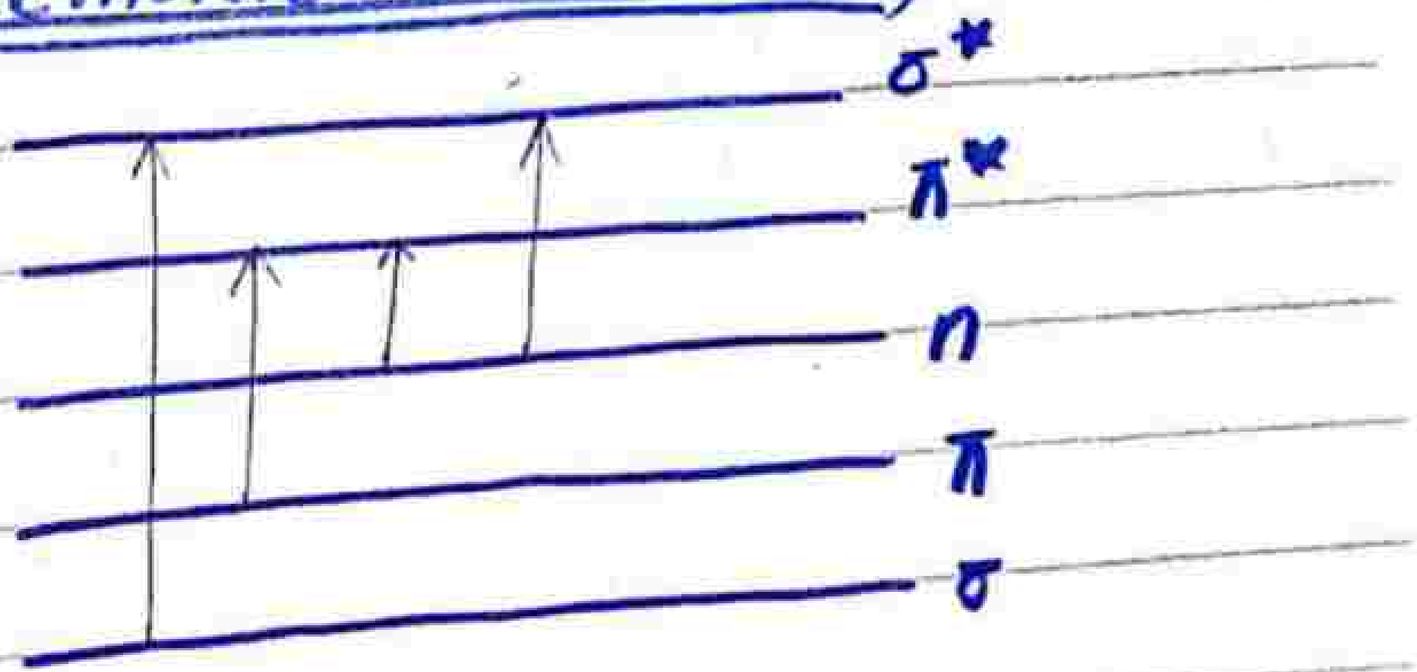
465nm

→ Lycopene in Tomato (Highly Conjugation)

→ β-carotene in Carrot

450nm

(ELECTRONIC TRANSITIONS)



(i) **n - Transition**

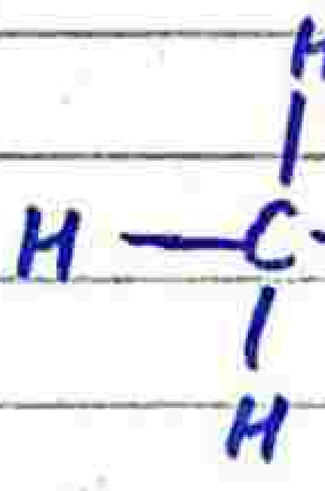
It is due to lone-pair or non-bonding electrons

(ii) **σ - Transition :-**

It is due to the σ - electrons/ bonds.

(iii) **π - transition .**

Unsaturation / multiple bond(s) results the π transition.



it shows only ($\sigma - \sigma^*$)

* Transitions :-

(i) $\sigma - \sigma^*$

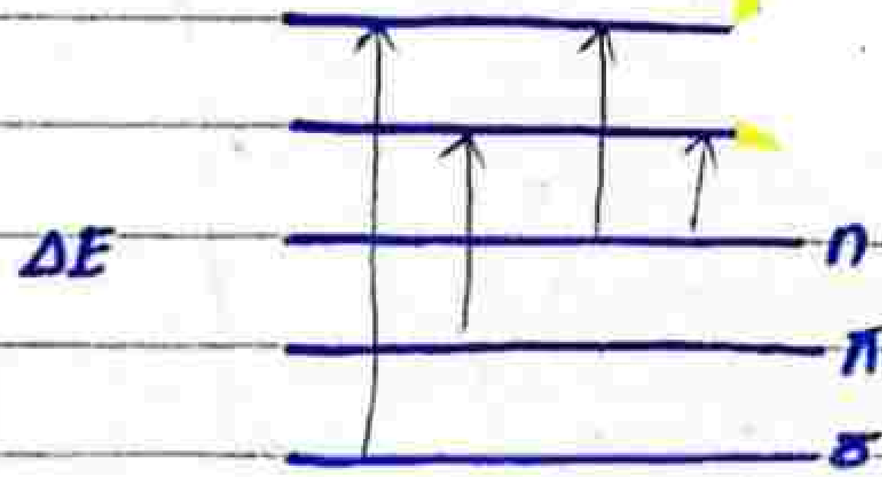
(ii) $n - \alpha^*$

(iii) $\pi - \pi^*$

(iv) $n - \pi^*$

(Lecture No. 3)

* Electronic transitions :-



$\sigma - \sigma^* > n - \sigma^* > \pi - \pi^* > n - \pi^*$

Easily transition occurs

→ Practically not possible because much higher energy is required.

(1) $\sigma - \sigma^*$:-

Methane, propane, Octane, all alkanes + Cyclo-alkanes.

(2) $n - \sigma^*$:-

lone pair and Sigma bond is required

$H_2\ddot{O}$, $\ddot{N}H_3$, $CH_3\ddot{O}H$, $CH_3-\ddot{O}-CH_3$

(3) $\pi - \pi^*$:-

Containing unsaturation, Alkenes, Alkynes

$CH_3-\overset{\overset{O}{\|}}{C}-\ddot{O}H$, $CH_3-\overset{\overset{O}{\|}}{C}-H$, $CH_3-\overset{\overset{O}{\|}}{C}-CH_3$

(4) $n - \pi^*$:- Containing lone pair and unsaturation.

Transitions

$\pi - \pi^*$
$n - \pi^*$

Allowed Transitions

High probability of occuring

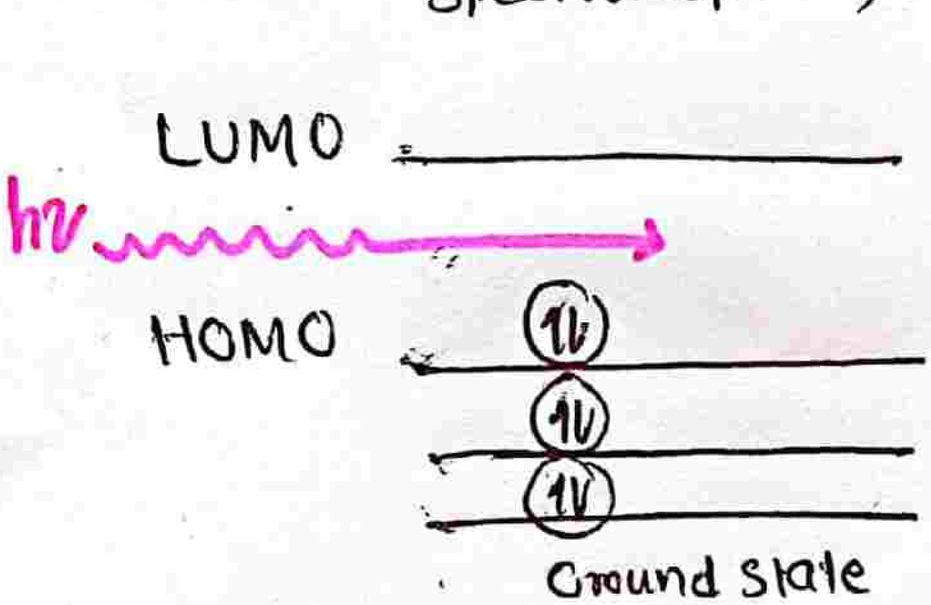
promotion of $\bar{e}$ from HOMO → LUMO (permitted in UV-spectroscopy)

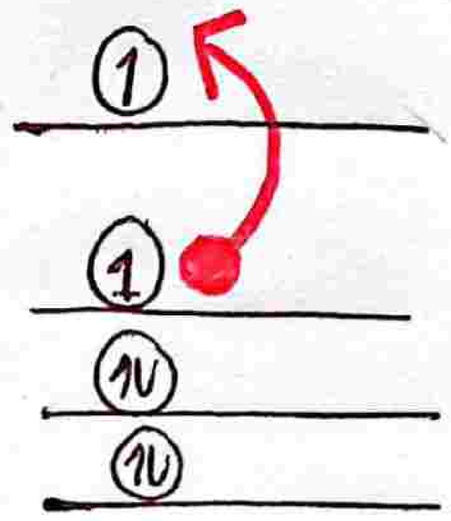
Forbidden transitions

minimum or no probability to occuring

e.g $\sigma - \sigma^*$ Forbidden

V.V. High Energy of photon Required

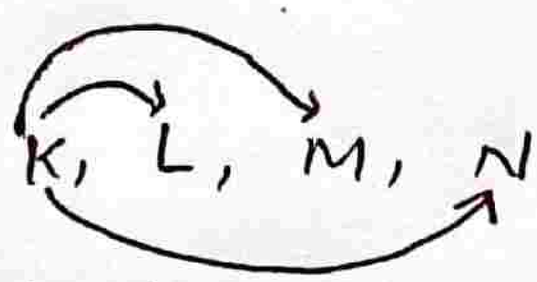




V.V.imp **SELECTION Rules:**

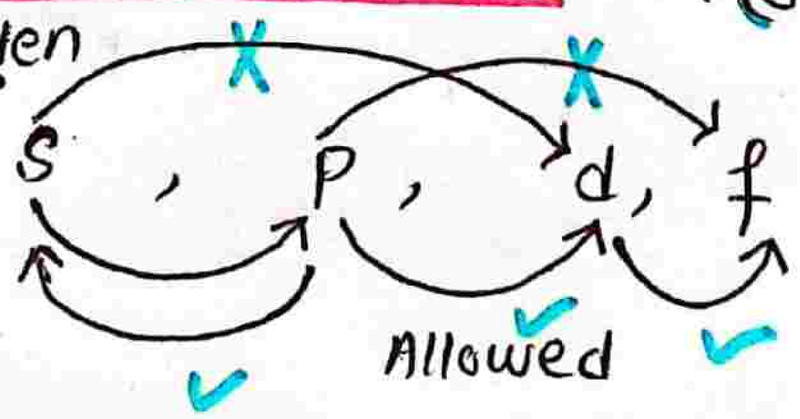
① **Energy level transitions:** Δn (Anything) Energy levels

All are Allowed transitions

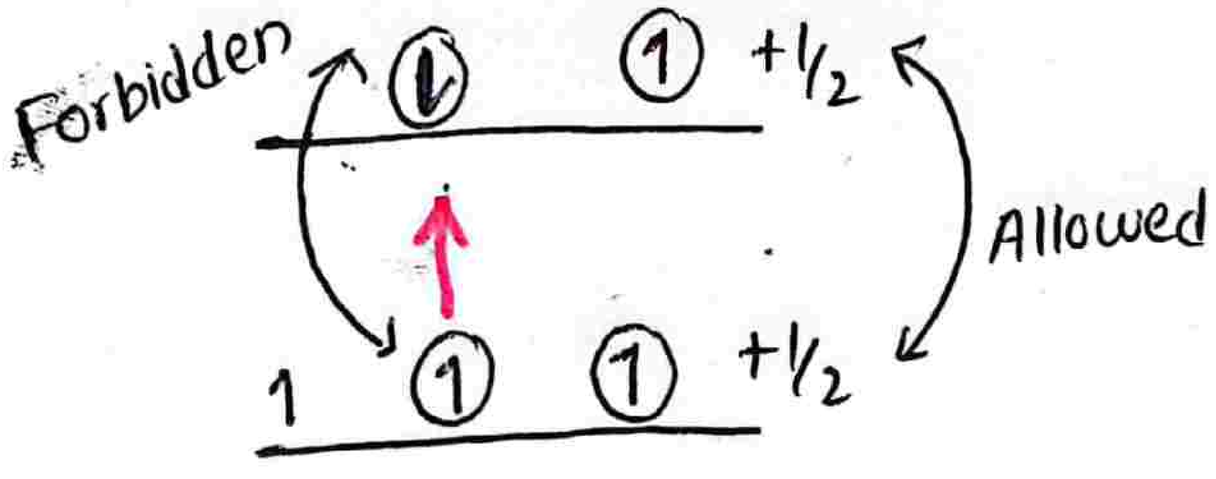


(Allowed)

② **Laporte selection Rule:** → (Orbitals) → $\Delta l = \pm 1$ only



③ **Spin-selection Rule:** ⟶ ($\Delta S = 0$)



$\Delta S = +1/2 - (+1/2) = 0$

* Blw same spins → Allowed
  e.g singlet-singlet, triplet-triplet
* Blw Diff-spins → Forbidden
  e.g S → D , T → S
  OR D → T

-: ABSORPTION THEORY :-

(1) Lambert's law + Beer's law :-

→ Lambert's takes width of solution or width of Cuvette ⇒ Sample cell

→ Beer's took conentration of solution as parameter

* Lambert says :-

width of solution ∝ Absorbenie

$$A \propto l$$

* Beer says :-

Absorbenie ∝ Conentration

$$A \propto C$$

$$A \propto lc = \boxed{A = \varepsilon lc}$$

∵ ε = Absorptivity coefficient/coefficient of extinction. It tells about the strength that how much absorption has occured.

* **Unit :-**

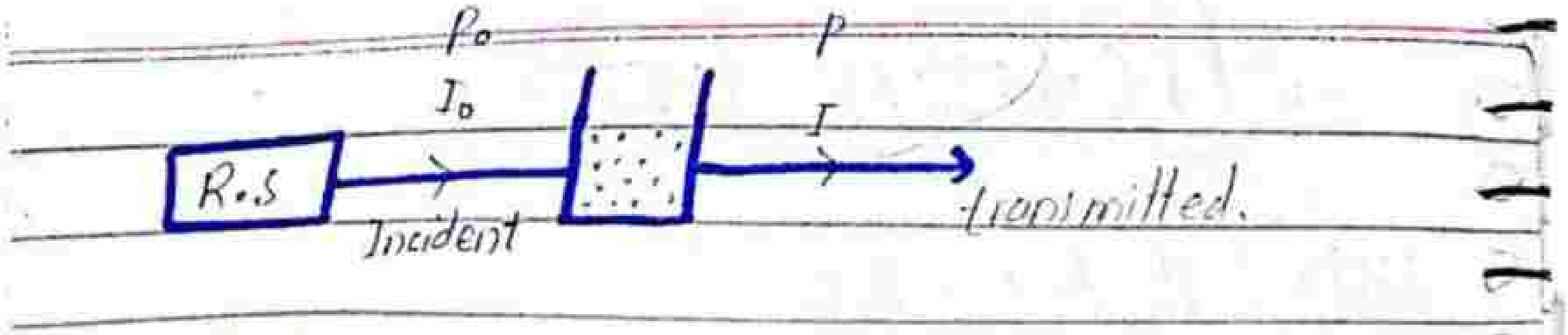
$$\varepsilon = \frac{A}{lc} = \frac{\text{No unit}}{cm \times gL^{-1}}$$

$$\boxed{\varepsilon = cm^{-1} g^{-1} L}$$

* Molar Absorptivity coefficient :-

$$\varepsilon = \frac{A}{lc} = \frac{\text{No unit}}{cm \times mol\,L^{-1}}$$

$$\boxed{\varepsilon = cm^{-1} mol^{-1} L} \quad / \quad \boxed{cm^{-1} mol^{-1} dm^3}$$



Modes ⟶ Absorption / Transmittence

$$T = \frac{I}{I_0} = \frac{P}{P_0}$$

P = Power of transmitted light

P_0 = Power of incident light.

$$A = \log \frac{1}{T}$$

$$A = -\log T'$$

$A = 2 - \log$ (percent

$\log (\%\ T) = 2 -$

$\%\ T =$ Ant

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

$$T = \frac{I}{I_0} = \frac{P}{P_0}$$

$A = \log \frac{1}{T}$ $\left(\frac{1}{T} = T'\right)$

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

Also $A = 2 - \log (\%\ T)$

$2 - A = \log (\%\ T)$

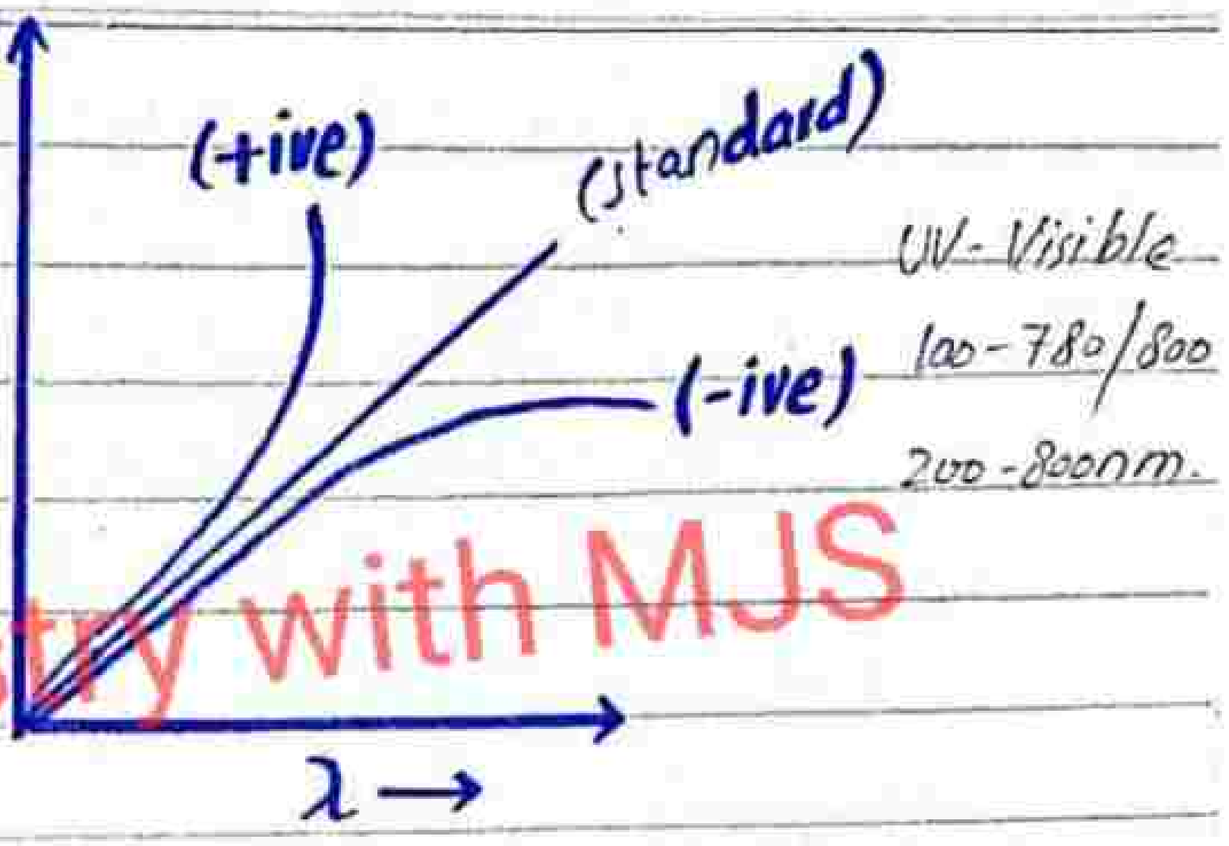
$\%\ T =$ AntiLog $(2 - A)$

★ **Numericals:-**

→ A sample in 1cm cell transmits 80% light at certain wavelength. If the absorptivity is 2, what will be conc.?

→ Molar absorptivity of analyte is 2×10^4 calculate percent transmittence and width of cuvette is 5cm and conc is 10^{-6} M.

When a beam of monochromatic radiation is passed through a solution of an absorbing medium, the decrease in intensity of light with is directly proportional to the incident light as well as the absorbing medium.



→ It gives absorbence value at each wavelength (201, 202, ... 800nm)

→ If straight line occurs, it follows Lambert Beer's law.

↳ This law is applicable on the solution which is neither too concentrated, nor too dilute.

* Limitations + Factors Affecting B-L-Law :-

1. Concentration :-

Lambert beer's law is applicable to dilute solution. If solution is highly concentrated molecules get aggregated and come into crystalline form and actual absorbance does not occur. Actual standard values does not come and deviation occurs.

* Too much diluted → A - Less
(pre - concentrable)

Too much concentable → A higher
(solvent is added)

2 = **Radiation Source:**

Radiation source should be momochromat because we need fix λ_{max} max. Bassically. radiation source is polychromatic thesefore, we have to pass it from prism which converts it into monochromatic same wavelengthe.

3- **Interfering Substances:**

If interfering substance is presnt in solution, then it absorbs uv-light and hence analyte does not absorb this light and max vale does not match with standed.

4 = **Strag Radiation:**

Due to instrument error light does not

pass thraghd the analyte and reach to detector Detedor gives the signal.

5. **Stabillity of sample:**

Same compounds are unstable so you have to run it immedialely on Vn-spentrophlometer But, if you run it after something it (may) give diffent result Due to light intesity, they get decompaeed. Therefore, we have to look in dark.

6. **Effed of Temperature:**

Temperature should be constand. if temperature of envisoment change then nature of anayte changes and it's got dissociated.

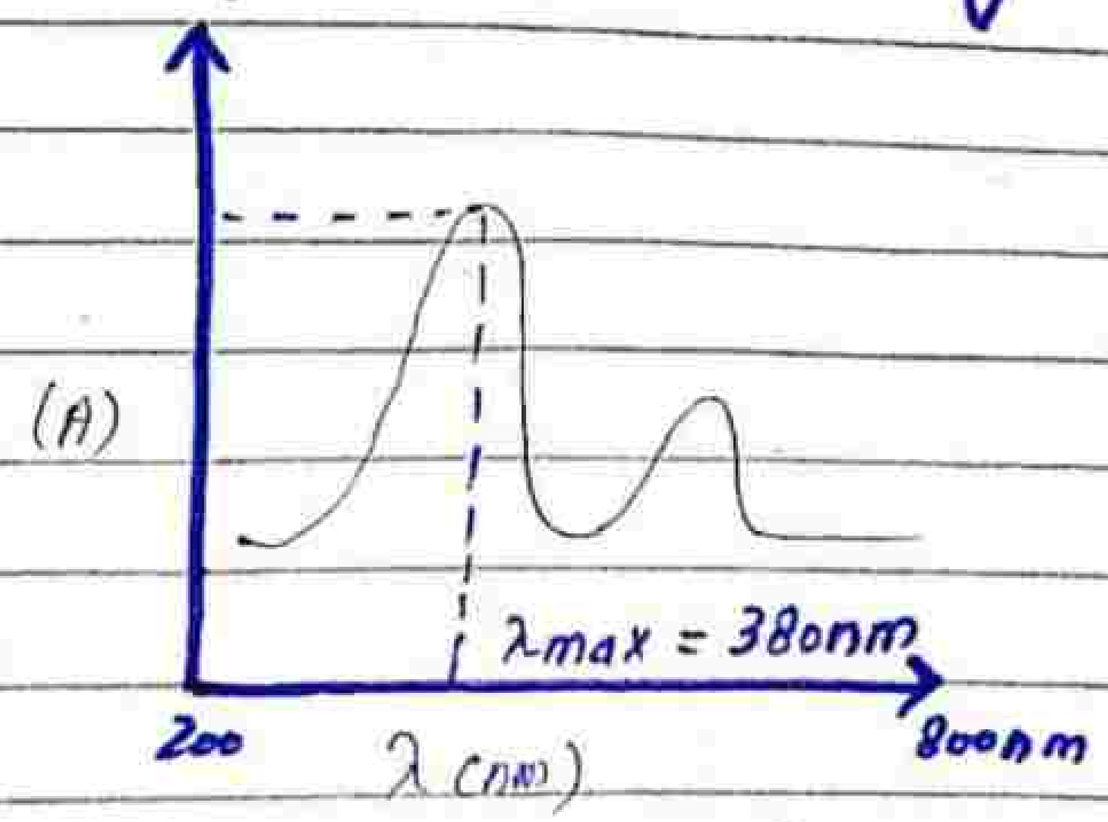
7* **Mismatch cells/Non-calibrated instrument.**

If instrument is not calibrated then result will be inaccurate.

Lecture No. 4

7-June-22

* Factors Affecting Absorption Intensity (λ_{max})



Characteristic wavelength = (λ_{max})

(1) Conjugation :-

- Aromatic
- Acyclic Diene System
- α,β-unsaturated Carbonyl Sys.

It is important factor which affect the absorption intensity.

10-400nm → (UV) Colorless Compounds.

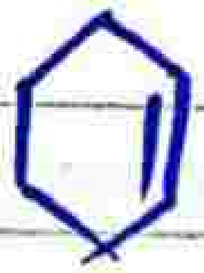
$-C=C-C=C-$ λ_{max} = 217nm

(1,3-butadiene)

As conjugation increases, the energy difference lowers

$CH_2=CH_2$: λ_{max} = 180nm

Ethene

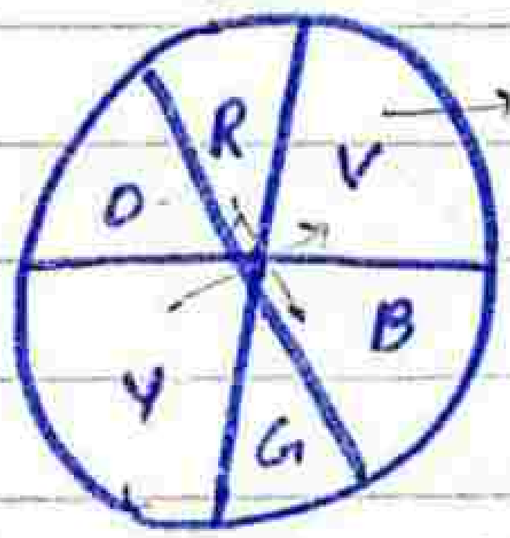


Cyclohexene

(λ_{max} = 182nm)

→ High (10 – 12 double bond) unsaturation is required for colored compounds.

* Colour Theory:-



→ 400 – 450nm.

(Munsel wheel)

λ increasing

V = Violet → Lowest wavelength. (400 - 450nm)

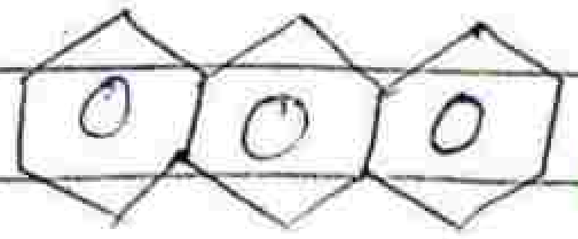
B = Blue

G = Green

Y = Yellow

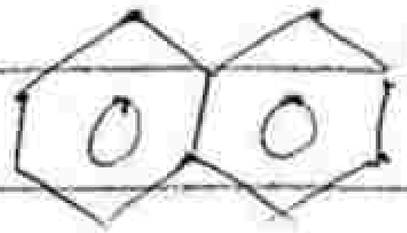
O = Orange.

R = Red → (Highest wavelength) (700 - 750nm)



(Anthracene)

λ_{max} = 400 - 450 nm

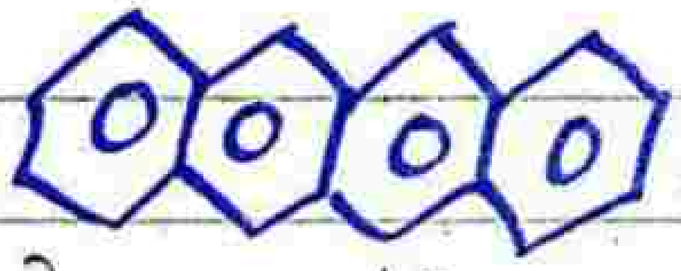


(380)

(Naphthalene)

λ_{max} = 314nm

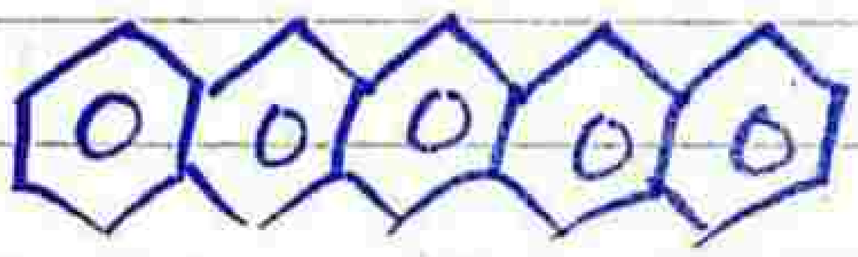
Colorless Compounds bcz no sufficient conjugation present.



(Tetracene)

λ_{max} = 480 (Naphthalene)

(Yellow) → Confirm



(Pentacene)

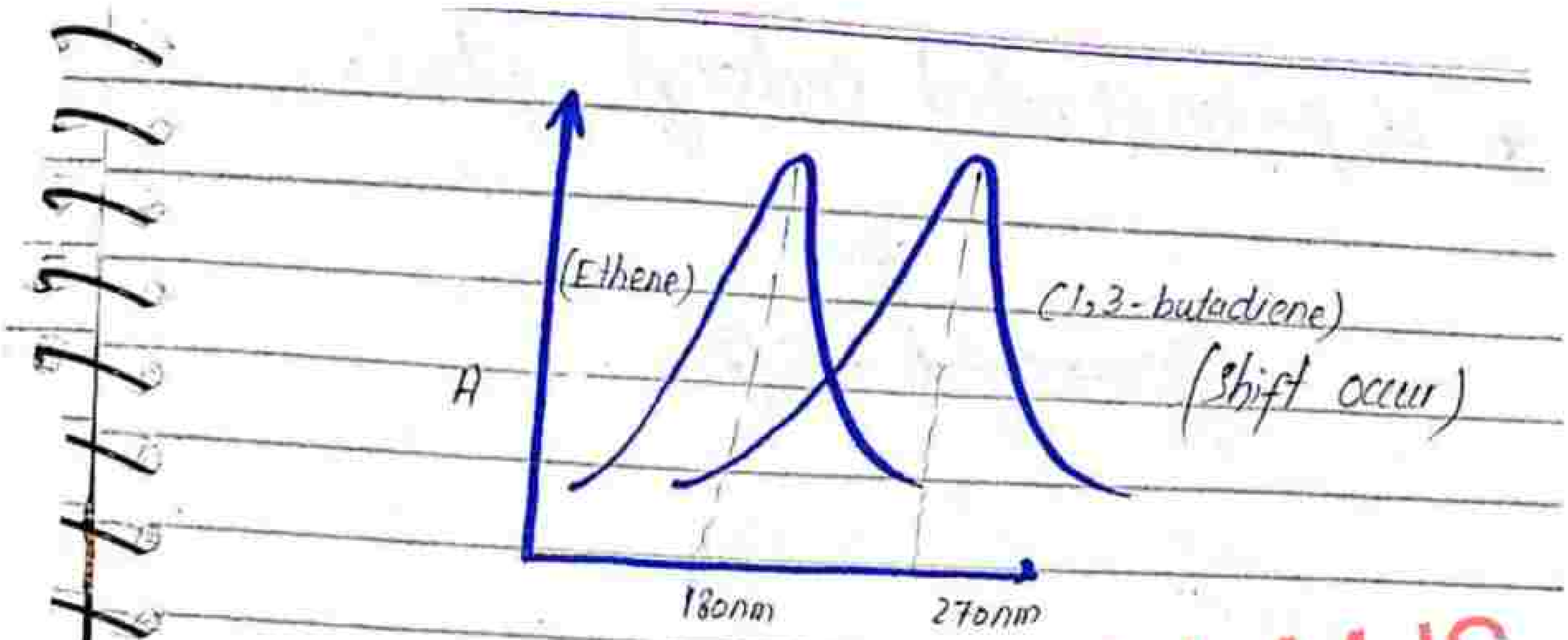
blue color

Lycopene ⟶ colored compound

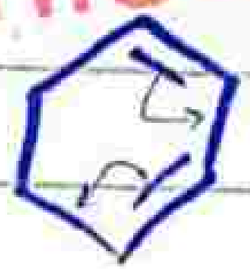
λ_{max} = 507nm (Red)

β-carotene ⟶ 497nm (Red)

(Red-orange carrots)



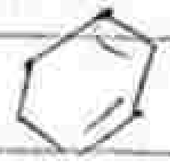
★ Diene



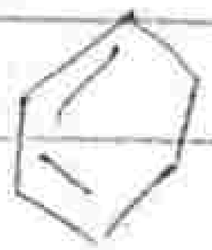
λ_{max} = 253nm

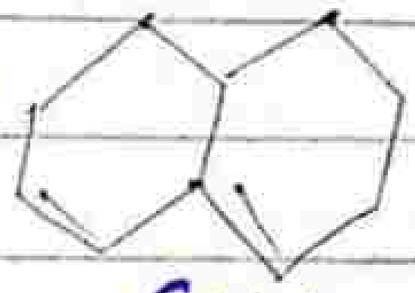
★ Polyene / Diene System :-

It has two type.

(i) Homoannular System 

(ii) Heteroannular System

(A) 

(B) 

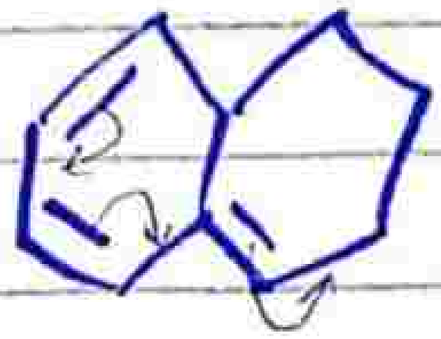
(Homo)

↳ Same ring Conjugation

(Hetero)

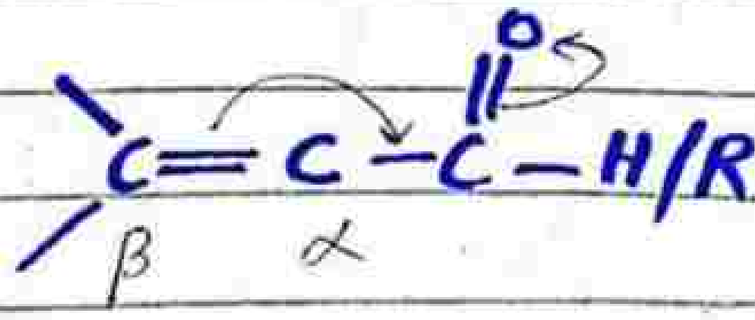
↳ Different ring

E↓ λ_{max} ↑

(iii) 

(Homo + Hetero)

* α, β- Unsaturated Carbonyl system:-



λ_{max} ↑ with increase in conjugation.

(2) Chromophores

→ The groups which are when attached with some groups, shifts its λ_{max} to visible. But it is older term.

But recently

→ " Those groups which absorb UV-Visible light, and attached to some compounds "

→ In actual, there are group which absorb UV/Visible

$>C=C<$ (Alkene)

$-N=N-$ (Azo)

$-C\equiv C-$ (Alkyne)

$-N^{+}(=O)-O^{-}$ (Nitro)

$>C=O$ (carbonyl)

$-C\equiv N$ (Cyano) Nitrile gp.

These groups can cause conjugation and increase the λ_{max}

* Auxochromes :-

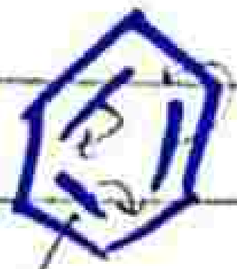
The groups which contain non-bonding electrons, and further extend the conjugation

They are attached to chromophores and conjugation exted and shifts λ max $\longrightarrow$

* $-\ddot{N}H_2$, $-\ddot{O}H$, $-\ddot{S}H$ (Thiols), $-\ddot{O}R$ (Ethers)

X (Halides), $-O-C(=O)-CH_3$

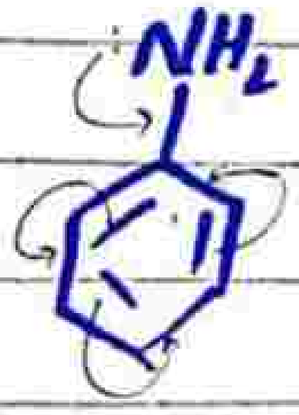
→ They do not absorb UV/visible, they just help increase in conjugation.



(3- shifts)

$\lambda_{max} = 182nm$

Double bonds = Chromophores.



λ_{max} = Visible

(4- shifts)

NH_2 = Auxochrome.

$CH_3-CH_2-CH=CH-\ddot{O}-R$ (Auxochrome)

↳ Chromophore.

Shifts in Absorption Spectra

★ **Red Shift:** (Bathochromic shift)

★ Shifting from $\lambda_{lower} \longrightarrow \lambda_{higher}$

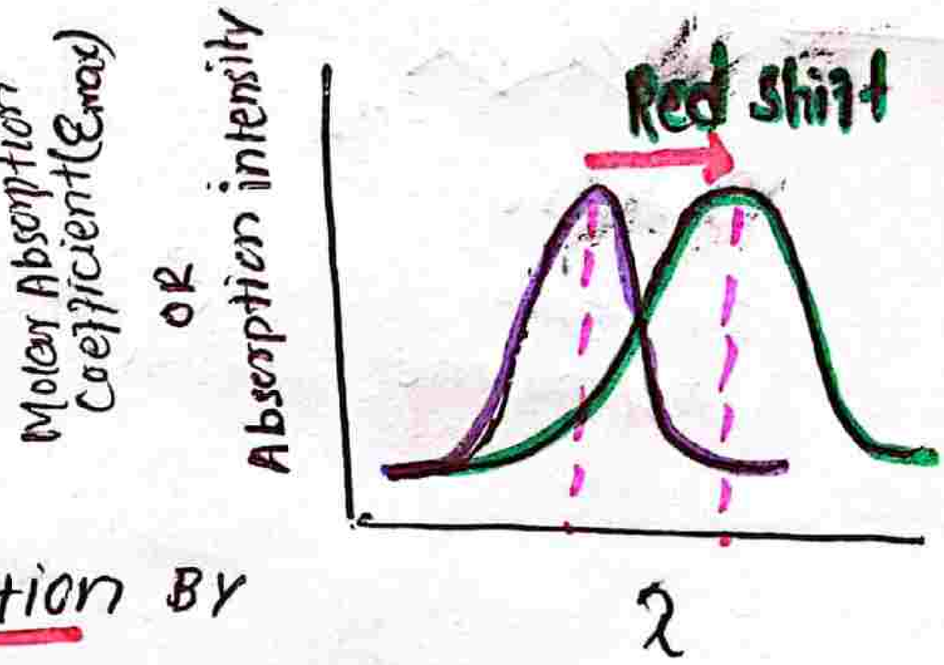
★ This shift is due to the Auxochrome groups- OR chromophores OR due to the solvent Effect.

↓

use the solvent of lower polarity

↓

more will be the Red Shift



★ increase conjugation BY two or more chromophoric groups.

★ **Blue shift:** (Hypsochromic Shift)

$\lambda_{higher} \xrightarrow{\text{shifting}} \lambda_{lower}$

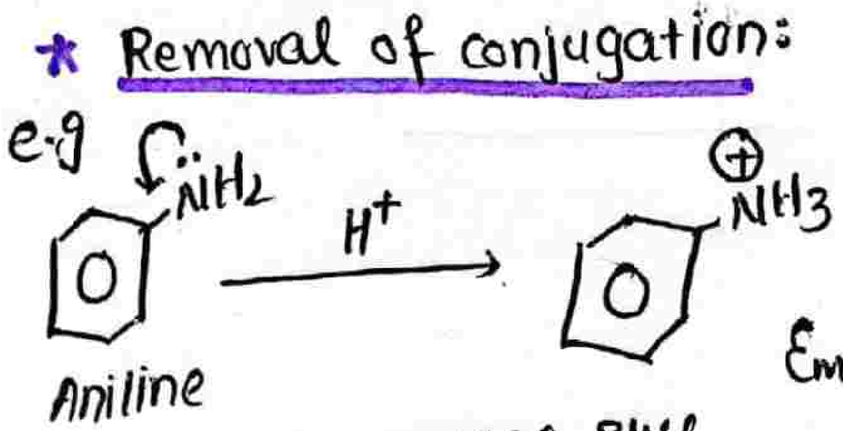
★ Removal of conjugation causes Blueshift.

★ change in polarity OF solvent (polar solvent)

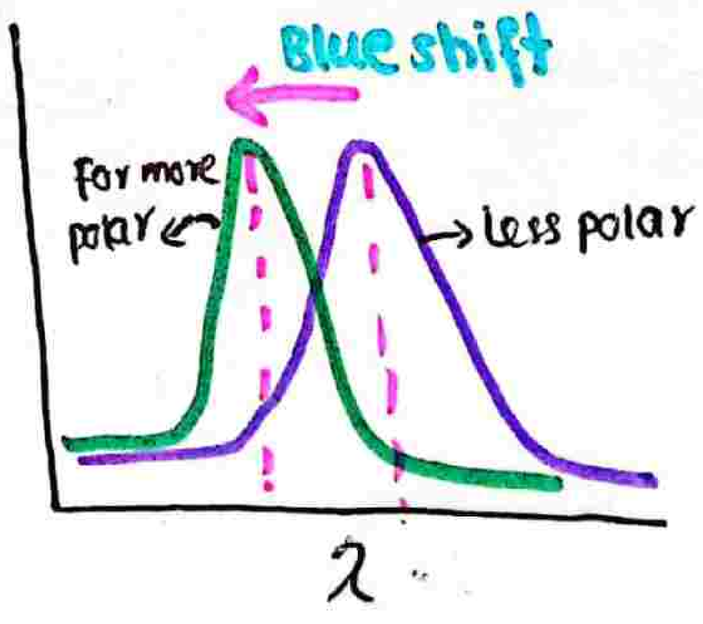
↳ when ground state interacts more strongly with the polar solvent, polar solvents → stabilize the ground state more than Excited state, so overall energy gap BTW excited & ground State increases Resulting lower λ → Blue shift.

★ Removal of conjugation:

e.g



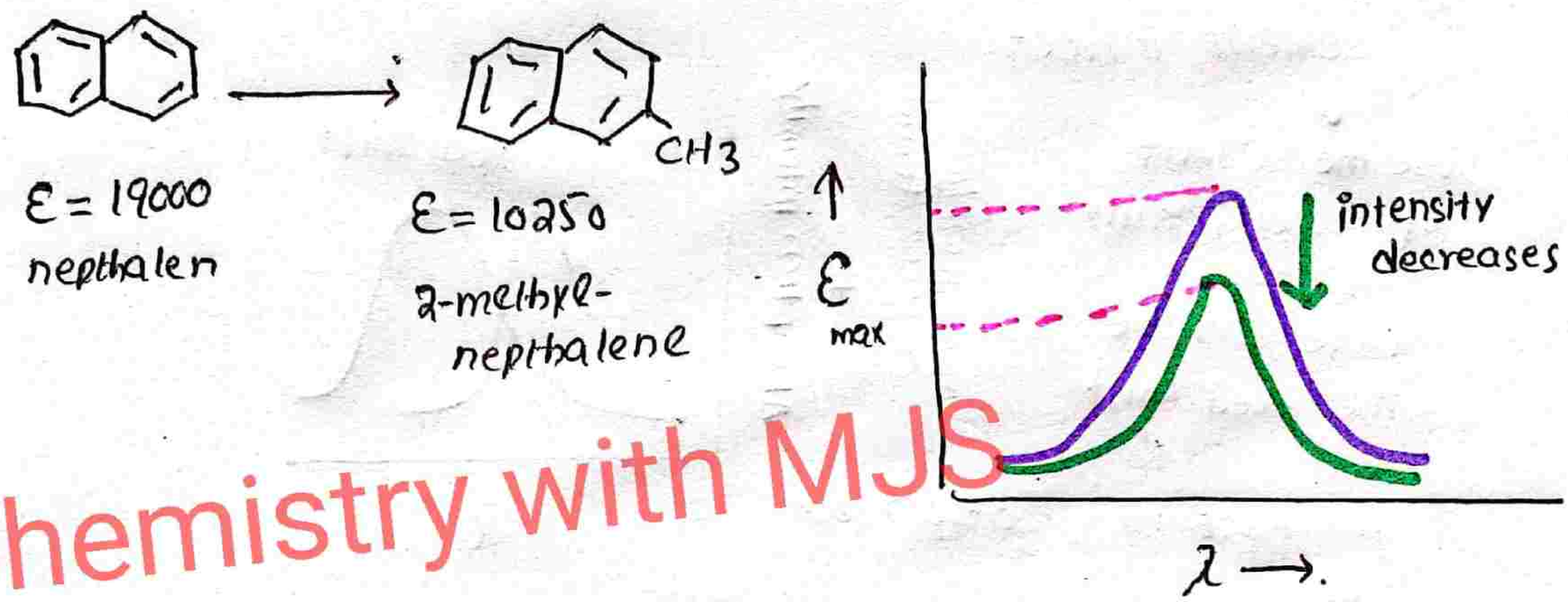
causes Blue shift



★ **Hypochromic shift:** → Related to Absorption intensity (ε_{max}) → molar Absorptivity

Absorption (I) ⟶ Lowest
(ε) (highest) (ε)

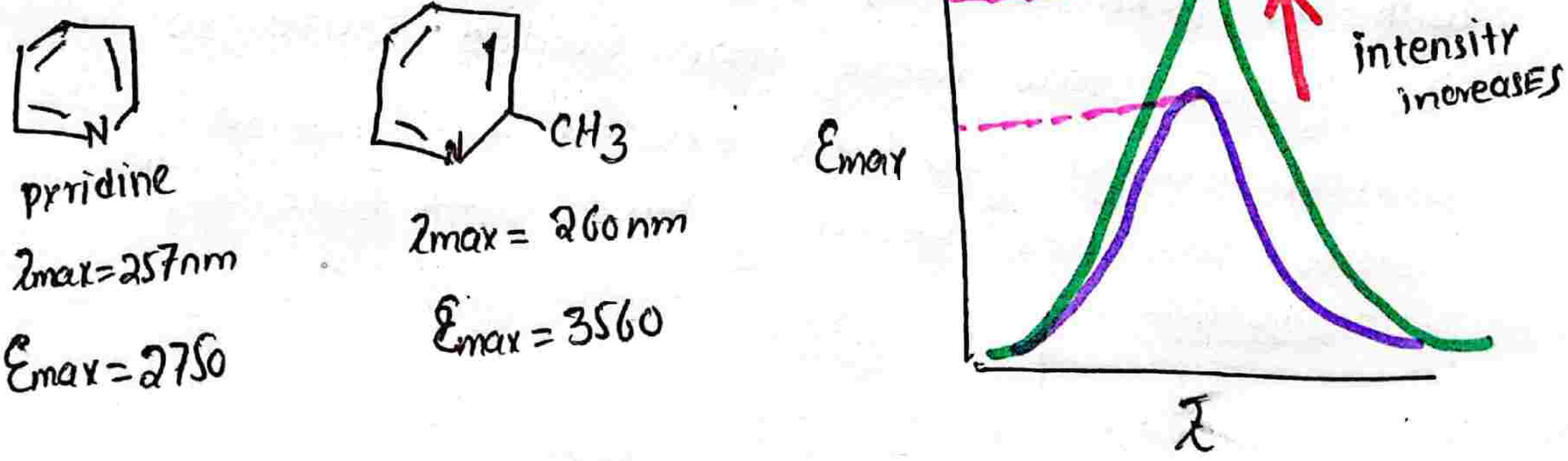
★ Introduction of any group to the compound which is going to Alter the molecular pattern OF the compound Result → hypochromic shift.

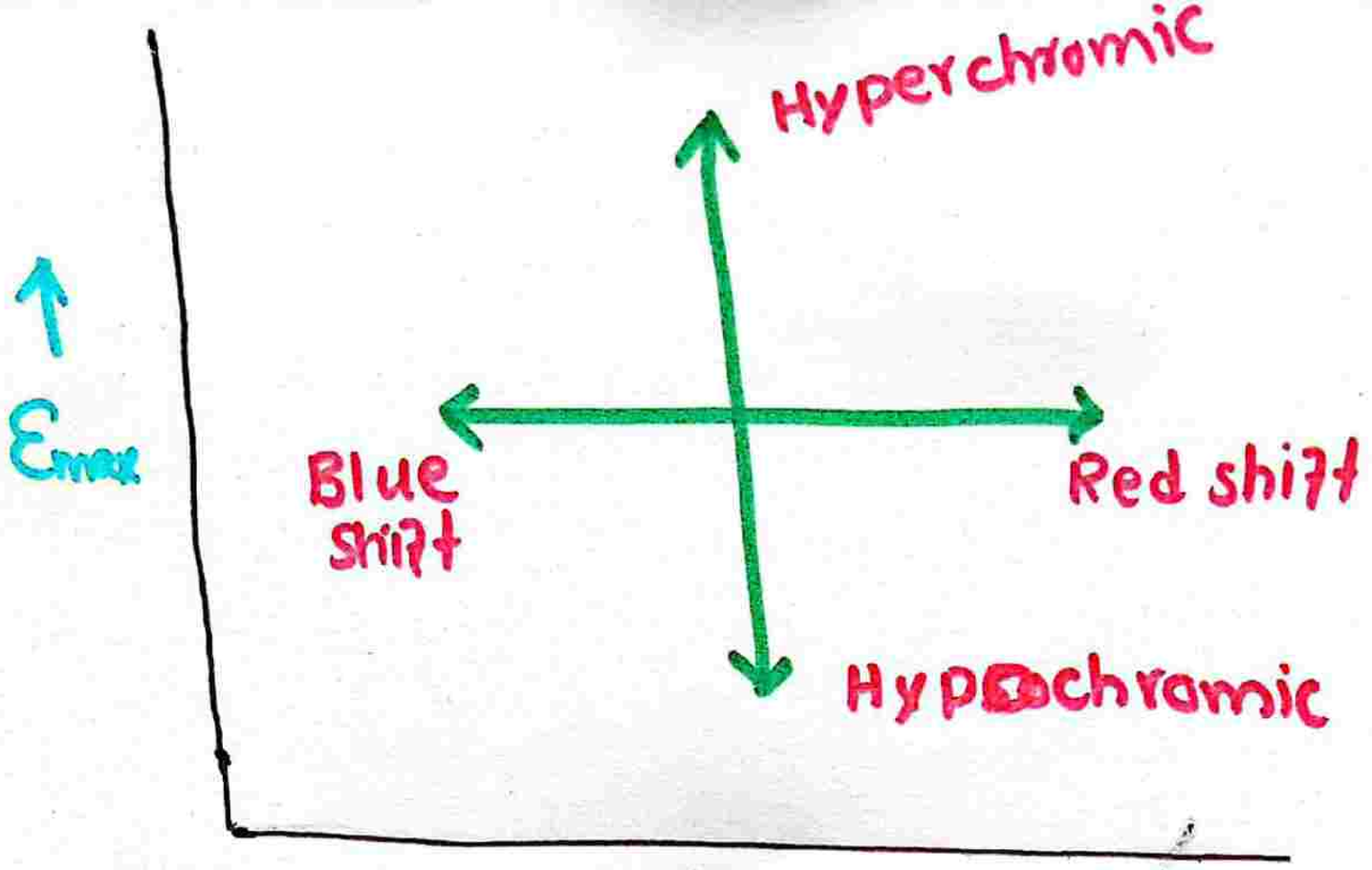


★ **Hyperchromic shift:**

$\varepsilon_{lower} \longrightarrow \varepsilon_{higher}$

★ Hyperchromic shift is generally due to the introduction of Auxchromes → which helps to absorb more radiations thus Absorption intensity increases.



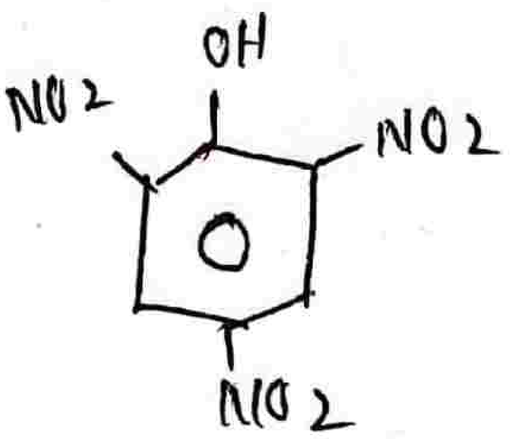


★ Dyes System:

Dyes are the organic colored compound. They show highly extended conjugation system → so Absorb the light in visible Region.

Dyes

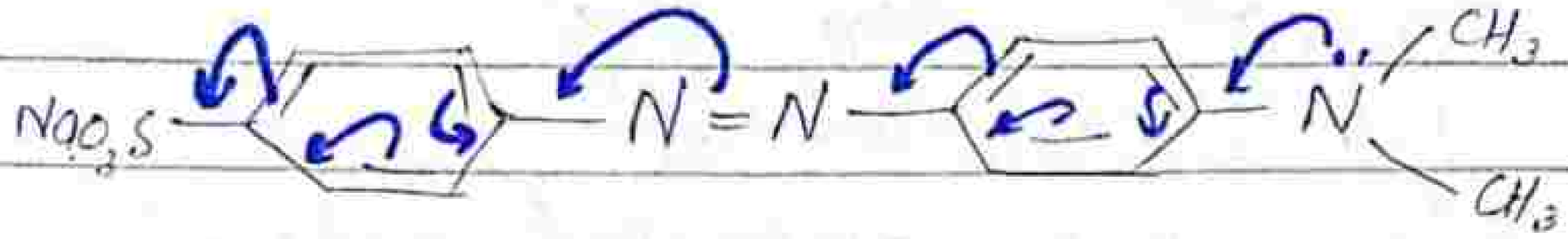
- **AZO-DYES** (−N=N−)
  - e.g
  - Methyl-orange (indicator)
    - Red color → Acidic Soln.
    - yellow color → Basic solution.
- **Nitro Dyes** (−NO_2)
  - ↓
  - picric acid



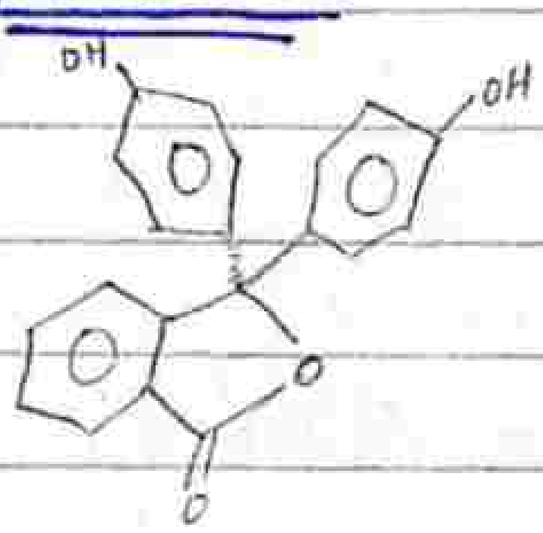
(yellow colour)
↓
Quinonoid structure.

Picric acid is colored Compound.

* **Methyle** :-



* **Phenophthalene**:-



* **Effect of Solvent**:-

To change / vary absorption intensity different solvents are used.

H_2O
Cyclohexane
THF
Ethers
Alcohols.

Polarity matters alot.
Solvent should not be react with analyte / should be inert.

★ $n-\pi^*$ transition: → non-bonding orbitals ↓ more stabilized due to H-bonding, thus more(E) required 2↓ ↓ Blue shift.

↳ This band undergo Blue shifts, since ground state with 2 electrons receive greater stabilization than excited state with only one electron.

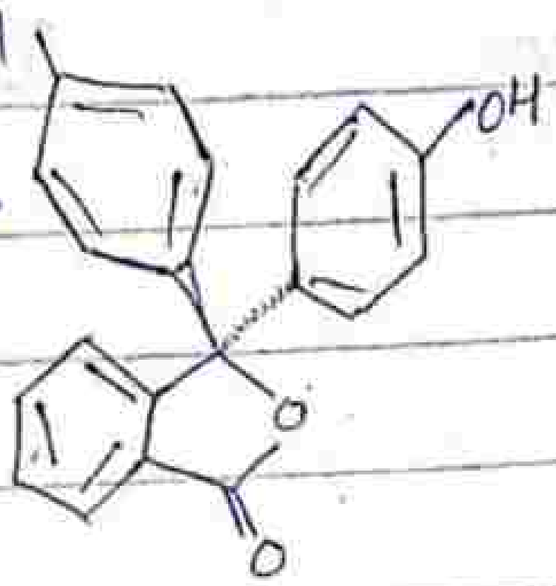
★ $\pi-\pi^*$ transitions:

- As solvent polarity is increased this band undergo Red shift.
- This is so, since excited state is more polar than the ground state, hence stabilization is greater relative to the ground state in polar solvents.
- Transition OF polar bond like C=O but not ethylene, are Affected by solvent polarity.

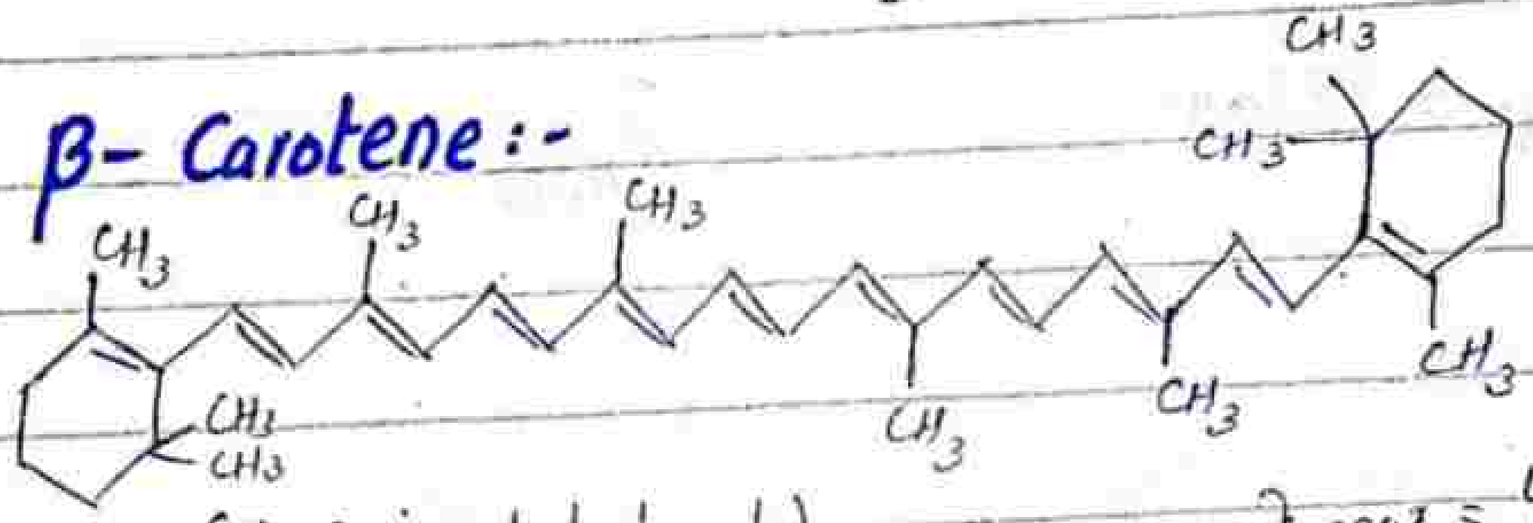
Solvents	$\pi-\pi^*$	$n-\pi^*$
Hexan	230 nm	329 nm
H_2O	243 nm	305 nm

Methyl Orange :-

Phenolphthalein :-



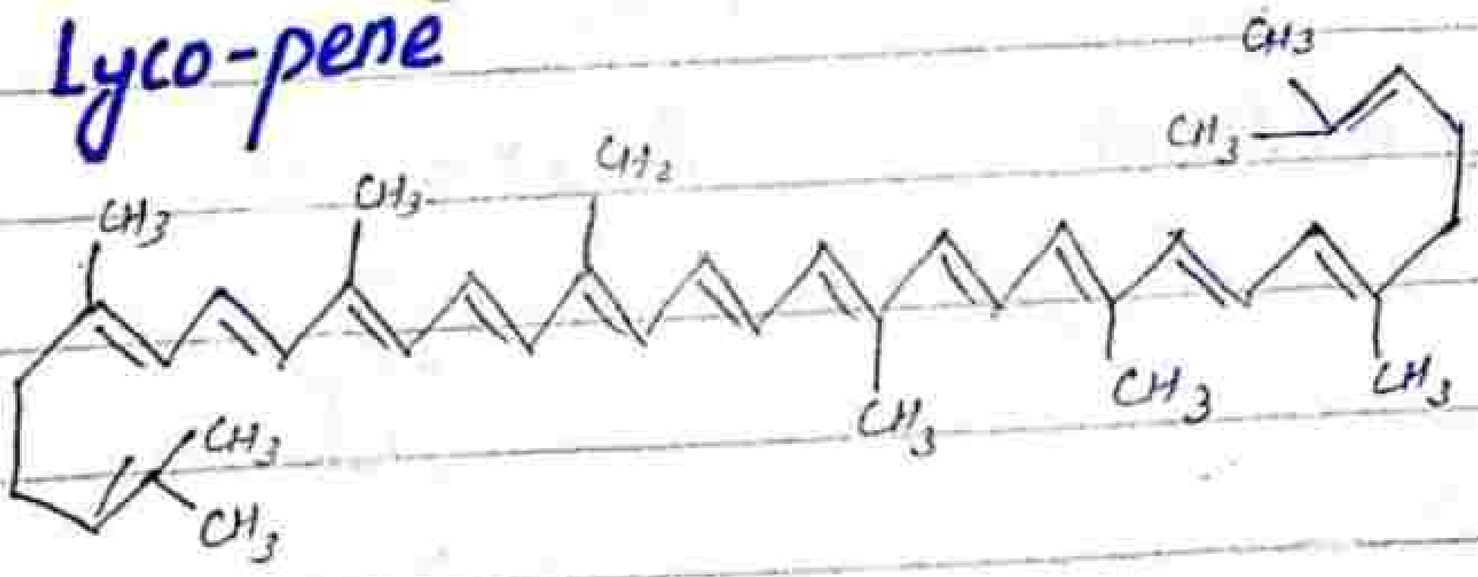
β- Carotene :-



(11- conjugated bonds)

$\lambda max = 497 nm$

Lyco-pene

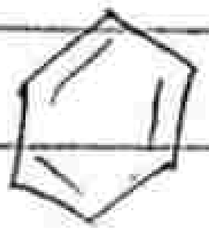


(11- conjugated bonds)

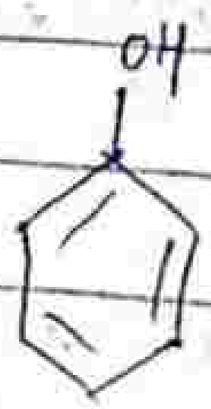
$\lambda_{max} = 505 nm.$

Scanned with CamScanner

(1)

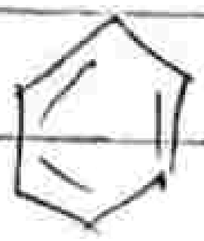


$\lambda max = 178nm$



$\lambda max = 200 - 290nm$

(2)



$\lambda_{max} = 178nm$

$\lambda_{max} = 254nm$

(3) $CH_3 - CH = CH_2$

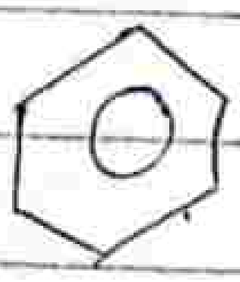
$\lambda max = 170nm$

$CH_3\,CH = CH\text{-}SH$

1 - Thiol

$\lambda max = 240nm$

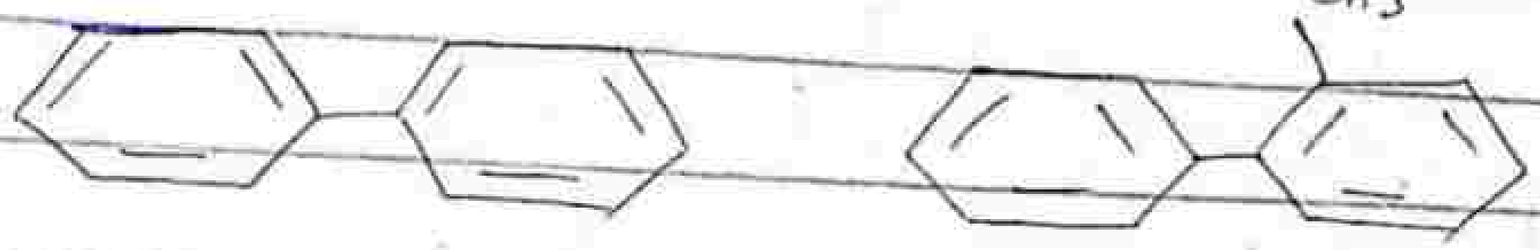
(4)



178nm

248nm

(5)



$\lambda_{max} = 250$ $\lambda_{max} = 257$

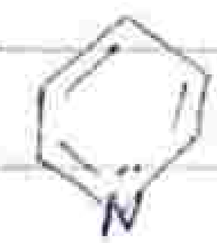
(6) $CH_2 = CH_2$ $CH_2 = CH - \overset{\oplus}{N}R_3$

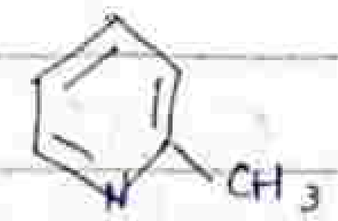
$\lambda_{max} = 174nm$

(7) $CH_3 - \overset{O}{\overset{\|}{C}} - OH$ $CH_3 - \overset{O}{\overset{\|}{C}} - NH_3$

$\lambda_{max} = 204nm$ $\lambda_{max} = 214nm$

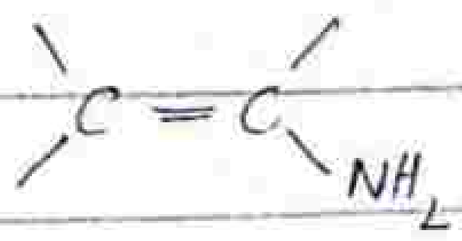
(8)





$\lambda_{max} = 257nm$ $\lambda_{max} = 262nm$

(9)



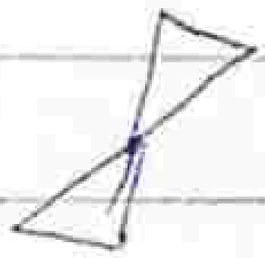
$\lambda_{max} = 190nm$ $\lambda_{max} = 230nm$

★ Instrumentation of UV-Visible :-

→ **Single beam** UV-VIS Spectrophotometre.
(At a time both sample and the reference are not taken.)

→ **Double beam** Spectrophotometre.
There is place of two sample + Reference.

* **Sample** cell
* **Reference** cell
* **Chopper**



* Expensive
Less stable.
Fast.

Single Beam Sp.	Double Beam Sp.
1- Less expensive.	1- Expensive
2- Stable.	2- less stable.
3- Time consuming Tedius.	3- Less time consuming.
4- Continuous Caliberation has to be done.	4- Caliberation is required at start Good Detector quality with high resolating power is required. (Sens. ↑) To boost up signal. Photomultiplyer tube detector. (PMT)

* Single Beam Spectrophotometre :-

(1) Radiation Source

UV or Visible. light.

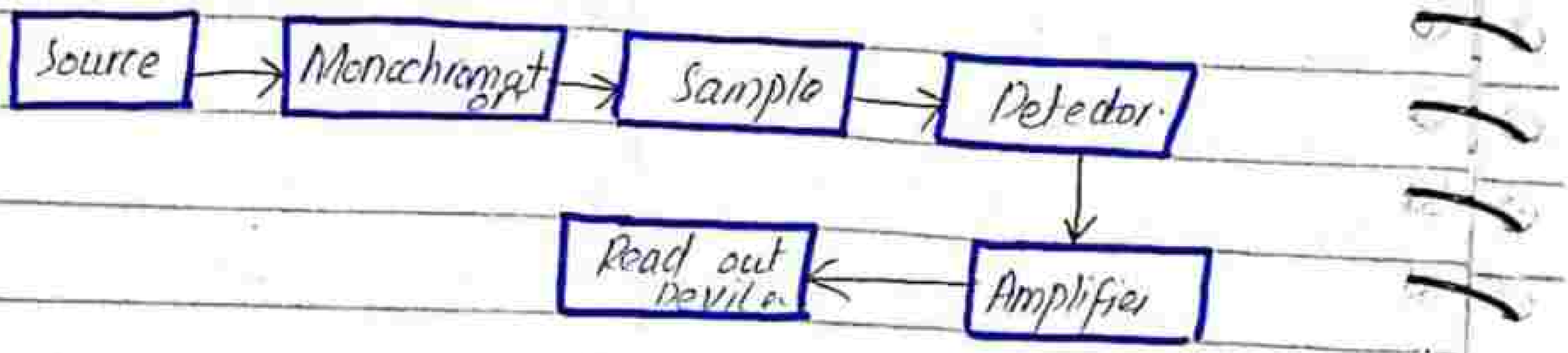
(2) Monochromator :-

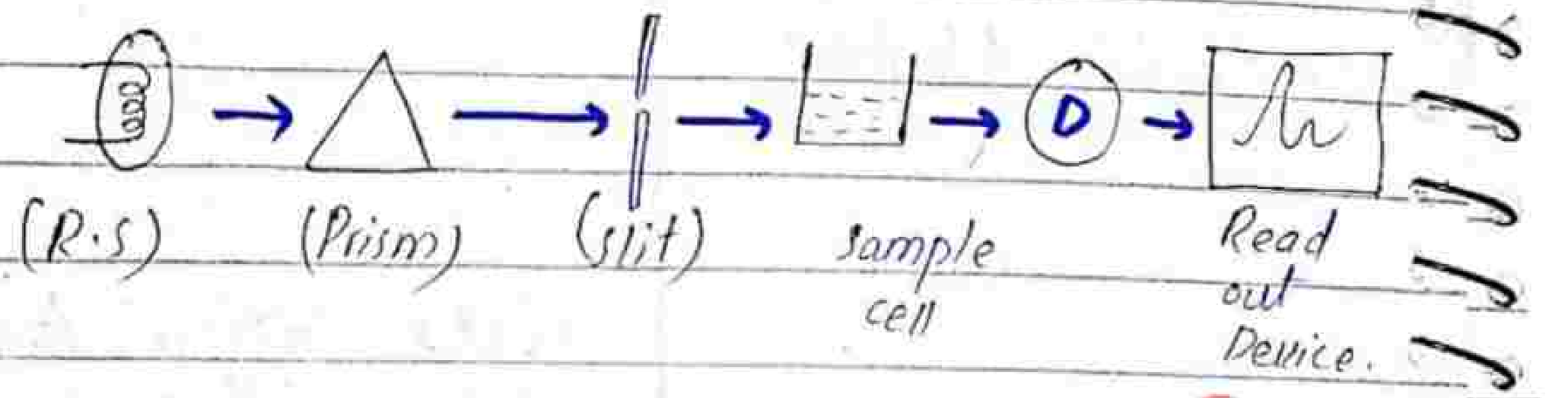
(3) Simple :-

(4) Detector :-

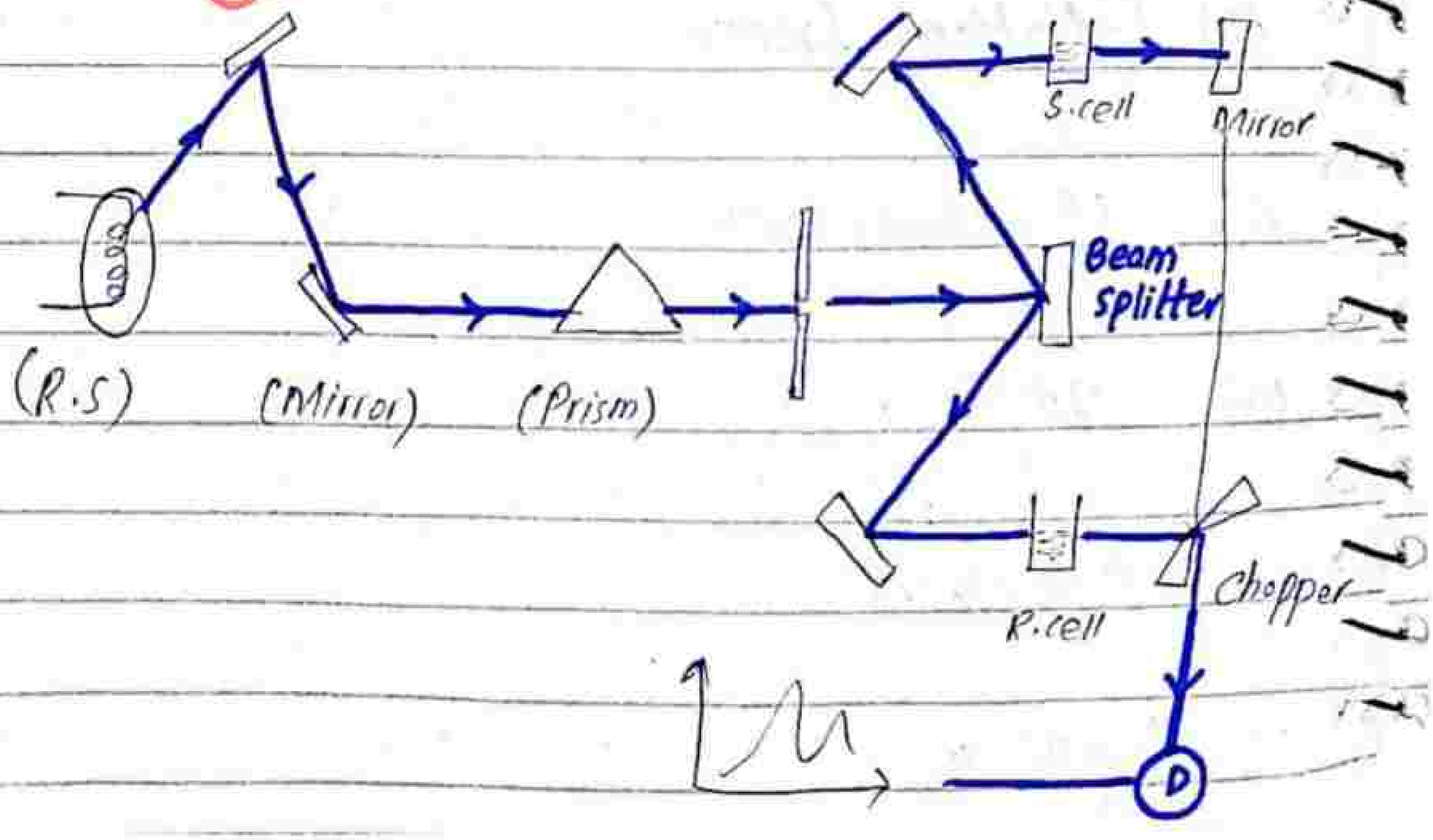
(5) Amplifier.

Read-out Device.





(2) Double- Beam



(1) Radiation Source:-

If colorless compound = UV source
If colored compound = Visible source.

→ **Visible =** Tungusten filament lamp or visible lasers.

→ **UV =** Deubrium lamp, H-Discharge lamp, Mercury discharge lamp. ,Xenon

(2) Mirror:-

Just give path towards Monochromator.

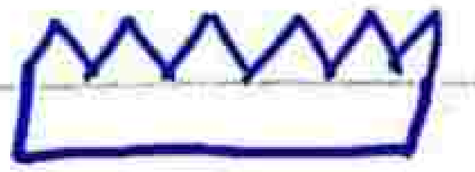
(3) Monochromator:-

It converts polychromatic light into monochromator light.

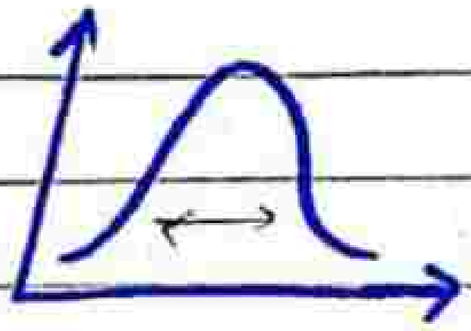
→ **Filtres :-** old / conventional and has less resolution for graph.

→ **Prism :** Resolution compared to filter is better. △. It is modified from filter.

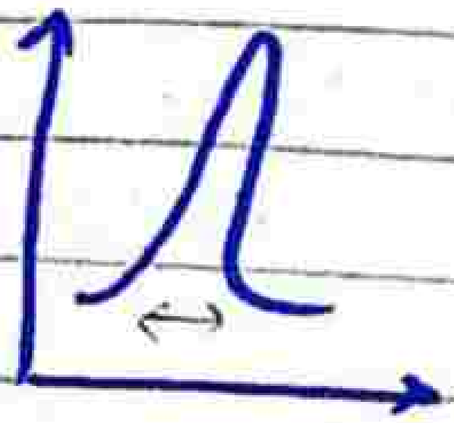
→ **Defraction grating :-** Engineer device and has grooves. Best resolution.. Reflect light at different angle.



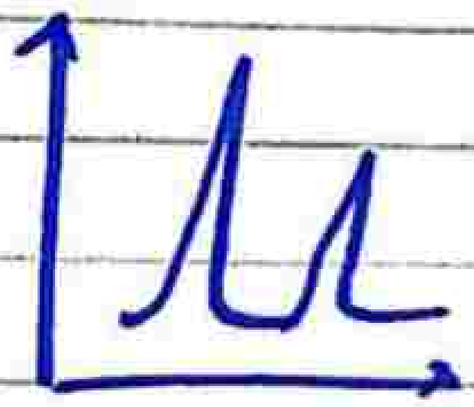
grooves = Separate glass



Broad peak
More width.
(Filter)



less broader



Sharp peaks
Good resolution.

* Beam Splitter:-

Divider the beam into two paths one reference and sample Thus intensity decrease.

* Sample cell / cell / Quvetts / Comparts :-

Quvette - 1cm

(1) Ordinary glass is used when visible is taken. (Na_2O)

(2) Quartz glass is used for both (UV + Visible).

★ **Chopper:-**

It rotates light at different angles at horizontal & Verticle angles.

★ **Detector:-** Light E → Electrical E

Basic function is to dectect signal. Converts light E into Electrical energy in the form of current.

(1) Photodectors

Phototuber

(2) Photomultiplier dectors. (e^- multiplier tube)

(PMT)

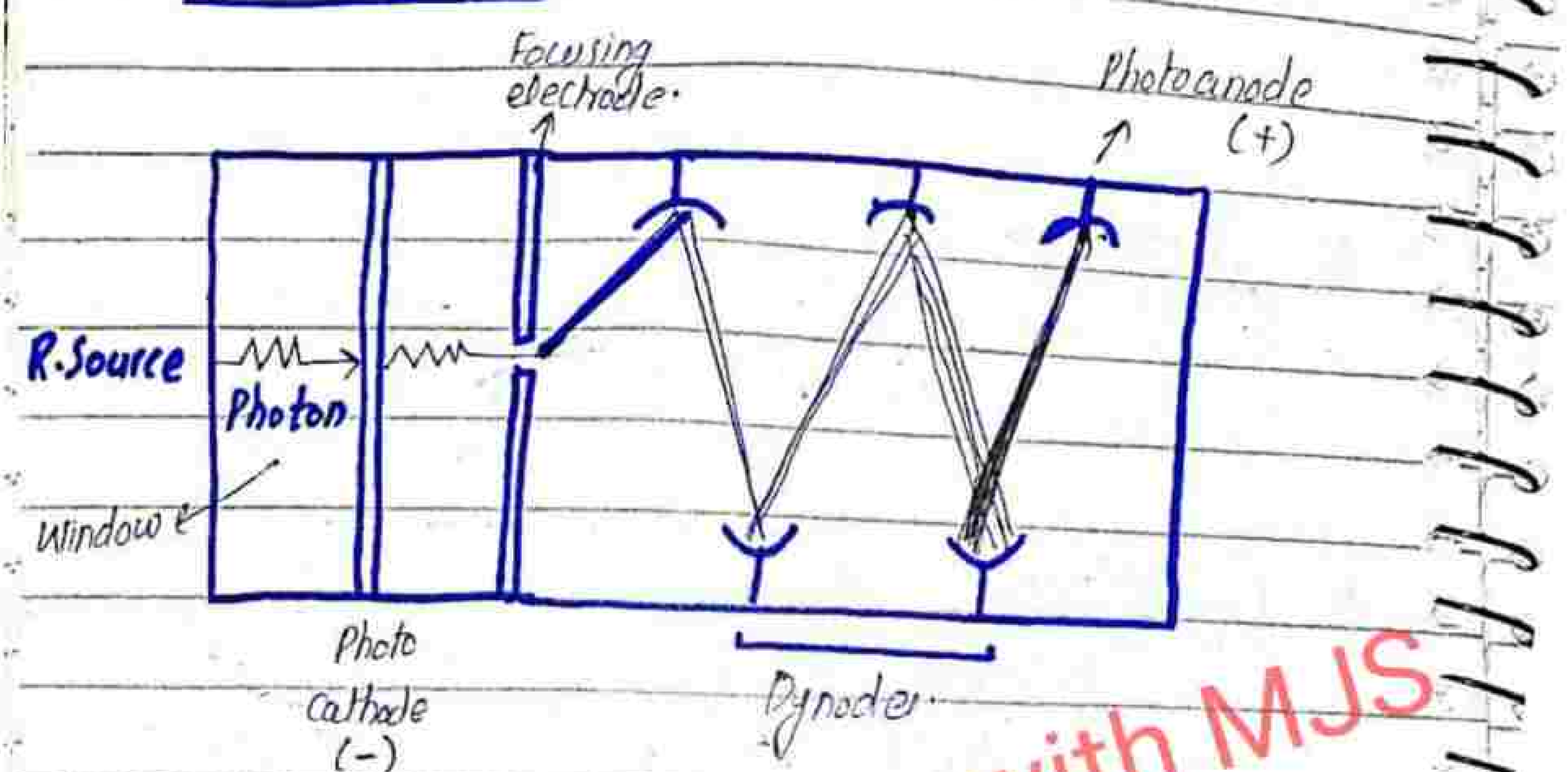
(3) Charged Coupled devices.

(CCD)

(4) Photodiode Array detector.

* PMT :-

* Construction :-



* Window :-

Window is glassy material (made with mixture of metals Na, K, Rb, MgF_2)

* Photo cathode :-

When photons strike cathode, e^- are released and move e^- towards focusing electrode.

* Focusing electrode :-

Focusing electrodes just provide path

* Dynodes:-

They are also called **electron-multipliers.** (Dynodes) They just multiply and amplify the signal.

* Photoanodes:-

Photoanode accepts the beam and converts into electrical signals. (+)

≈ Woodward - Feiser Rule

This is used to Find out the λ_{max} of the structure.

A) Conjugated Diene System

* Basic / parent values of
  - Alicyclic diene ⟶ 217 nm
  - Homoannular diene ⟶ 253 nm
  - Heteroannular diene ⟶ 214 nm

* Increments:
  - Alkyle Substitution = 5 nm
  - Ring Residue = 5 nm
  - Exocyclic = 5 nm
  - Extencled conjugation = 30 nm

Auxochromes

$-OR$ = 6 nm

$-SR$ = 30 nm

$-Cl, -Br$ = 5 nm

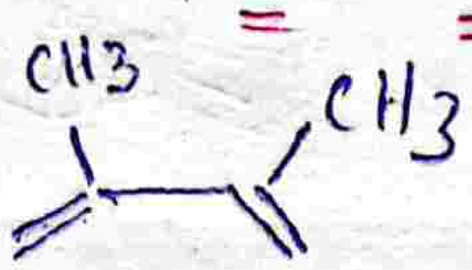
$-O-\overset{O}{\overset{\|}{C}}-CH_3$ = 0

WOODWARD FIESER RULE
Aliphatic + Aromatic + Diene

SYSTEM	BASIC VALUE
Aliphatic System	217 nm
Homoannular ring	253 nm
Heteroannular ring	214 nm
Alkyl subst.	5nm
Ring residue	5nm
Exo-dble bond	5nm
Extended conj.	30 nm
-Cl	5nm
-Br	5nm

Examples (predict the λ_{max})

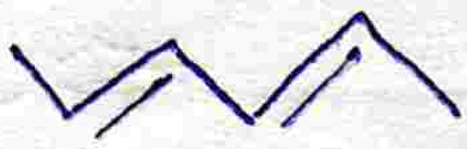
Alicyclic dienes

① 

B.V = 217

Alkyl(S) = 2×5 = 10

λ_{max} = 227nm

② 

Basic = 217

Alkyl(S) = 10

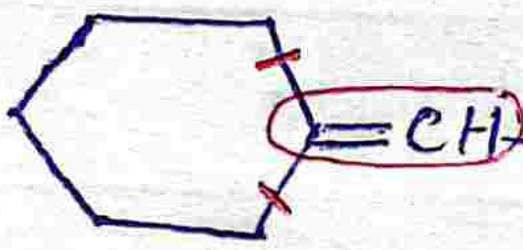
λ_{max} = 227nm

③ 

B.V = 217

Alkyl = 5

λ_{max} = 222nm

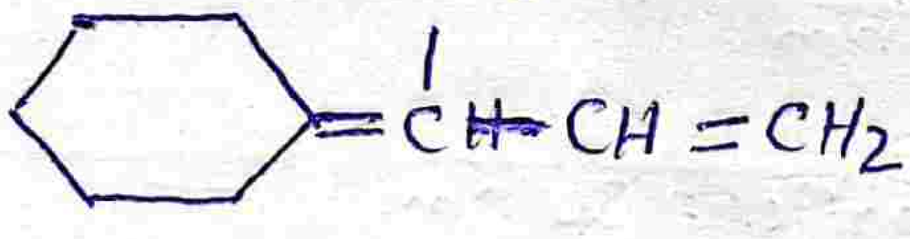
④ 

B.V = 217nm

Ring Residue = 10nm

Exocyclic Bond = 5nm

λ_{max} = 232nm

⑤ 

B.V = 217

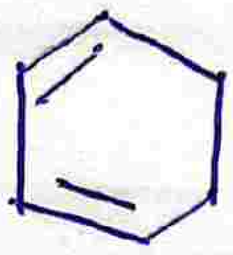
R.R = 10

Exocyclic = 5

Alkyl = 5

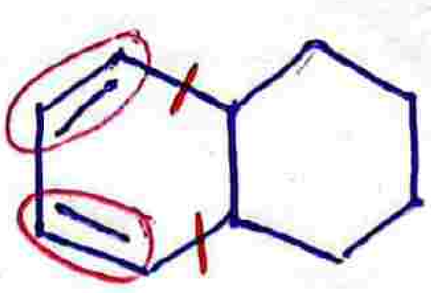
λ_{max} = 237nm

✓ : Homoannular & Heteroannular Dienes:

① 

Basic value = 253 nm

λ_{max} = 253nm

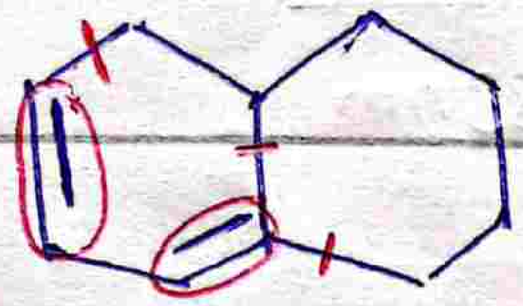
② 

B.V = 253nm

R.R = 10 nm

λ_{max} = 263nm

(3)



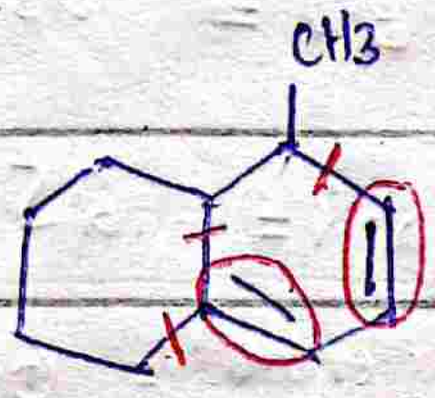
B·V = 253nm → (Homoannular)

R·R = 3x5=15nm

Exocyclic = 5nm

λmax = 273 nm

(4)



B·V = 253

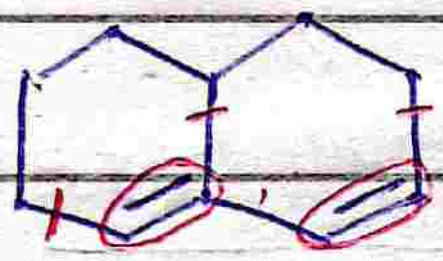
Ring Residue = 15

Exo = 5

Alkyl = 5

λmax = 278

(5)



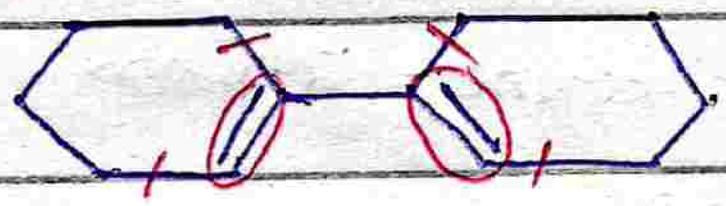
B·value = 214 nm (Hetero)

Ring·R = 15

Exo = 5

λmax = 234 nm

(6)

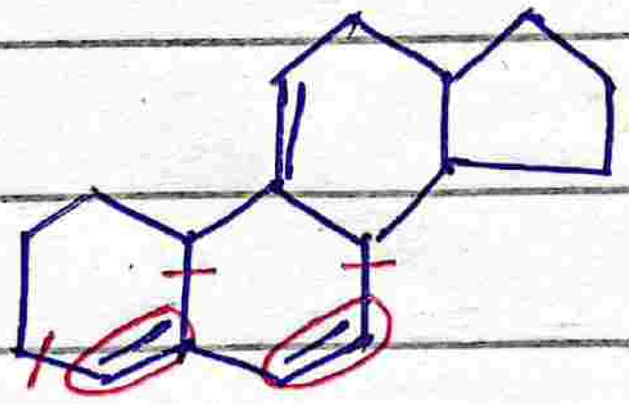


B·V = 214nm

R·R = 20 nm

λmax = 234nm

(7)



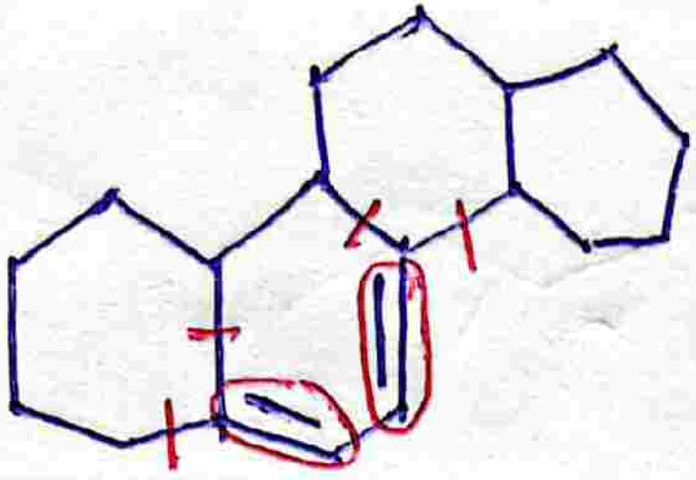
B·V = 214 nm

R·R = 15nn

Exo = 5

λmax = 234nm

(8)



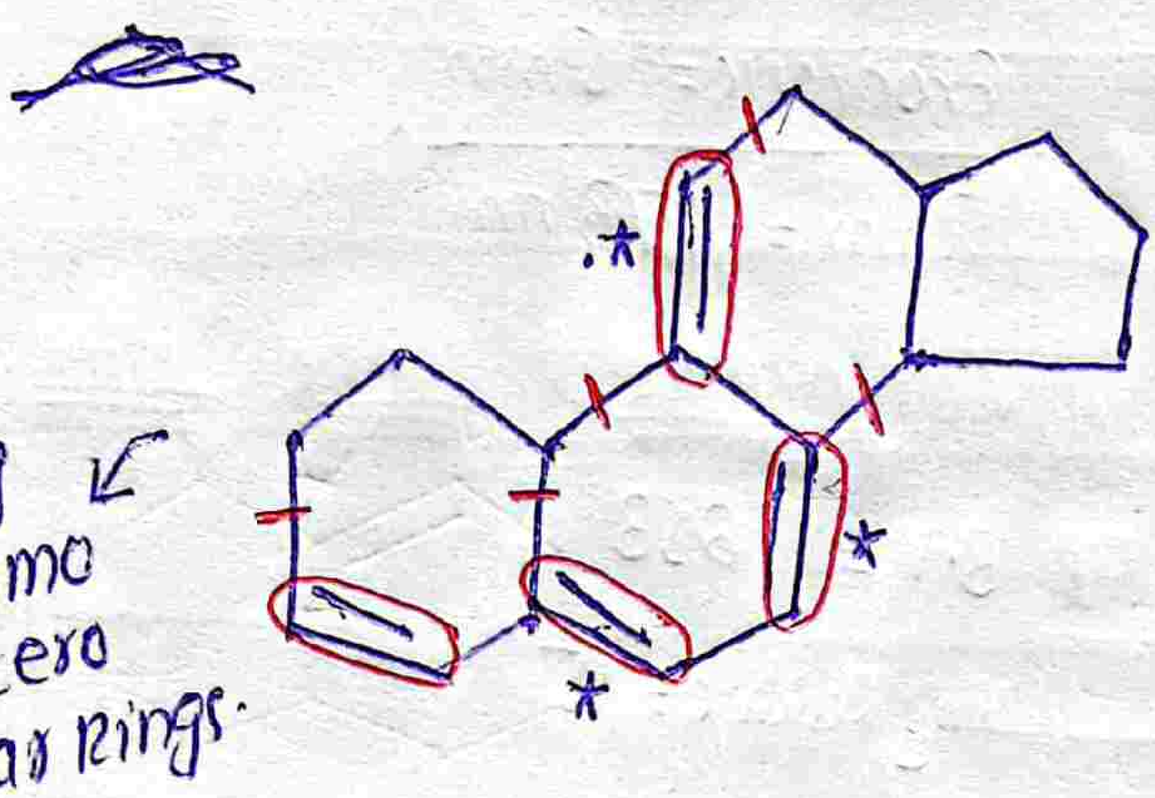
B·V = 253 nm

R·R = 20

EXO = 10

λmax = 283 nm

(9)



containing Both Homo & Hetero annular rings.
↓
So we prefer Homo

B·V = 253 nm

R·R = 25 nm

Extended conjugatio = 60 nm

Exocyclic Bonds (*) = 15 nm

λmax = 353 nm

B) α-β unsaturated Carbonyl System:

Basic values;

- Acyclic and Six-membered cyclic ketone = 215 nm
- Fife mem-cyclic ketone = 202 nm
- α-β-unsaturated Aldehyde = 207 nm
- Carboxylic Acids| Esters = 195 nm

Increment:

- Double bond Extended conjugation = 30 nm
- Homodiene component = 39
- Exocyclic = 5

Substituent

Substituents	α	β	γ	
Alkyl/Ring·R	10	12	18	nm
OH	35	30	30	nm
Br	25	30	25	nm

Examples

① $CH_3-\overset{\beta}{C}H=\overset{\alpha}{C}(CH_3)-\overset{O}{\overset{\|}{C}}-CH_3$

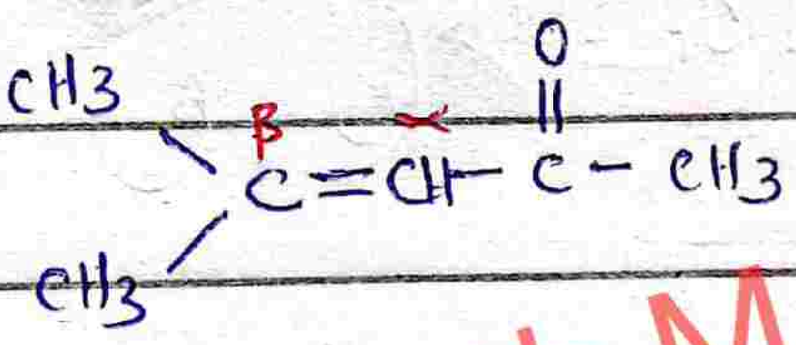
B·V = 215 (Acetone)

α = 10

β = 12

λmax = 237 nm

②

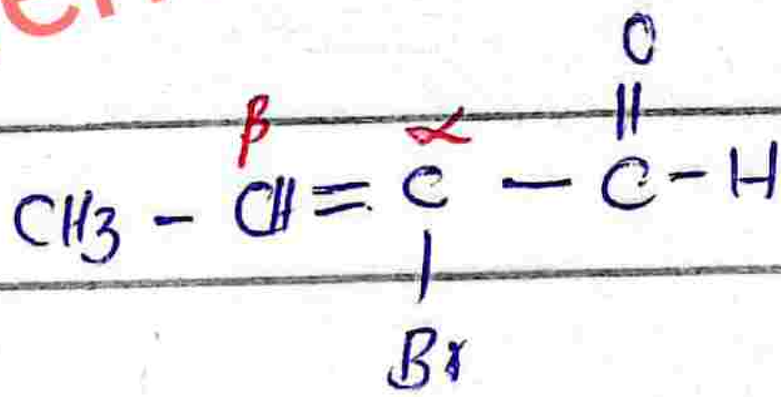


B·V = 215

β = 12×2 = 24

λmax = 239 nm

③



B·V = 207 (Aldehyde)

α-(Br) = 25

β(methyl) = 12

λmax = 244

Applications of UV-Visible Spectroscopy

COUISE CONTENTS:

1 * Natural product Research-

2 * pharmaceutical industry-

3 * Separation process-

4 * Enzyme Assay Study-

5 * Clinical Studies-

6 * Microbiology-

1) Natural products:

Natural product is a chemical compound/substance made by living organisms.

e.g Milk, starch, proteins, Nucleic Acids

Honey, Food Products

* UV-visible → describe the conformational transitions in proteins & Nucleic Acids.

* Constituents of Milk products are typically studied in UV (185-210nm) wavelength Ranges.

* UV-visible spectrum show the Adulteration in Milk by Fats, Sugars, & Foreign proteins.

* Concentration of proteins/Nucleic Acids in Solution can be easiey and

Accurately determined using Lambert Beer's law.

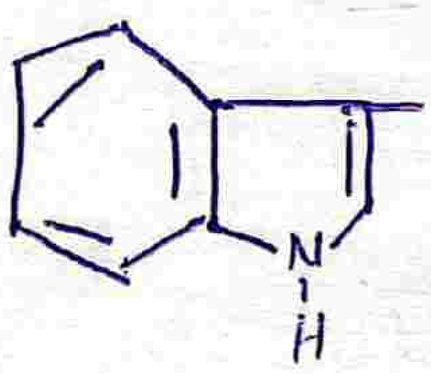
⊛ Adulteration of proteins: / concentration.

↓

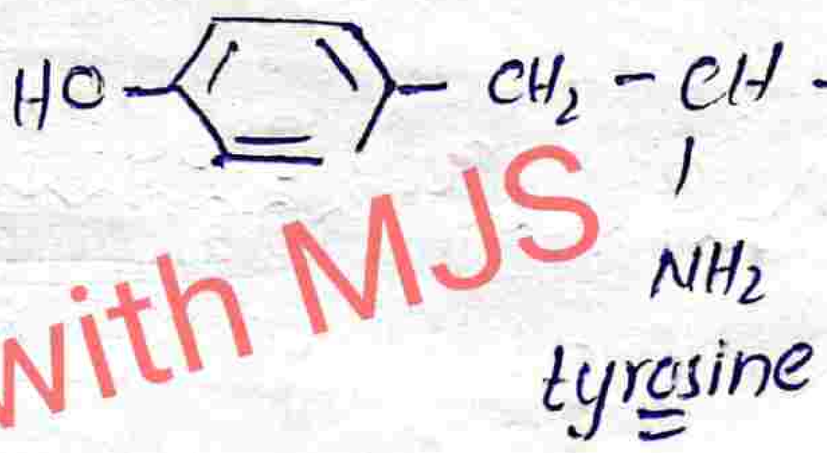
when there is some adulterated products are added to any solution. This adulteration is ~~due to the~~ analyzed by the UV-visible spectrophotometer.

e.g Tryptophan & tyrosine → They show the λ_{max} which can be identified.

λ_{max} = 275 & 280 nm BIW



tryplophan



tyrosine

⊛ Concentrations OF Nucleic Acids:

⇒ Concentrations OF Nucleic Acids in solution are routinely determined from their strong absorbance at 260 nm

They show absorbance in the region 240–275 nm

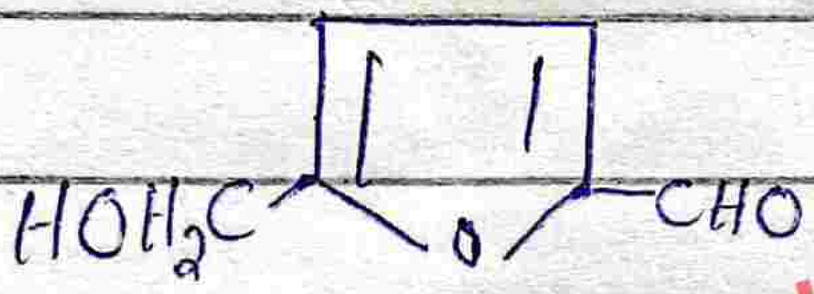
⊛ Concentration of phenolic compounds in wine:

phenolic compounds play important role in the colour, & Flavour OF wines. Their concentration is determined using UV-visible spectroscopy

④ Concentration of HMF in Food products:

* HMF is key factor to control the quality and flavours of foods, especially in honey. It is necessary to quantify the HMF using UV-visible spectroscopy.

HMF = λ_{max} = 284 nm



(HMF)

2) Pharmaceutical Industry:

① Dissolution testing of tablets:

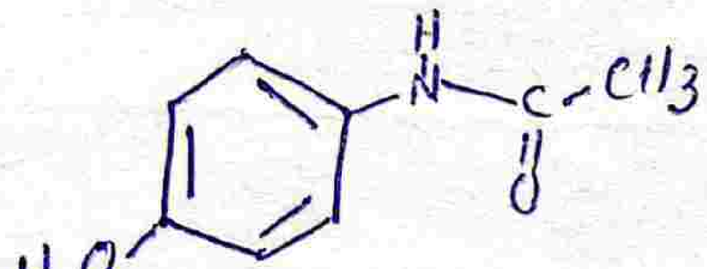
⊛ Many drugs which are present in the form of raw materials or in the form of formulation → they can be assayed/Analyzed by making a suitable solution of drug in a solvent and absorbance is measured at specific wavelength → (λ_{max} = 243 nm)

e.g paracetamol tablet → pain killer/ Reduce the Fever- but its acute overdose is fatal and toxic so its composition should be checked- How much amount of ingredients are present in this- This done by UV-visible.

② UV-visible spectrum provide valuable information about the active ingrediants or impurities present in the pharmaceutical compounds.

③ Many pharmaceutical compounds containing chromophores e.g including antibiotics, hormones, vitamins etc.

∴ Tetracycline → λ_{max} = 360 nm
or
∴ Ciprofloxacin



(paracetamol)

3) Separation process:

1) Separation BIW Different concentrations of Same/different Substances-

2) Separation OF Functional groups-

⇒ $-CHO$ 290 nm

⇒ $-COOH$ 208 nm

⇒ $-\overset{\overset{O}{\|}}{C}-NH_2$ 220 nm

3) Drug Separation:

↳ Many drugs are separated from the solvent by measuring the absorbance at the specific wavelength.

4) Clinical Study:

UV-visible spectroscopy has a lot of applications in clinical-

★ water Sample / Air Sample / Urine Sample / Blood Sample

⇒ Urine Sample:

∴ Quantification of uric acid (toxic) in urine Samples is done by UV-visible-

uric acid (λ_{max}) = 294 nm

⇒ Blood Serum:

Quantification of Haemoglobin in blood by UV-visible

∴ heme (λ_{max}) → 269.2 nm

5) Microbiology:

⊛ UV-visible → used to determine the structure, size and shape of Microorganisms found in given sample.

⊛ Chromophores are present in the microorganism → to determine the absorption of light by microorganisms.

* As microorganisms grow → spectra changes

* Greator the no. of Bacteria in any solution → Greator is the absorbance

⑥ Enzyme Assay Study:

Measuring Activity of Analyte

→ Using the absorbance, measuring the activity of Enzyme

$$\text{Reactant} \xrightarrow{\text{Enzyme}} \text{product}$$

⇒ The Rate of this Reaction can be accurately measured using UV-visible spectrophotometer.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

(Enzyme (Substrate) complex)

P → Bi-product

* The Bi-product can be quantified and observed by monitoring its maximum absorption wavelength.

: Others Applications of UV-visible:

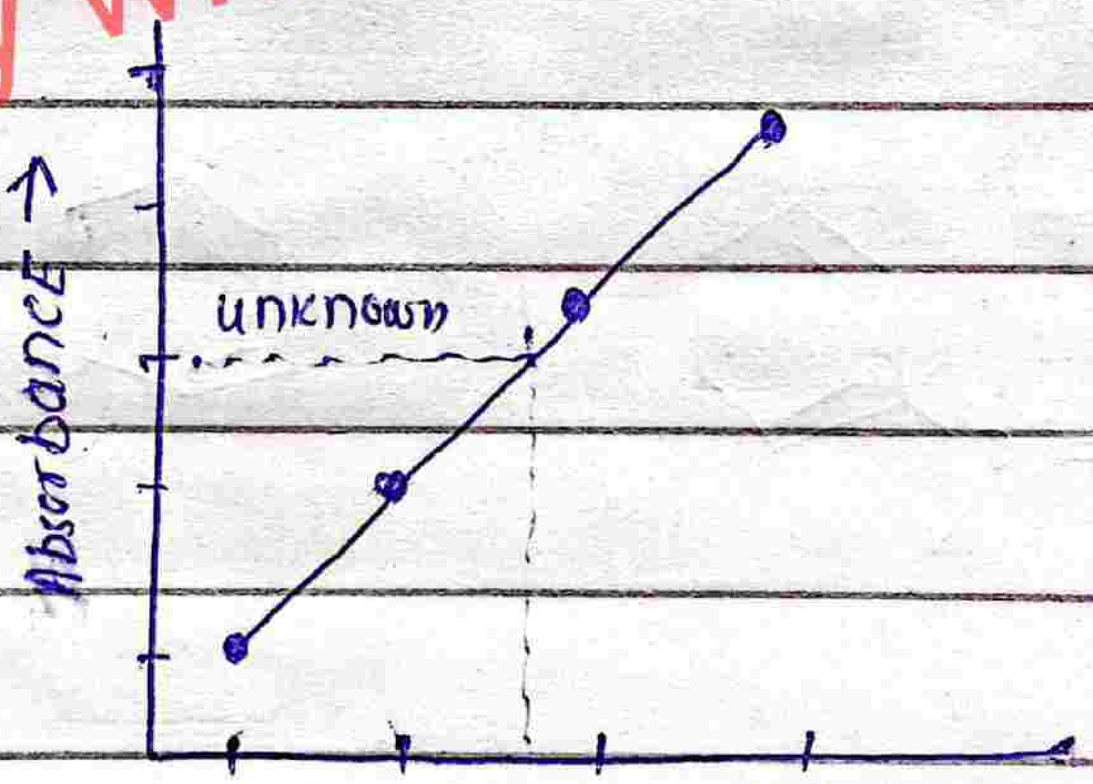
① Structure Elucidation: / unknown compound

⇒ compound of unknown structure is detetmined by compairing it with the UV-visible spectra of known structure (standard)

② Quantitative Analysis: / calibration Curve Method.

↳ We can quantify the Analyte in our Sample.

- Make the different standards of desired Analyte different concentrations and Absorbance is Noted. and graph is plotted.
- Then Run The unknown Analyte whose concentration is to be determined. in this way by drawing calibration concentration can be Find out



Standard Solutions: ( )

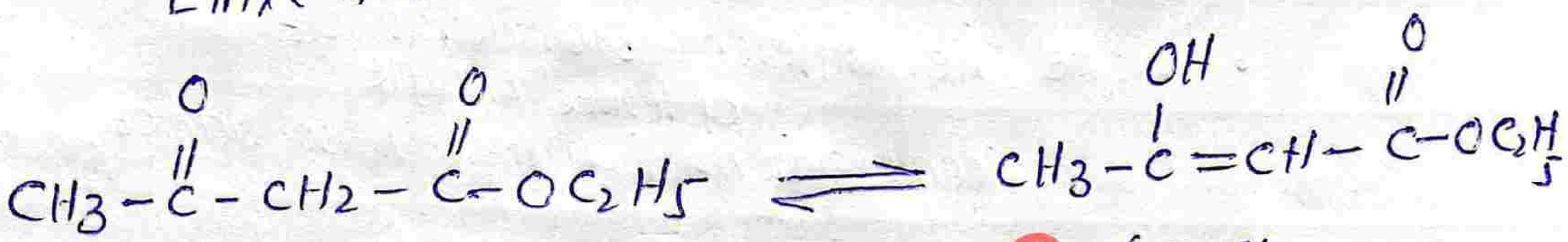
5 ppm, 10 ppm, 15 ppm, 20 ppm

3) Study of tautomeric forms:

UV spectroscopy used to determine the various tautomeric forms e.g keto & enol forms present in tautomeric Equilibrium.

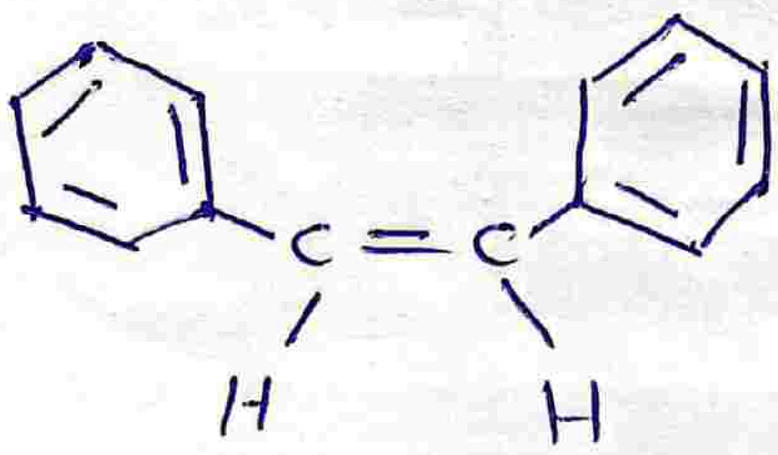
Example;

Ethyl Acetoacetate

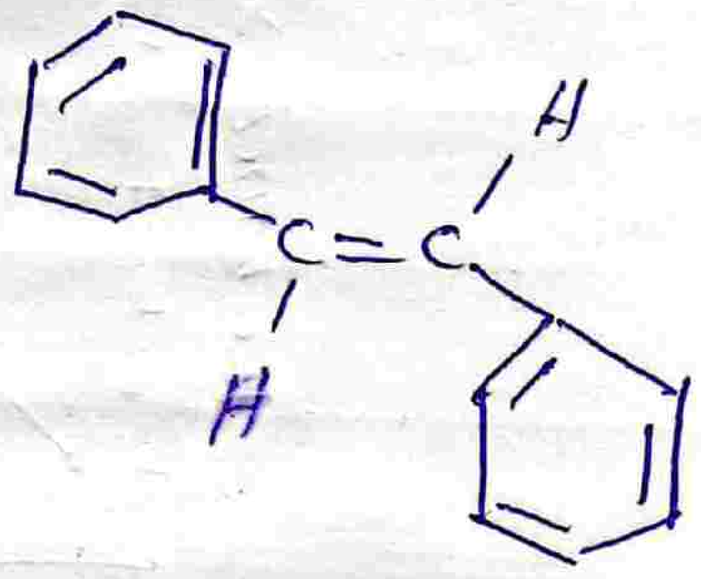


4) Determination of configurations (cis-trans)

* Cis-Alkenes absorb at different wavelength as compared to the trans-Alkenes.



cis-stilbene



trans-stilbene

(λmax = 283 nm)

(λmax = 295.5 nm)

* trans → Alkenes are more stable structure as compared to cis-Alkenes → so less Energy Reauired and λmax greator.

5) Detection of impurities:

⊛ UV absorption is one of the best method for the determination of impurities in organic molecules.

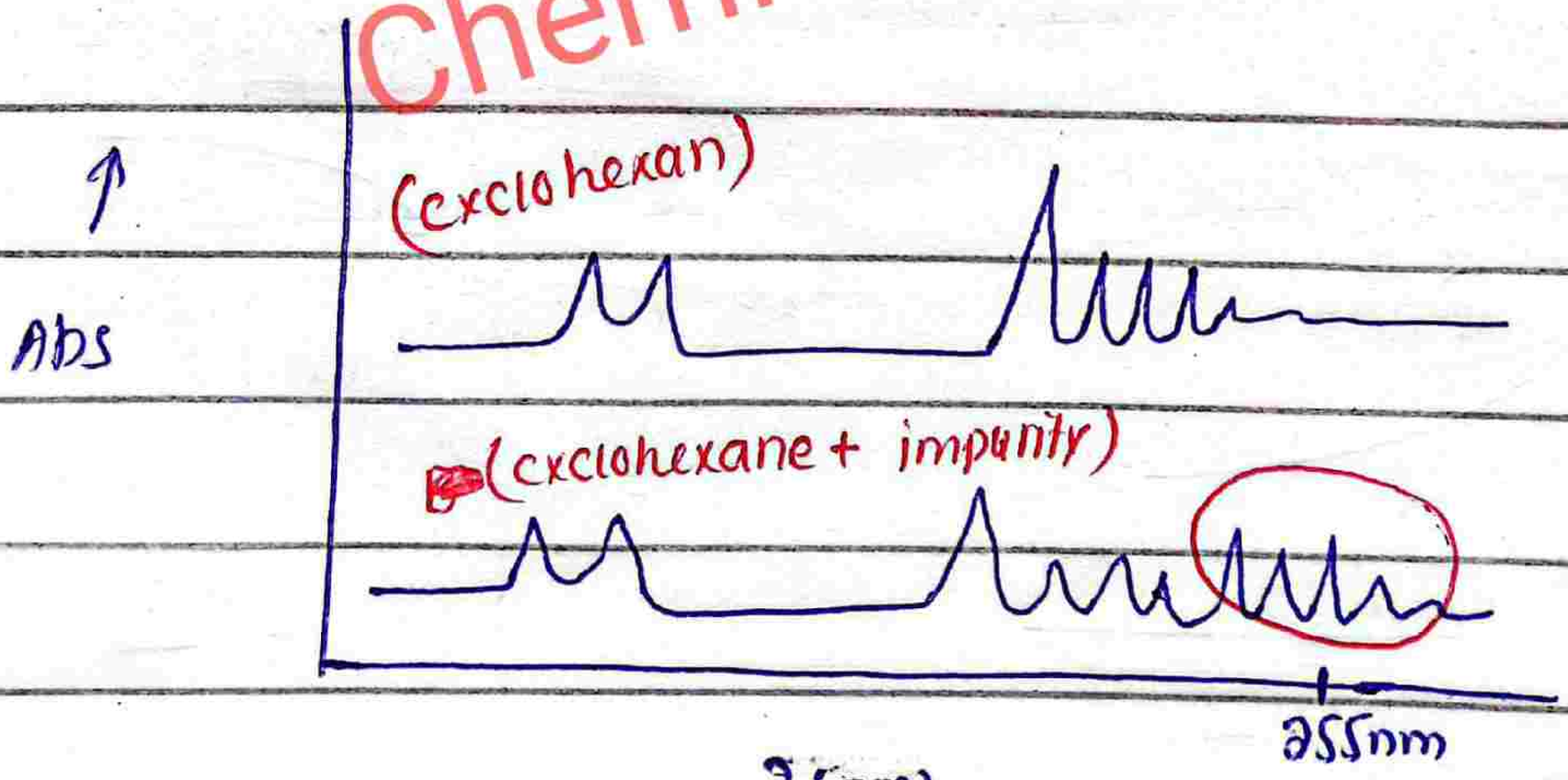
⊛ Additional peaks are observed, due to impurities.

⊛ spectra of organic compound is is compared/matched which indicate the variation due to impurity.

Example:

* Benzene is a common impurity in cyclohexane.

* Benzene as an impurity detected by its absorption at 255nm



6) Extent of conjugation:

Addition of unsaturation → increase of double bonds - shifting of λ increases. Also conjugation increases.

$CH_2=CH_2$ — $CH_2=CH-CH=CH$

$n=1$ — $\lambda_{max}=217\ nm$

$\lambda_{max}=171\ nm$

$R-(CH_2=CH)_n-R$ (coloured)

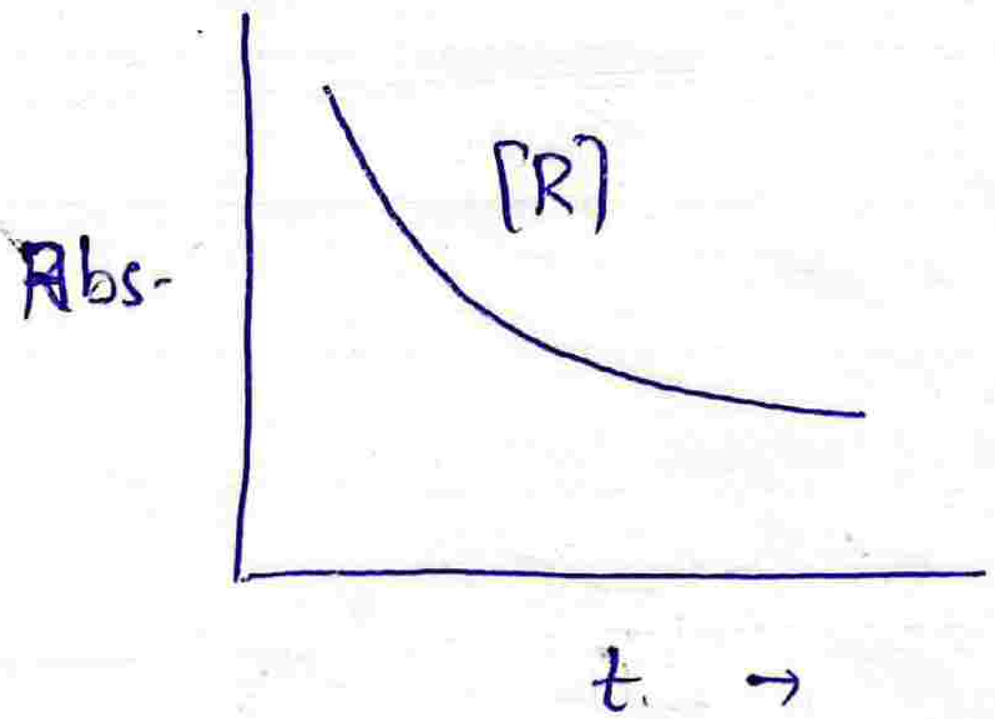
If $n=8$ $\lambda_{max}=420\ nm$

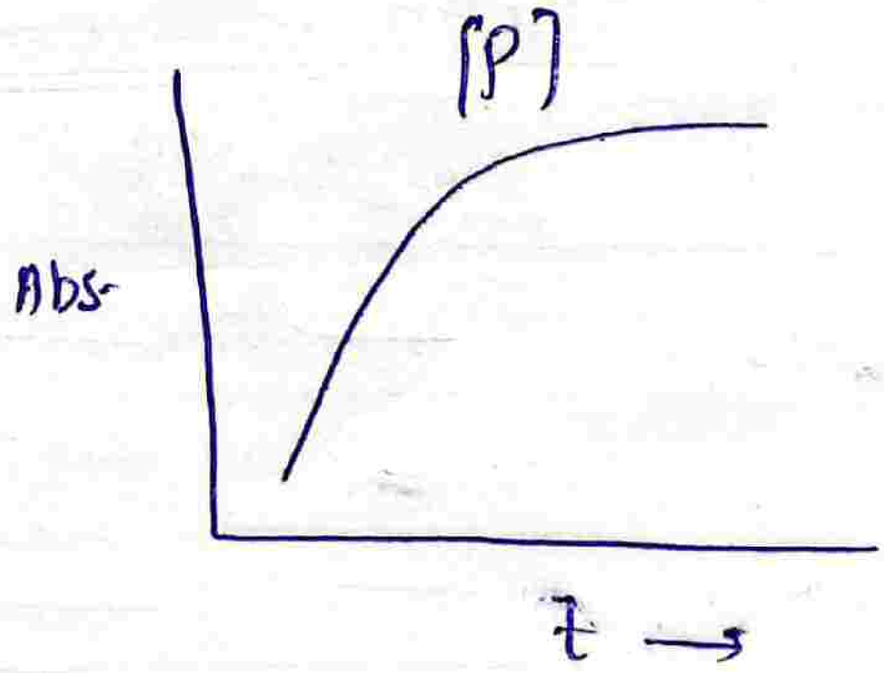
7) Kinetic Measurements:

⇒ UV-visible provide a powerful tool for the determination of Rate of a Reaction.

* R·O·R ⟶ measured by decrease in Absorbance when Reactant is absorbing specie.

* R·O·R → measured by increase in Abs- when products are absorbing.





Good Luck
MJS