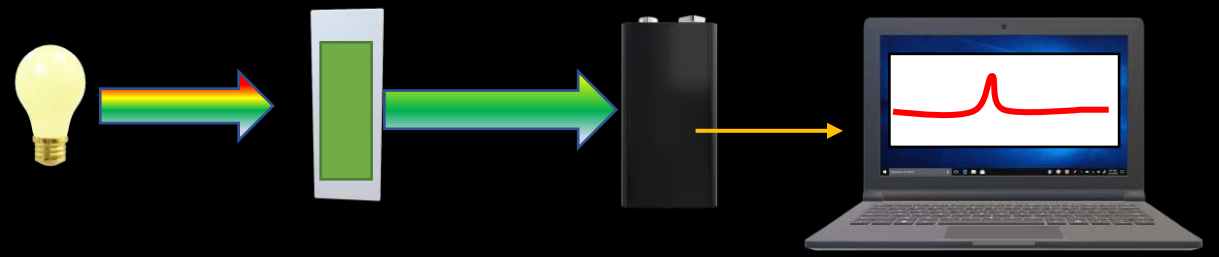
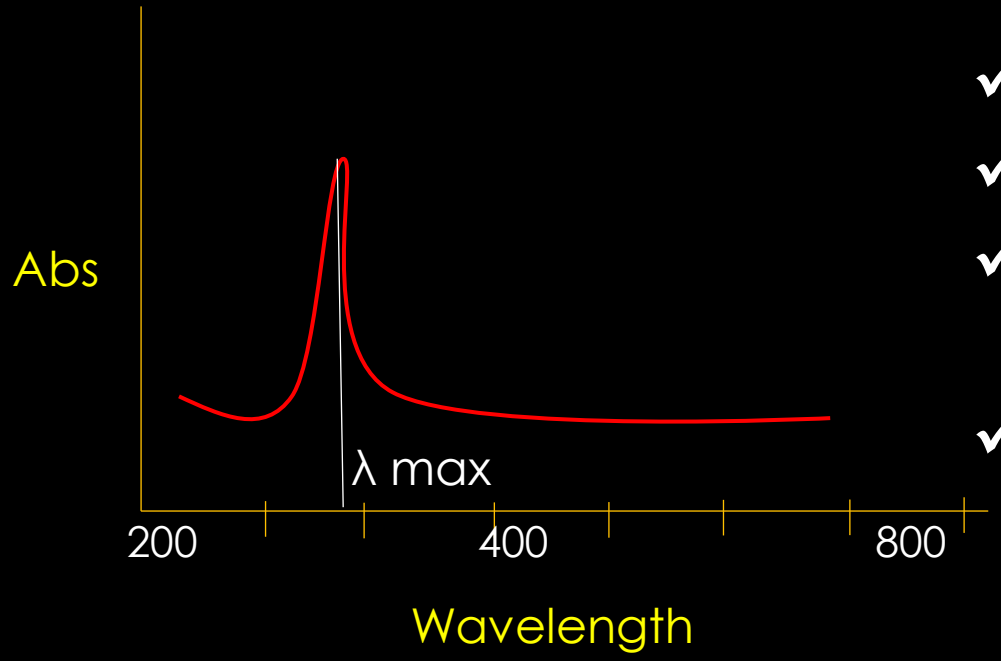
UV-Visible Spectroscopy (Part 1)





- ✓Spectroscopy
- ✓Basics of Spectra/EMR
- ✓Introduction to UV spectroscopy
- ✓Basic Principles

Spectroscopy
Instrumental Analysis

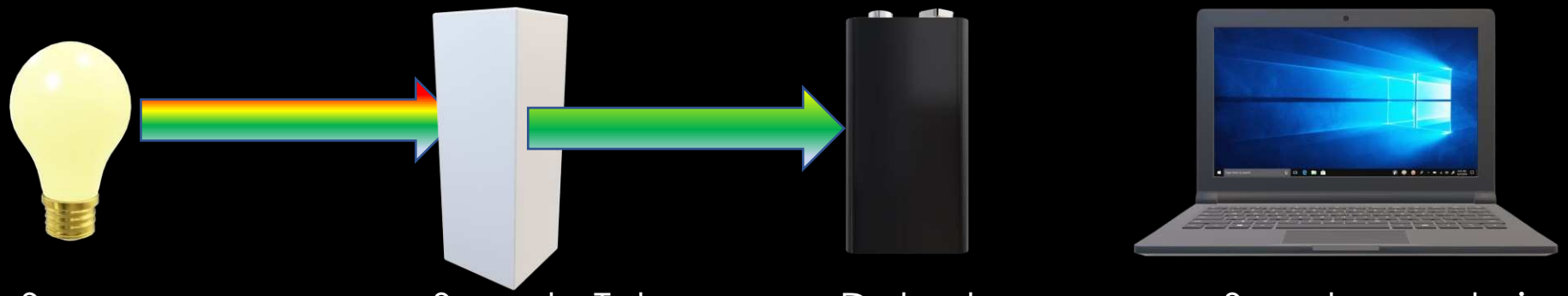
UV-VISIBLE SPECTROSCOPY

Spectroscopy:

- Evaluation of spectrum.
- It is derived from the spectrum, which mean a band of different colours formed due to difference in wavelength and skopin means examination or evaluation
- It is the study of the absorption and emission of light or spectrum or EMR and other radiation by matter.
- It is used to measure the energy difference between various molecular energy levels & to determine the atomic & molecular structures
- The instruments used in such studies are called ***spectrophotometer***.

Absorption Spectroscopy:

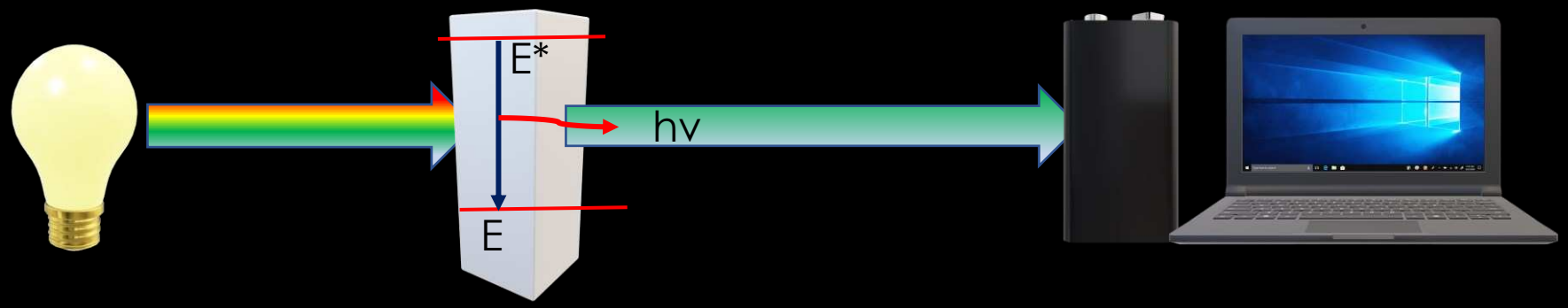
- **Absorption spectroscopy** measures how much light is absorbed by a sample over a range of wavelengths defined by the electromagnetic spectra
- E.g., UV absorption spectroscopy, IR absorption spectroscopy, Atomic absorption spectroscopy, NMR, Calorimetry



Source Sample Tube Detector Spectra analysis

Emission Spectroscopy:

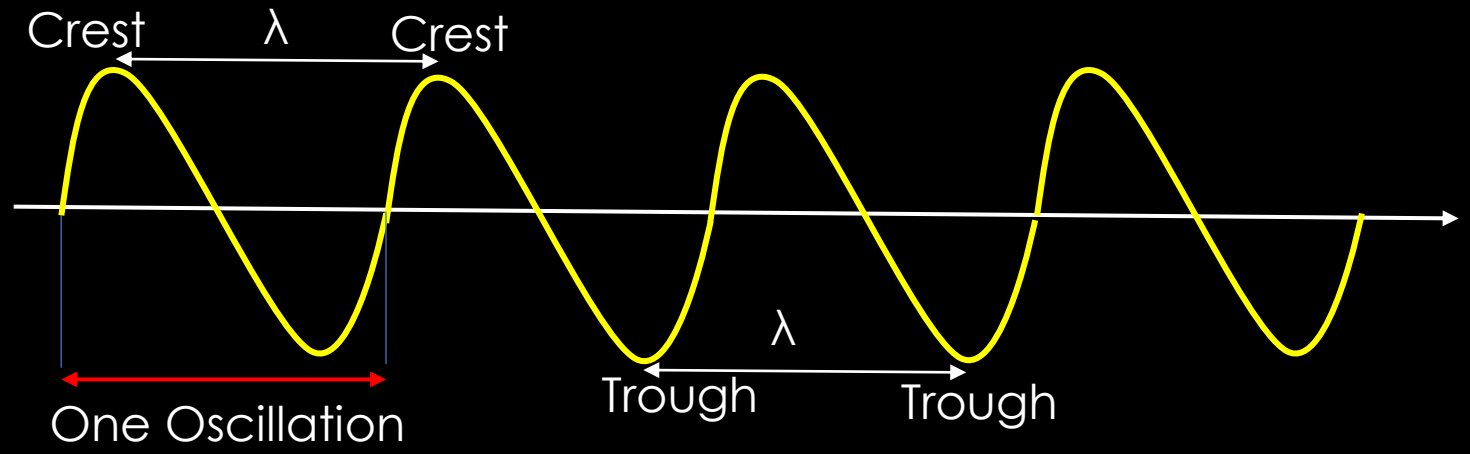
- **Emission spectroscopy** is a spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state.
- E.g., fluorescence spectroscopy, Atomic emission spectroscopy, flame emission spectroscopy (flame photometry)



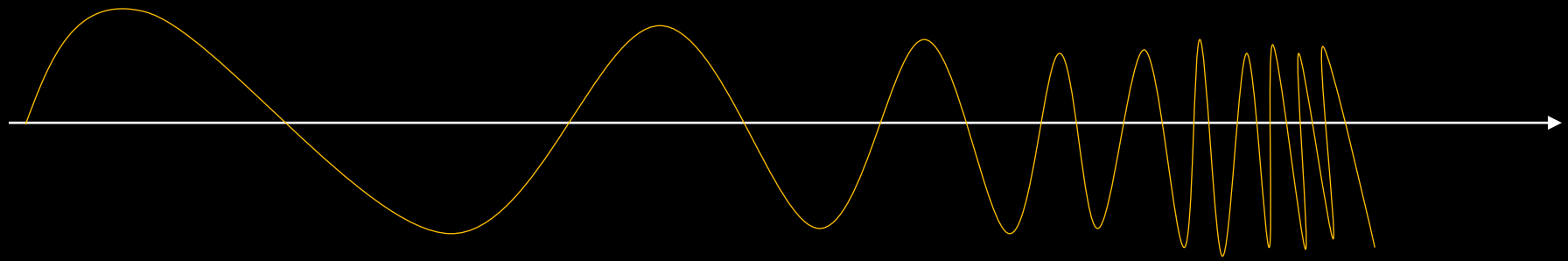
Source Sample Tube Detector Spectra analysis

UV-VISIBLE SPECTROSCOPY

Spectrum or EMR



Frequency is the no. of oscillation per second



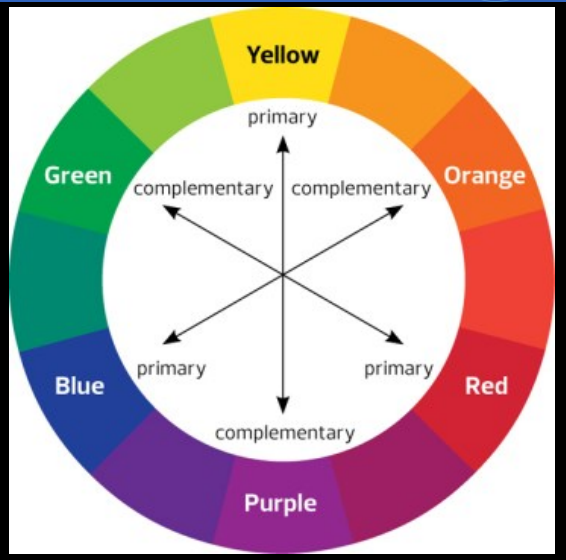
Radio—Microwave—Infrared—visible—UV—X-Ray—Gamma Ray

UV-VISIBLE SPECTROSCOPY

SPECTRUM or EMR

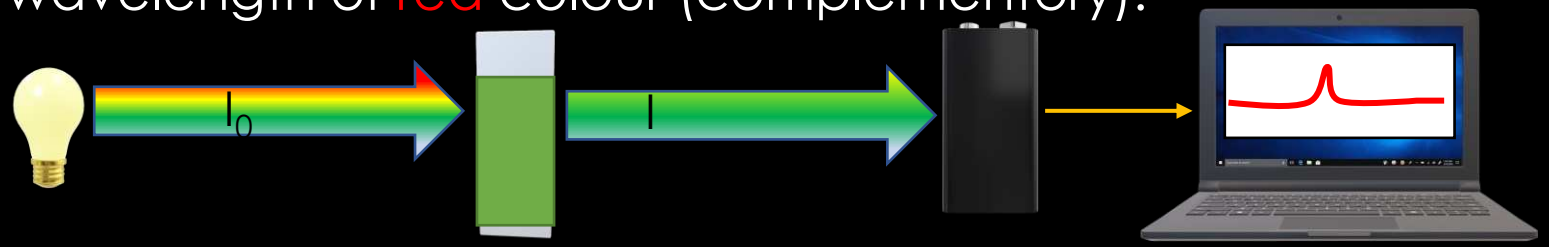
Type of Radiation	Wavelength	Type of molecular spectrum or Excitation
Radio waves	1-10^7 m	NMR (Spin orientation)
Microwave	0.1-1 m	Rotational
Infra Red	0.8-200 μm	
Far IR	15/25-200 μm	Vibrational fundamental or rotational
Mid IR	2.5 μm – 15/25 μm	Vibrational fundamental
Near IR	0.8 – 2.5 μm	Vibrational (overtones)
Visible	380nm – 780nm	Electronic (valence orbital)
Near UV	180nm – 380nm	Electronic (valence orbital)
Vacuum UV	10nm – 200nm	Electronic (valence orbital)
X-rays	0.1-10 nm	Electronic (core orbitals)
Gamma rays	10^{-10} cm	Mossbauer effect (Nuclear transitions) excited states of nuclei
Cosmic rays	**10^{-12} cm**	

UV-VISIBLE SPECTROSCOPY



INTRODUCTION

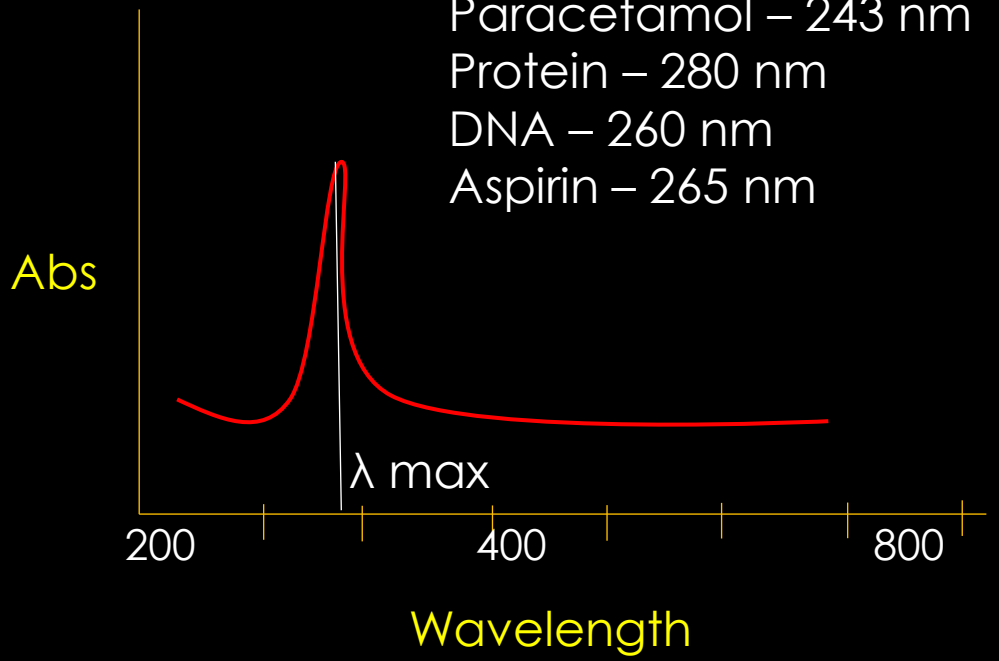
- UV Spectra- 180-380 nm or 200-400 nm
- VISIBLE: 380-780 nm or 400-800 nm
- When a UV-Vsible spectra pass through a solution, the compound present on the solution, absorb specific certain wavelength and transmit or leave unabsorbed wavelength which is detected by uv-visible spectrophotometer, and the methos is called uv-visible spectrophotometry of spectroscopy
- If we see the solution green it means compound is absorbed wavelength of red colour (complementory).

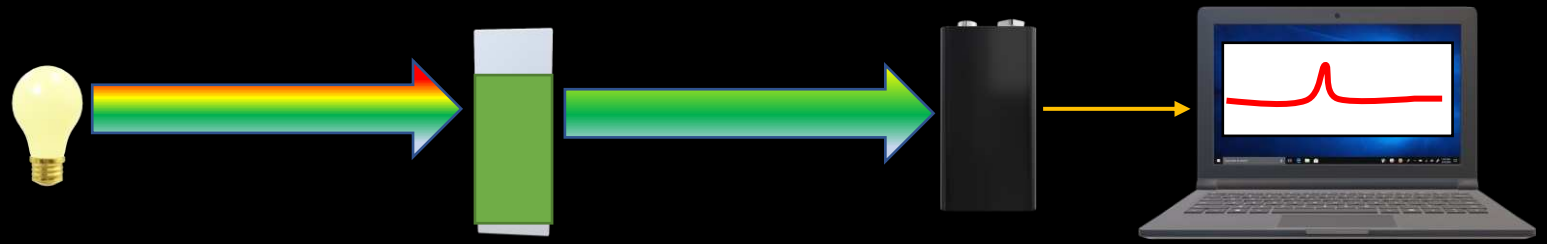


Source Sample Tube Detector Spectra analysis

INTRODUCTION

λ max
Paracetamol – 243 nm
Protein – 280 nm
DNA – 260 nm
Aspirin – 265 nm





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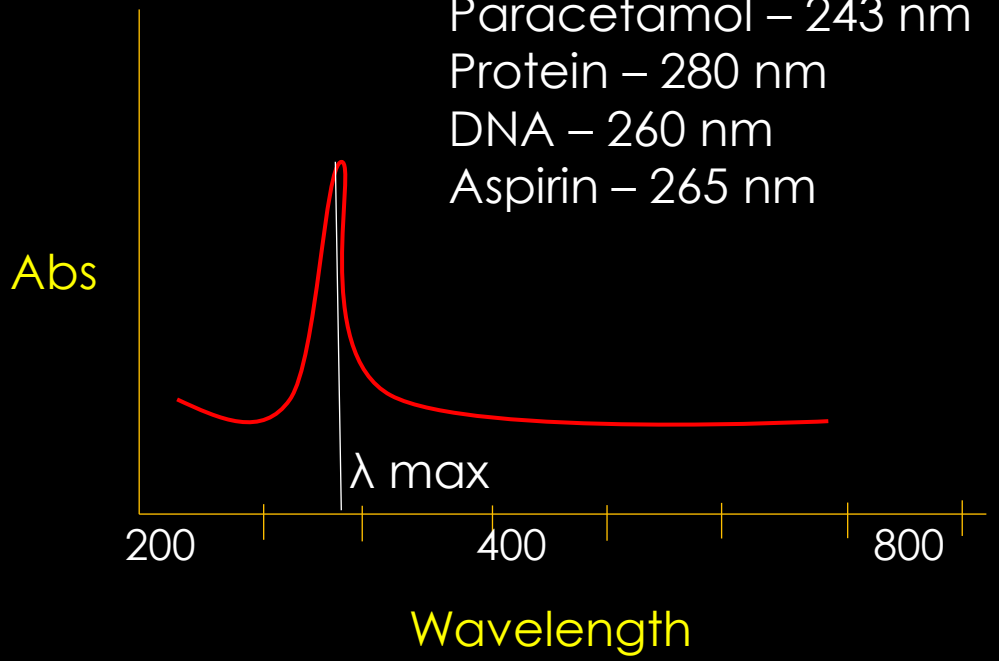

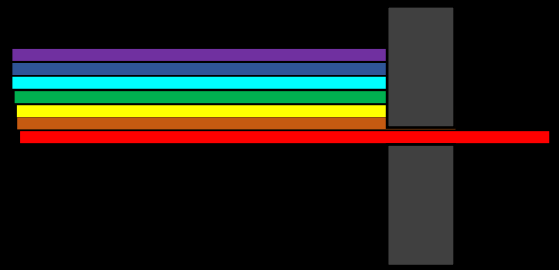

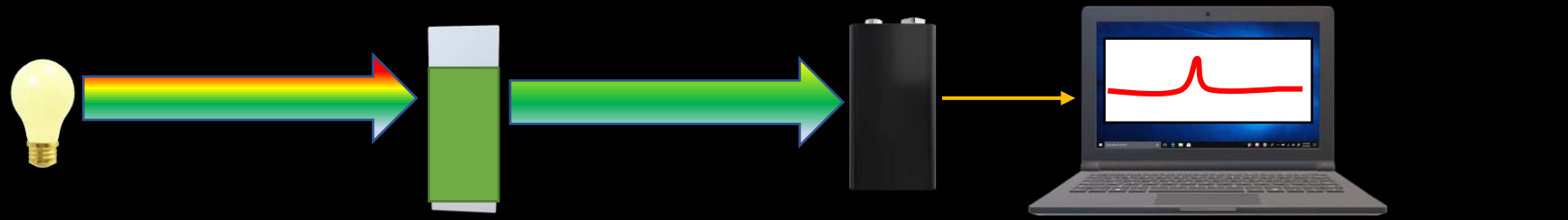

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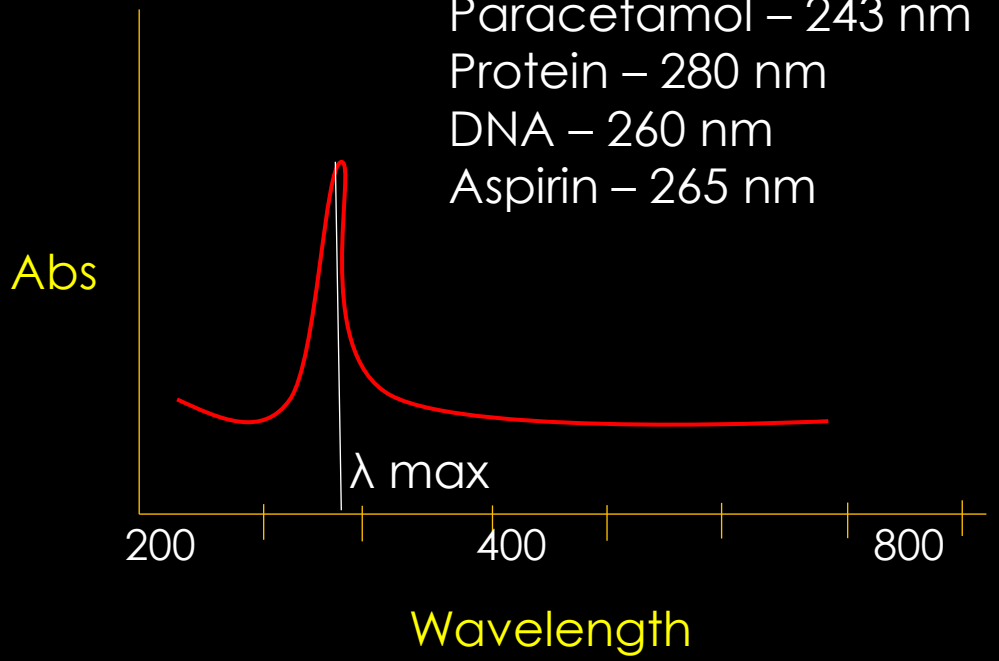

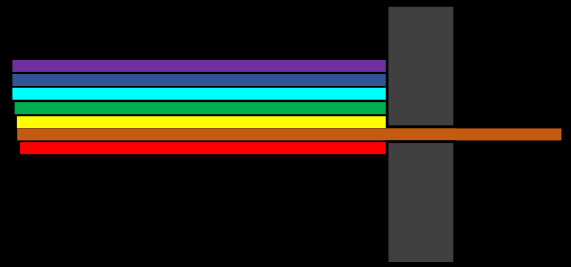

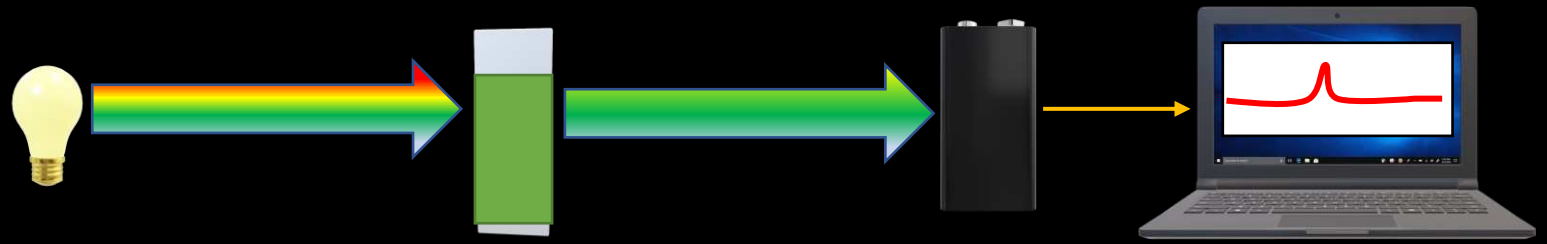

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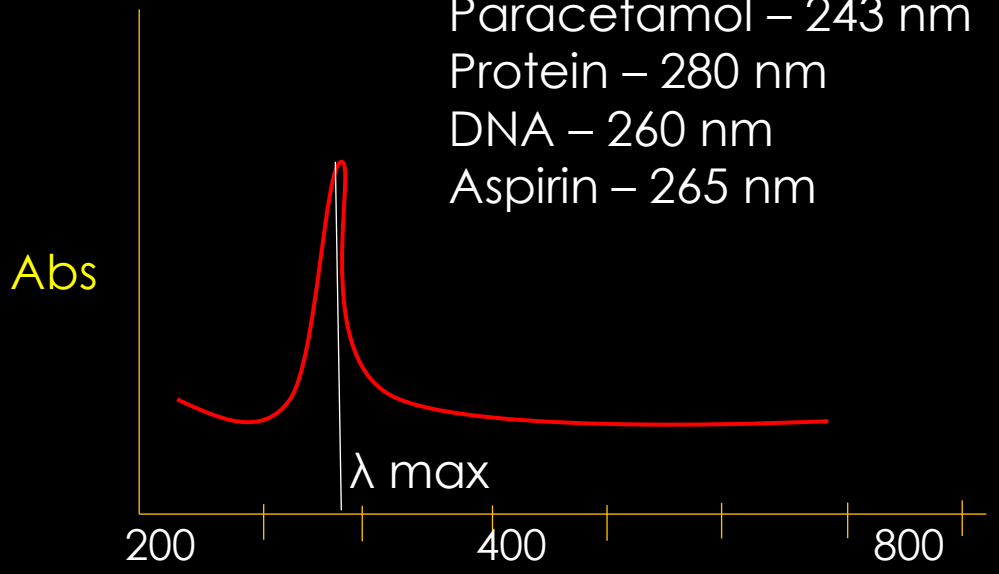

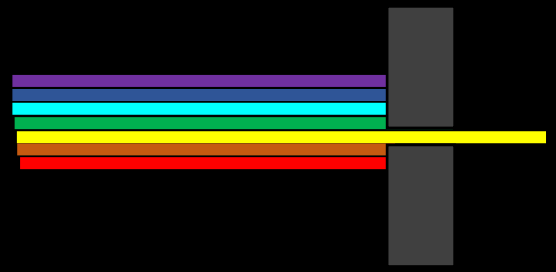

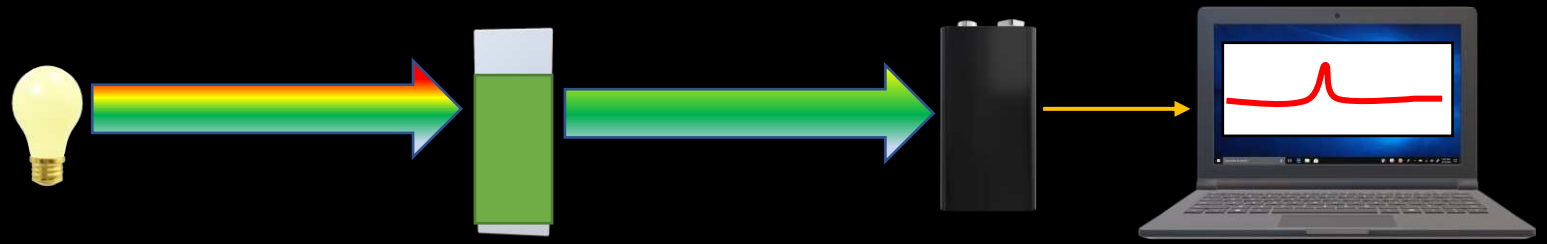

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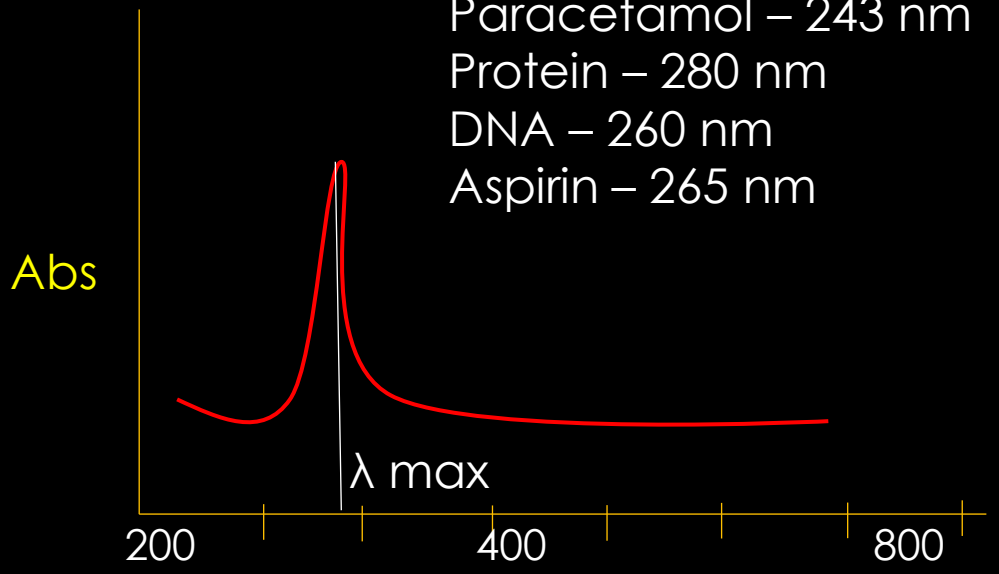
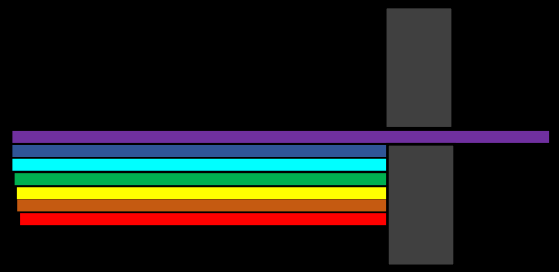
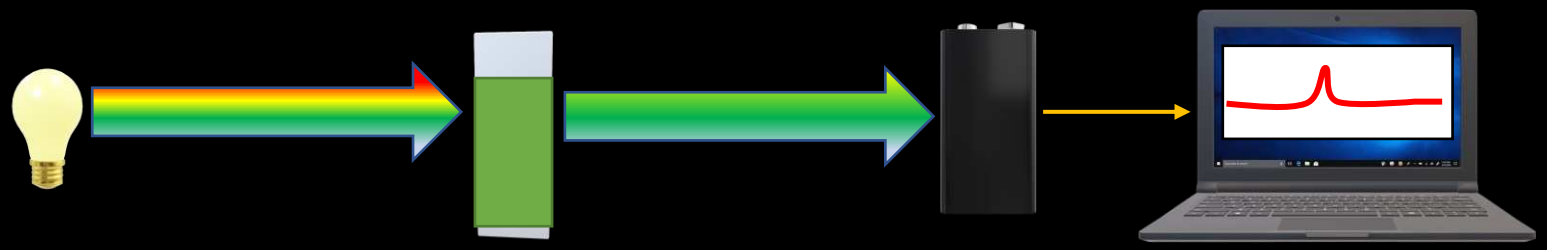




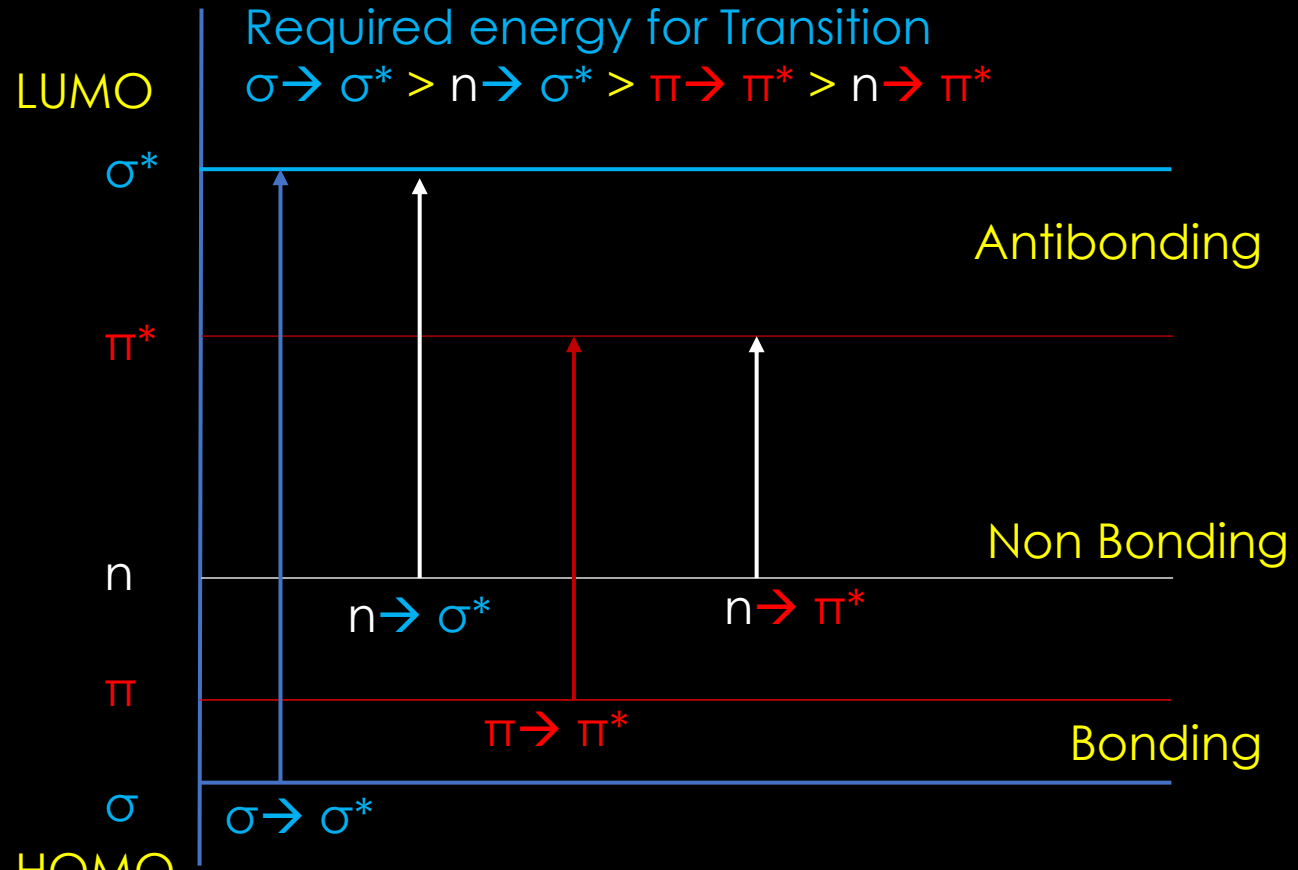


Source Sample Tube Detector Spectra analysis

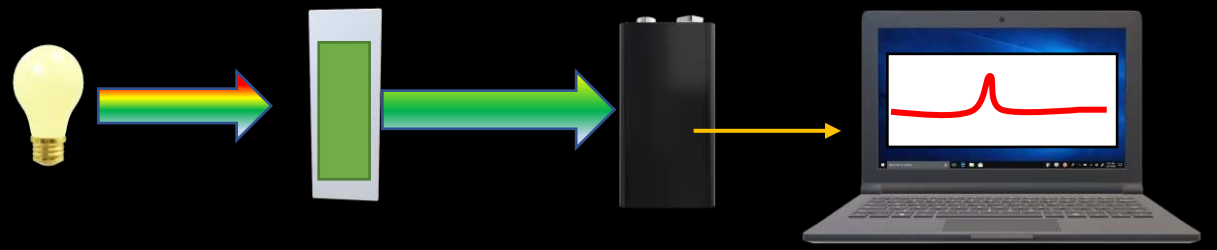
UV-VISIBLE SPECTROSCOPY

PRINCIPLE

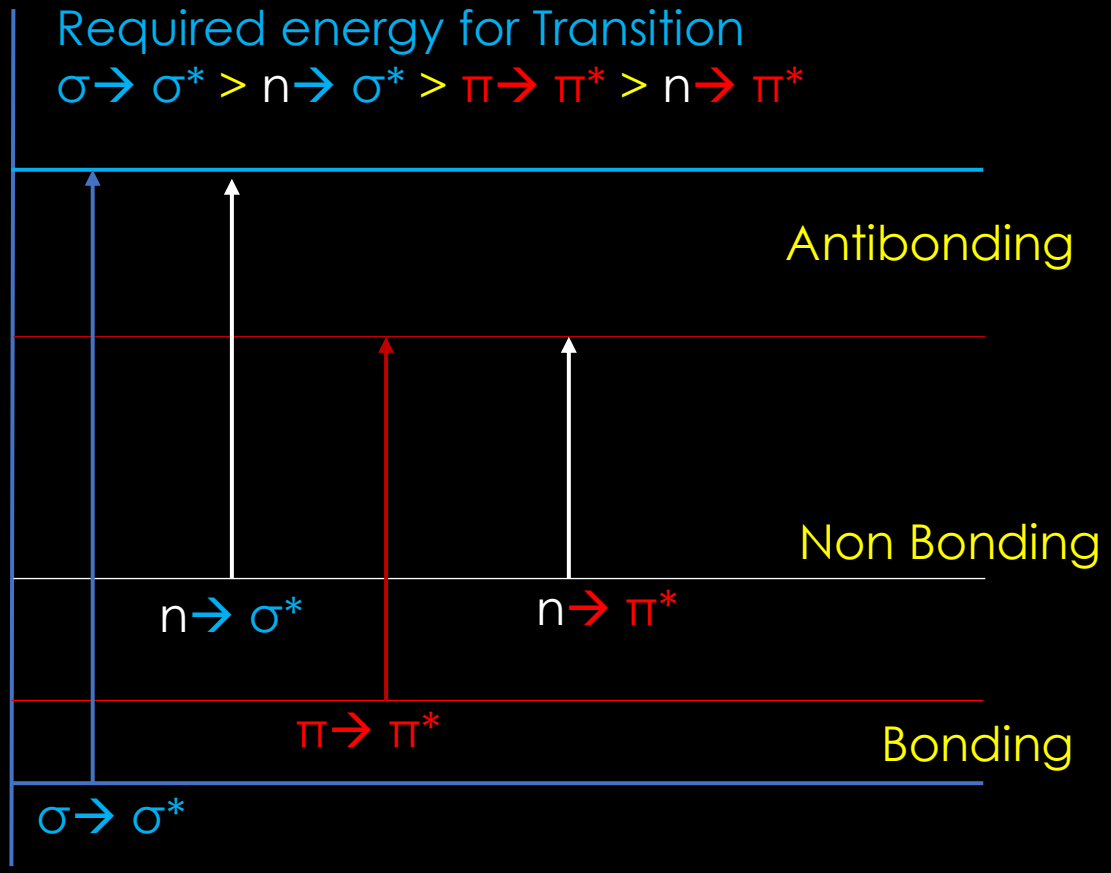
- When a compound absorbs uv-vivible spectra, the electrons are promoted from ground state to an excited state. This can be due to absorption of particular energy, which involves promotion of one electron from HOMO (highly occupied molecular orbital) to LUMO (lowest unoccupied molecular orbital)
- It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.



UV-Visible Spectroscopy (Part 2)



Electronic Transitions



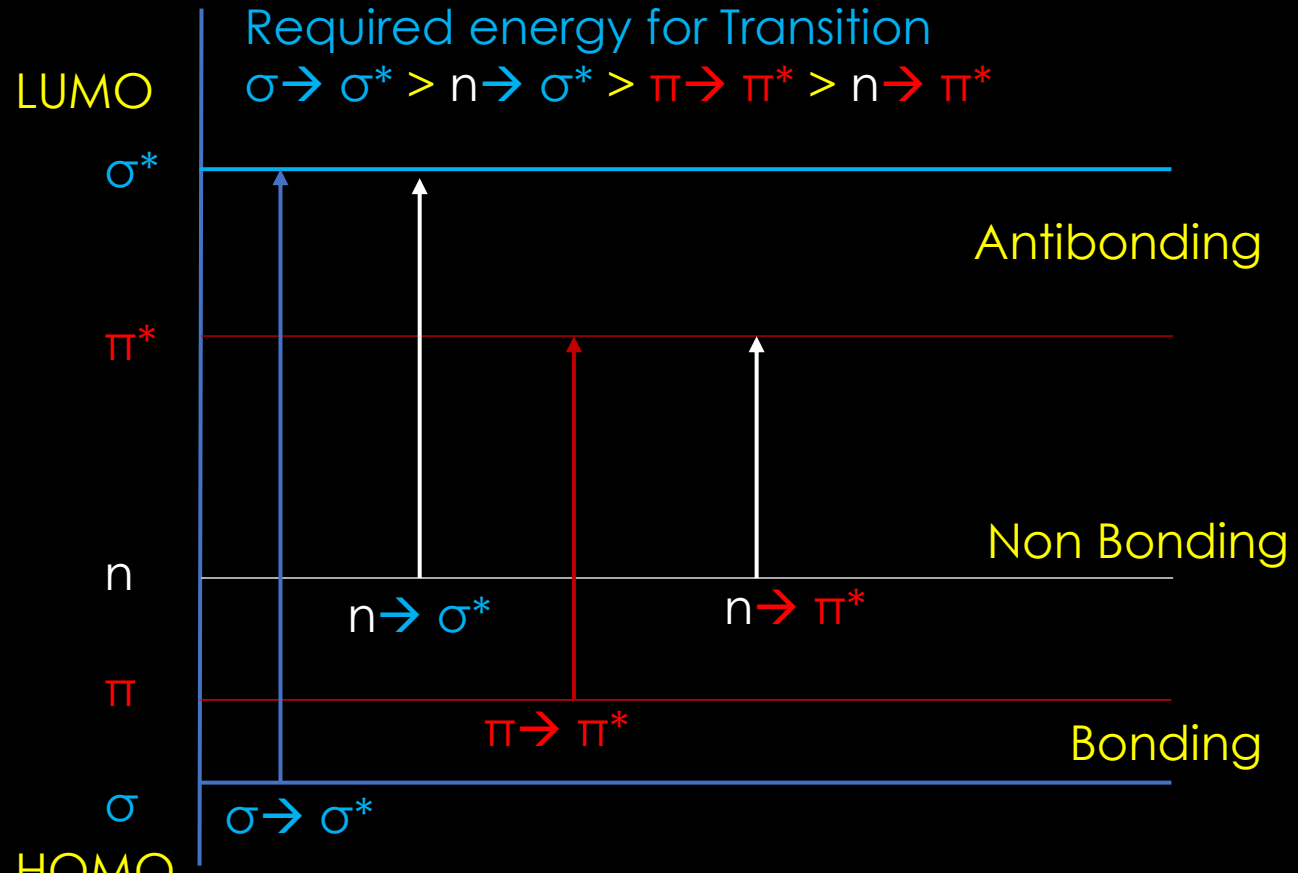
Spectroscopy
Instrumental Analysis

UV-VISIBLE SPECTROSCOPY

Electronic Transition

- There are mainly Four types of Electronic Traansitions:

1. σ→ σ*
2. n→ σ*
3. π→ π*
4. n→ π*



ΔE = (Eel + Evib + Erot)excited - (Eel + Evib + Erot)ground

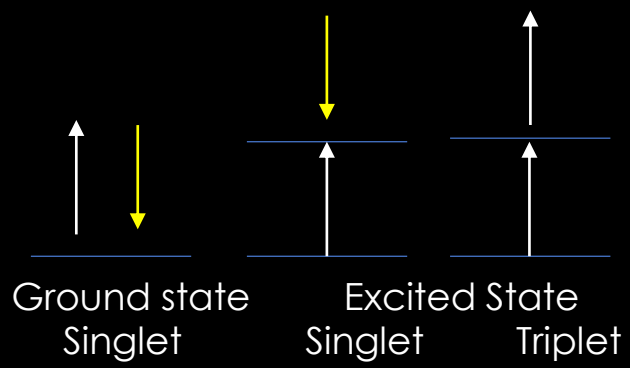
$$\Delta E = hc/\lambda$$

$$\Delta E\ (eV) = 1240/\ \lambda\ (nm)$$

$$\lambda\ (in\ nm) = 1240/\ \Delta E$$

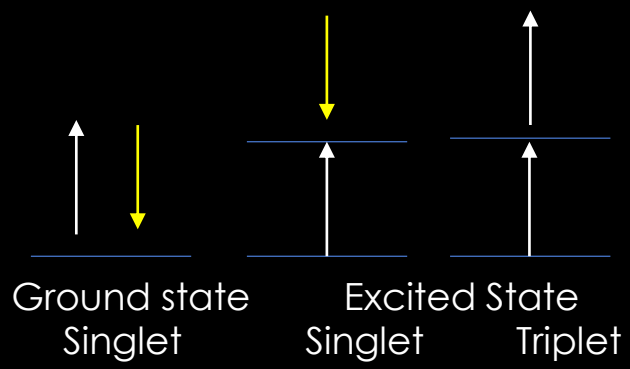
Electronic Transition Theory

- Electron gets promoted from the ground state to the higher energy state after absorption of uv-visible spectra
- In the ground state, the spins of the electrons in each molecular orbital are essentially paired.
- In the higher energy state, if the spins of the electrons are ***opposite*** and ***unpaired***, then it is called as an **excited singlet state**.
- On the other hand, if spins of the electrons in the excited state are ***parallel*** and ***unpaired***, it is called as an **excited triplet state**.
- The triplet state is always lower in energy than the corresponding excited singlet state. Therefore, triplet state is more stable as compared to the excited singlet state.



Electronic Transition Theory

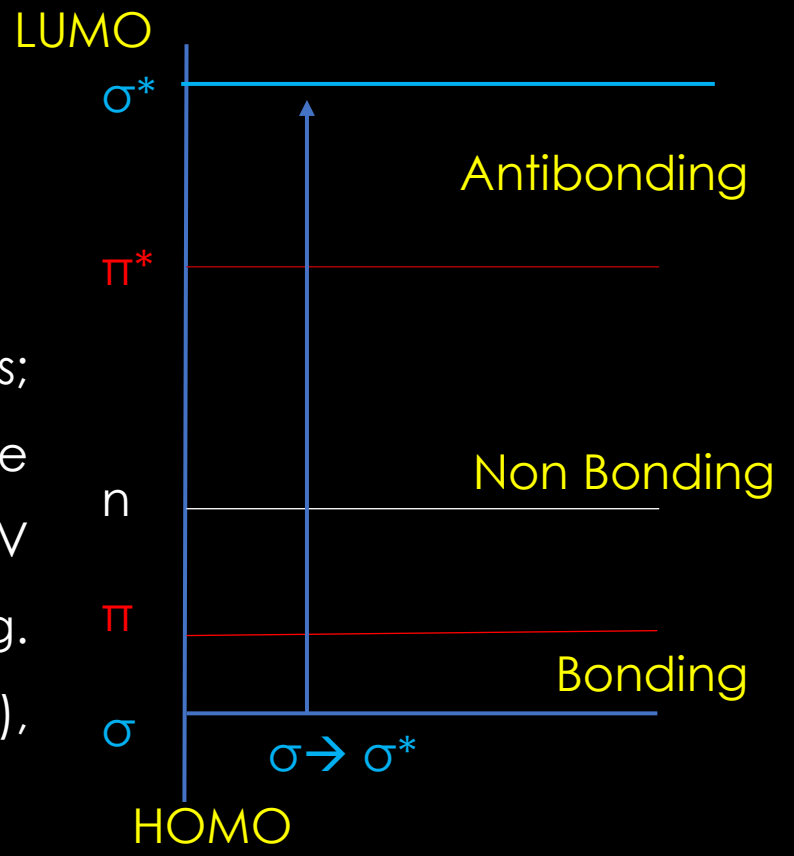
- In the triplet excited state, electrons are farther apart in space & thus, electron-electron repulsion is minimized.
- Normally the absorption of UV or visible light results in singlet ground state to excited singlet state transition, i.e. excitation proceeds with the retention of spins.
- An excited singlet state is converted to excited triplet state with the emission of energy as light. The transition from the singlet ground state to excited triplet state is symmetry forbidden.



Electronic Transition

1. σ→ σ*

- It is a high energy process since a-bonds are very strong.
- It is observed with saturated compounds (especially hydrocarbons; CH4, C2H6), in which all the valence shells electrons are involved in the formation of sigma bonds do not show absorption in the normal UV region, so they absorb far vaccume UV region. i.e. 120nm – 180nm. e.g. methane (122 nm), ethane (135 nm), propane (135 nm), cyclopropane, etc.
- The usual spectroscopy techniques can not be used be bellow 200 nm because oxygen present on air begin absorb strongly. Therefore, entire path length must be evacuated. Thus bellow 200nm is commonly called vacuume uv region.

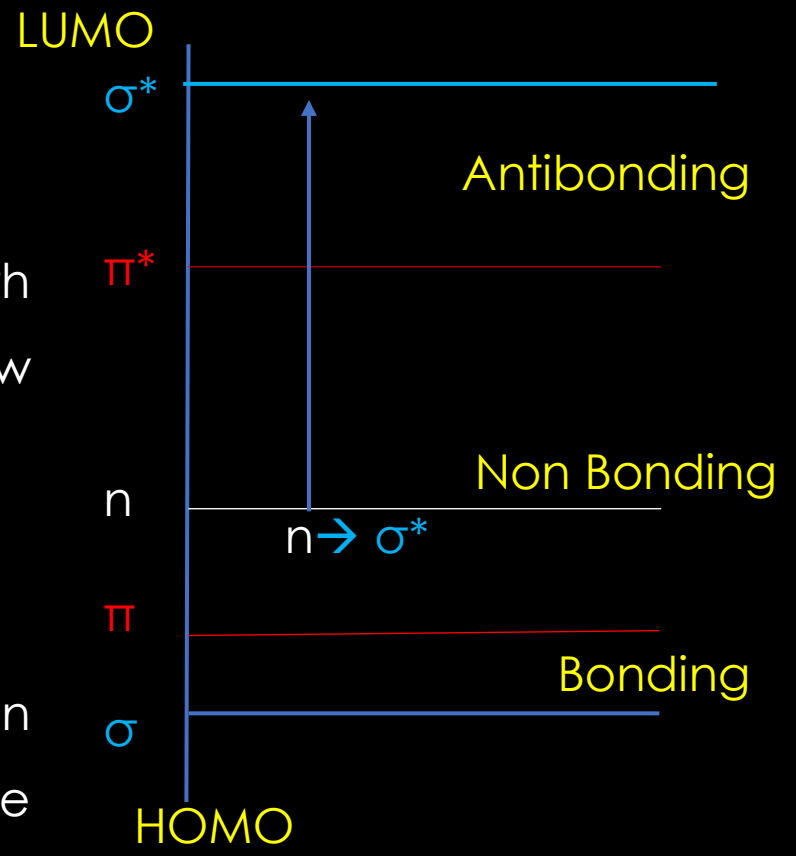


Electronic Transition

2. n→ σ*

- It occurs in saturated compounds containing one hetero atom with unshared lone pair of electrons (η-electrons) and comparetevely low energy than σ→ σ*
- E.g. saturated halides, alcohols, ethers, amine, etc.
- Region 180-260 nm
- In saturated alkyl halides, the energy required for such a transition decreases with the increase in size of the halogen atom (or decrease in the electro-negativity of the atom).

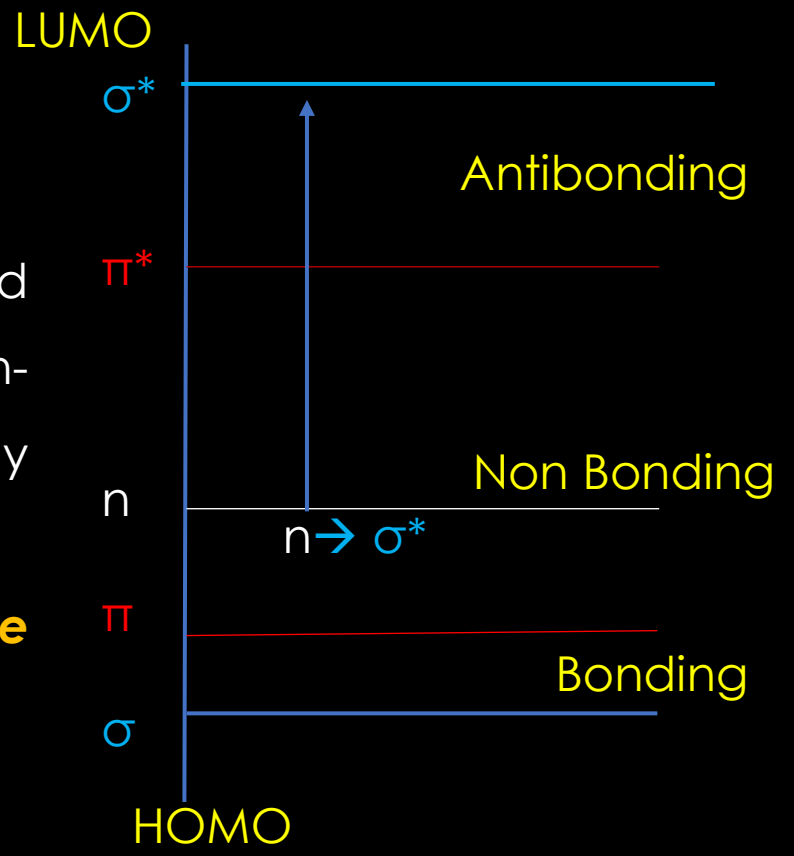
Ex. Methylene chloride – 173 nm, water – 191 nm, Methanol – 203, ethanol – 204 nm, Ether – 215 nm, methyl iodide – 258 nm



Electronic Transition

2. n→ σ*

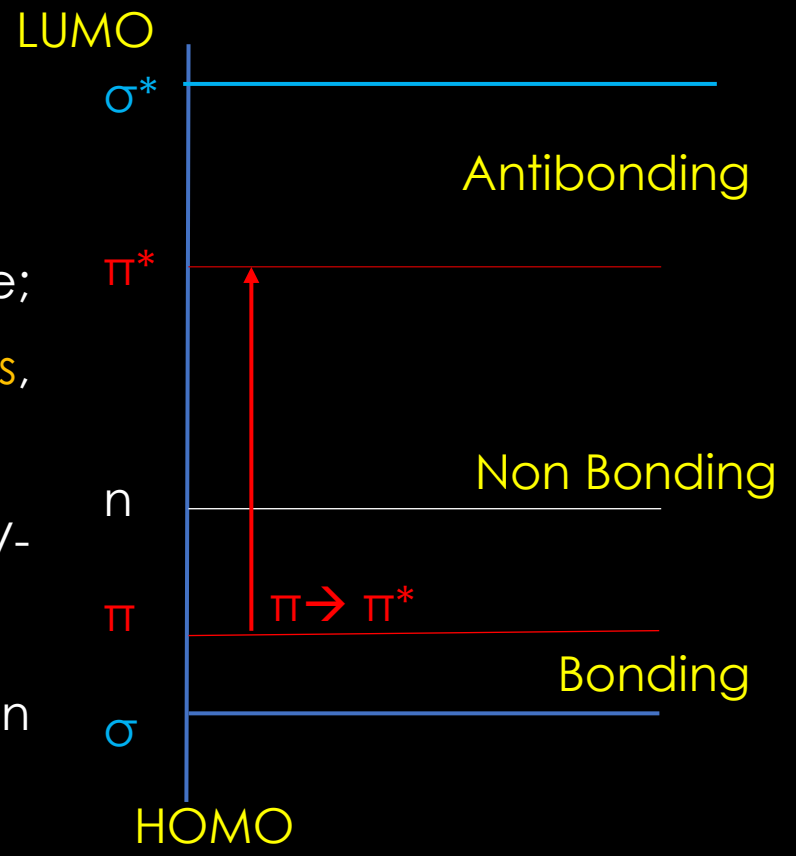
- It is sensitive to Hydrogen bonding. Alcohol as well amines form H-Bond with the solvent molecules, such association is due to presence of non-bonding electron on heteroatom, thus transition requires higher energy (lower wavelength).
- ***H-bonding shifts uv aborption to shorter wavelength by increasing the polarity of solvent***



Electronic Transition

3. π→ π*

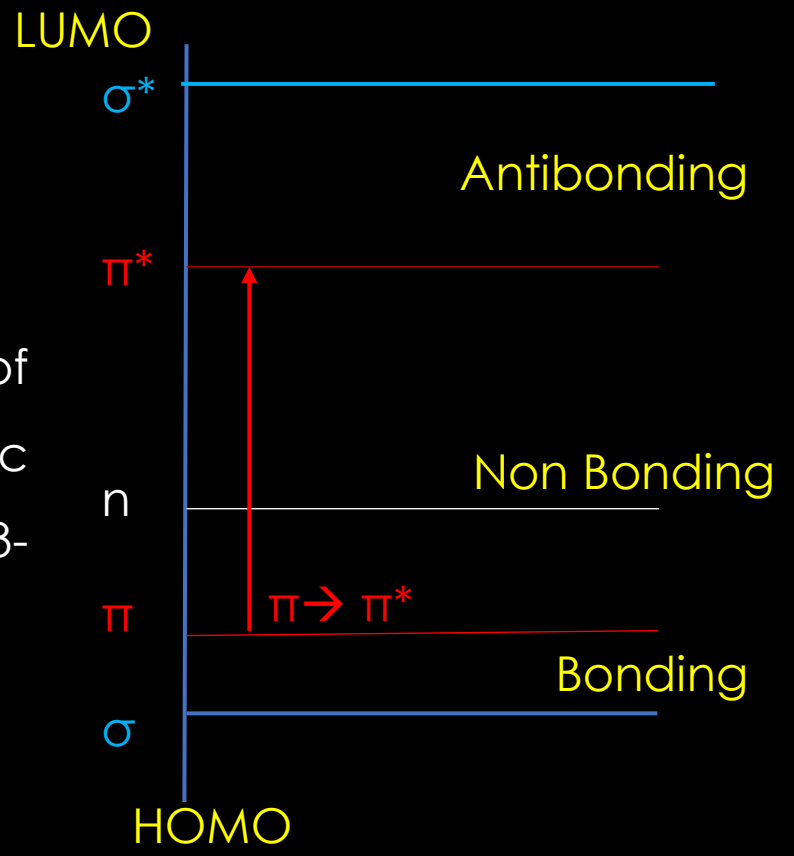
- This type transitions occur in the unsaturated centers of the molecule; i.e. in compounds containing double or triple bonds like alkenes, alkynes & also in aromatic compound.
- Absorption usually occurs within the region of ordinary UV-spectrophotometer.
- The excitation of π-electron requires smaller energy & hence, transition of this type occurs at longer wavelength.
- A π-electron of a double bond is excited to π*-orbital. E.g. alkenes, alkynes, carbonyl compounds, cyanides, azo compounds, etc.
- This transition requires still lesser energy as compared to η→σ* transition.
- ***H-bonding shifts uv aborption to longer wavelength* (Red shift) *by increasing the polarity of solvent***



Electronic Transition

3. π→ π*

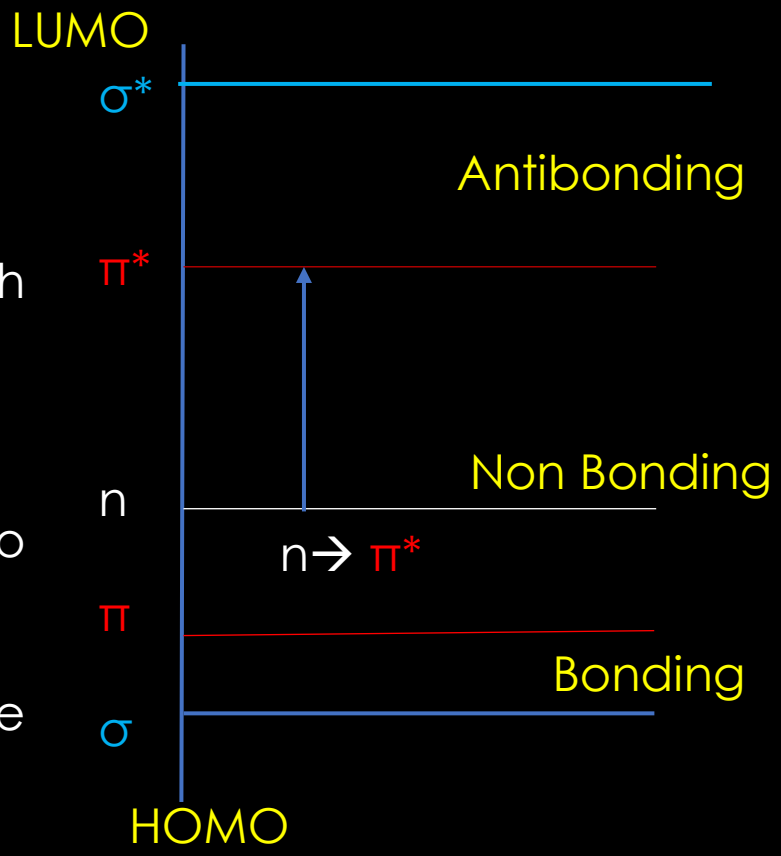
- In unconjucated alkenes absorption appear at around 170-190 nm.
- In carbonyl compound – 180 nm (π→ π*, intense), the value of excitation coefficient is high. The introduction of alkyl group to olefinic linkage (C=C) produces Bathochromic (Red shift) shift of the order of 3-5 nm per alkali group.
- There are three bands appear in this transitions
- B-Band (Benzenoid bands) – Aromatic & Heteroaromatic system
- E-Bands (Ethylenic bands)- Aromatic System
- K-Bands (Conjugates System) – Conjugated system



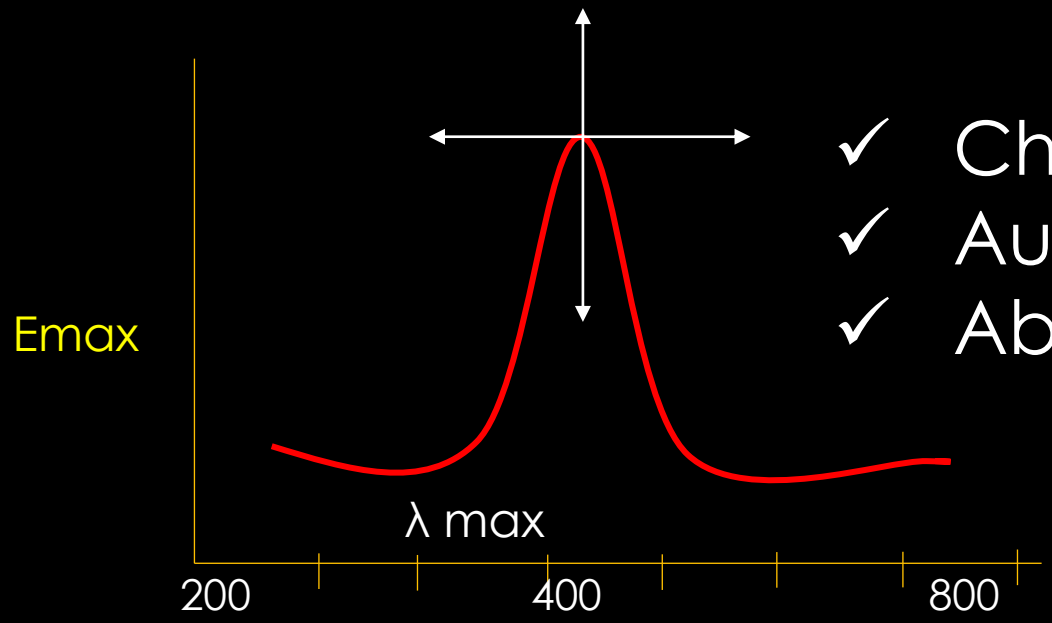
Electronic Transition

4. n→ π*

- The compound containing double or triple bonds along with heteroatom like O, N, S, X show this type of transition
- E.g., aldehyde & Ketones (range 270-300 nm)
- An electron of unshared electron pair on hetero atom gets excited to π*-anti- bonding orbital.
- This type of transition requires least amount of energy out of all the transitions, & hence occurs at longer wavelength.
- **R-Band (Radikalarting German) transition**
- Saturated carbonyl compounds show both type of transition: low energy n→ π* (weak, R-band) and high energy transition n→ σ* (intense) & π→ π* (intense)



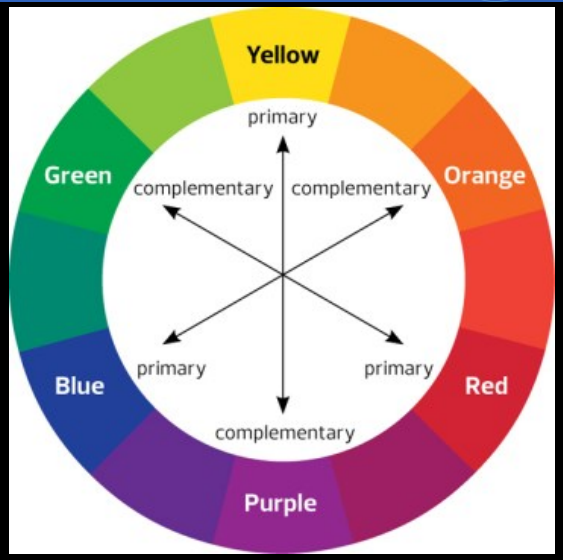
UV-Visible Spectroscopy (Part 3)



- ✓ Chromophore
- ✓ Auxochrome
- ✓ Absorption shift

Spectroscopy
Instrumental Analysis

Chromophore Concepts



- 'Chrome'- Color and "Phore"- Groups.
- The chromophore is a group of compounds that absorb light at wavelength range of 380-780 nm (visible light).
- The appearing color is depends on wavelength of light absorbed by compound.
- A compound containing chromophore is called chromogen
- E.g., nitro compound shows yellow color, mean nitro group is the chromophore which impart yellow color. Similarly aryl conjugated azo group is a chromophore for providing color to azo dyes
- So it is defined as any isolated covalently bonded group that shows s characteristic absorption in the uv-visible region.

Chromophore Concepts

- Types:
- 1. Independent Chromophores: when a single chromophore is a sufficient to produce color to the compound.
- Such compunds contains π and n electron and undergoes π→ π* and n→ π* transition.
- E.g., nitroso (-NO), azo (-N=N-), nitro (-NO2), carbonyl (C=O) and o/p quinoid groups, etc.
- Benzene (colourless) & Nitro-benzene (Yellow color)

Chromophore Concepts

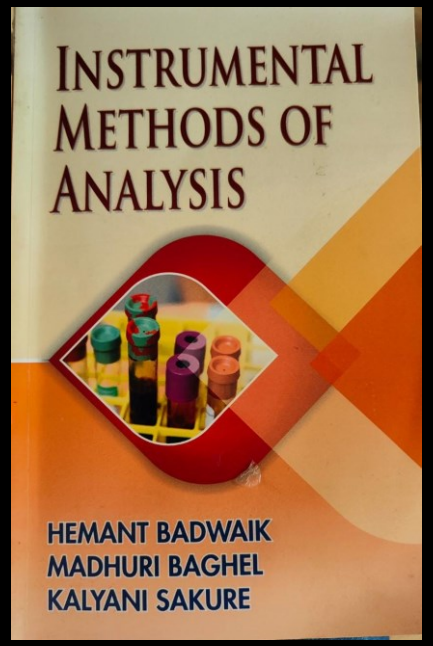
- Types:
- 2. Dependent Chromophores: when a single chromophore is a not sufficient to produce color to the compound and require more than one chromophore to produce color.
- Such compunds contains π electron and undergoes π→ π*
- E.g., carbonyl (C=O), ethylene (C=C), and acetylene (C≡C) groups, etc

R-CO-R	R-CO-CO-R	R-CO-CO-CO-R
Acetone (colourless)	Diacetyl (Yellow)	Triketopentane (Orange)

Chromophore Concepts

Chromophores with their Corresponding Transition and λ_{max} Values

Chromophore	*Transition*	*Absorption maxima (nm)*	ε_{max}	*Solvent*
C = C	$\pi \rightarrow \pi^*$	175	150000	Hexane
C ≡ C	$\pi \rightarrow \pi^*$	220	150	Hexane
C = O	$\eta \rightarrow \sigma^*$	160	18000	Hexane
	$\pi \rightarrow \pi^*$	180	10000	Hexane
	$\eta \rightarrow \pi^*$	285	15	Hexane
N = N	$\pi \rightarrow \pi^*$	338	5	Ethanol



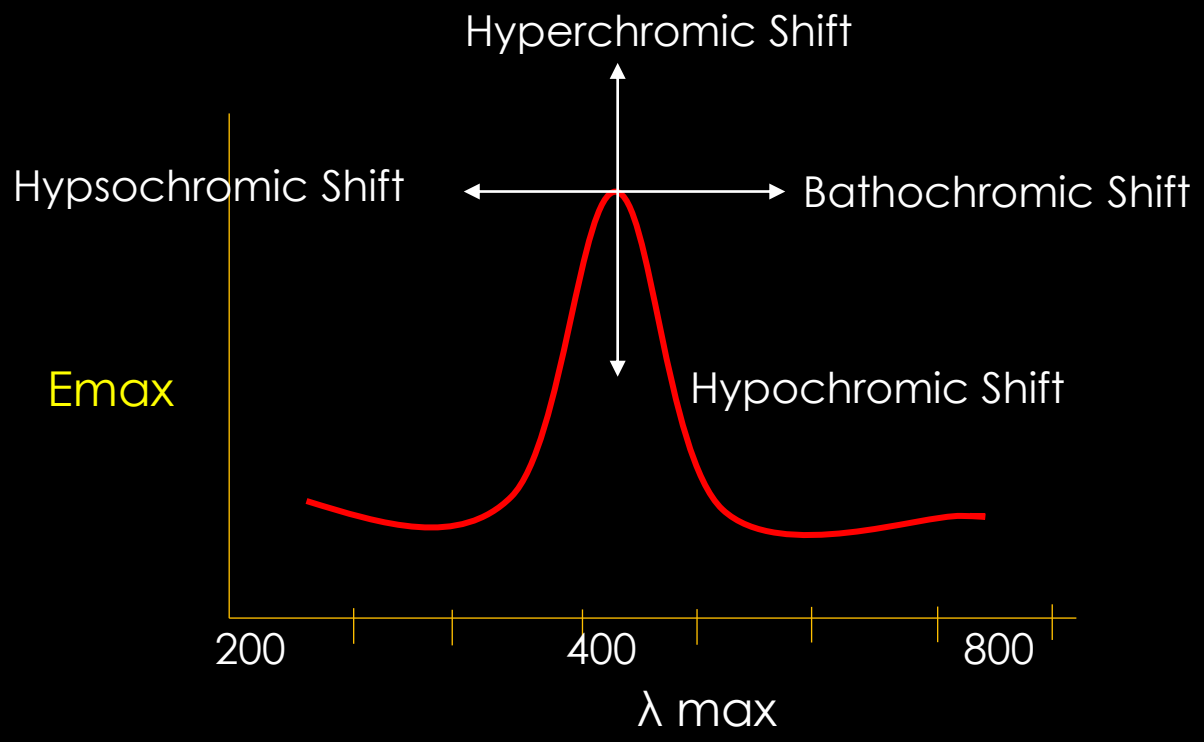
Auxochrome Concepts

- Auxochromes are the saturated group with nonbonded electron, which do not act as a chromophore but it presence to chromophore, they alter both wavelength (red shift) and intensity of absorption.
- It is called color enhancing group
- It does not show characteristic absorption above 180 nm
- E.g., -OH, -OR, -NH2, -NR2, -SR
- This effects is due to its ability to extend the congulation of a chromophore by the sharing of non-bonding electron

$CH_2=CH_2 - NH_2 \rightarrow CH_2 - CH=NH_2$

Benzene (255 nm) Aniline (280 nm) Anilinium (204 nm)

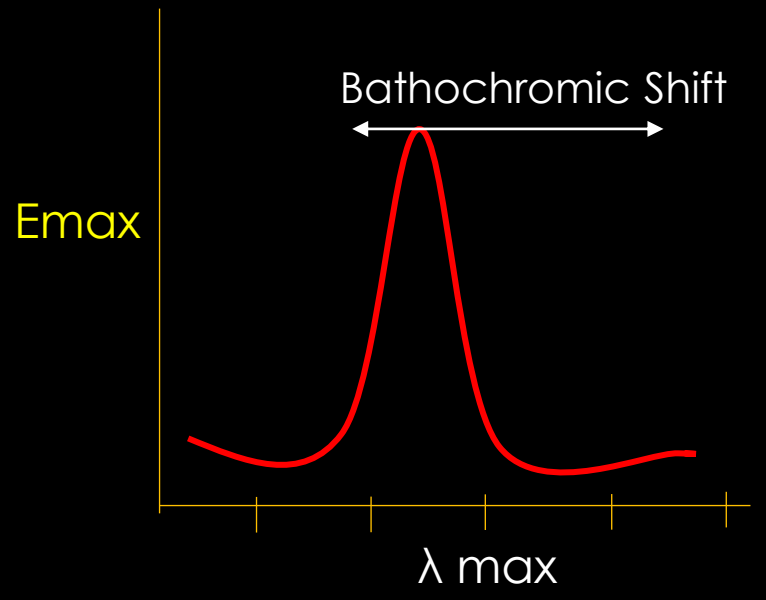
Absorption and Intensity Shift



- ✓ Changes in chemical structure or the environment lead to changes in the absorption spectrum of molecules and materials.
- ✓ There are several terms that are commonly used to describe these shifts

Absorption and Intensity Shift

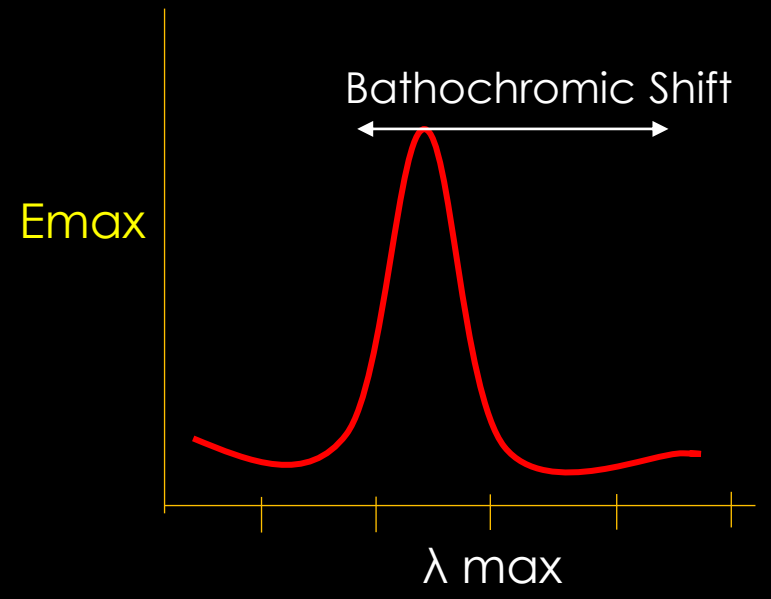
- **Bathochromic Shift (Red Shift):** It refers to shift of wavelength towards longer wavelength due to presence of an auxochrome and or by changing solvent.
- E.g., The n→ π* transition for carbonyl compounds experiences bathochromic shift when the polarity of solvent is decreased. A red shift is noted when phenol by changing the solvent from water to CCl4.



Absorption and Intensity Shift

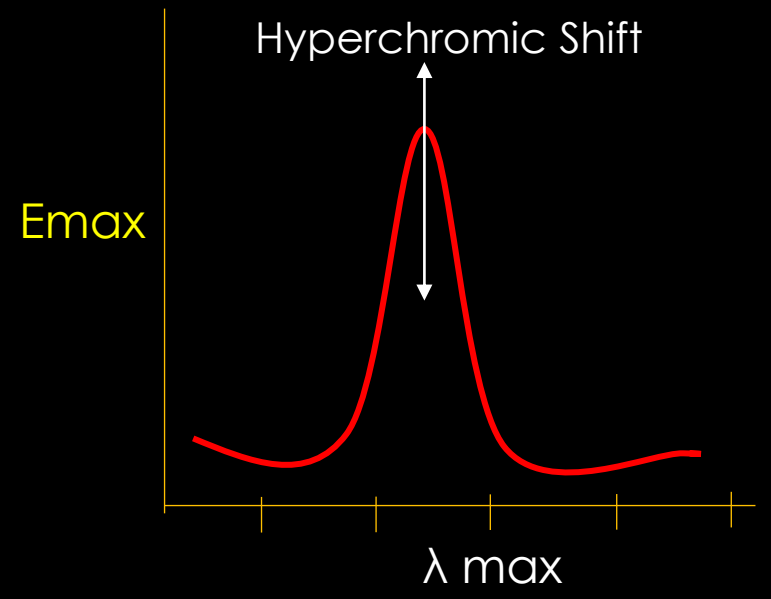
- **Hypsochromic Shift (Blue Shift):** It refers to shift of wavelength towards shorter wavelength due to removal of conjugation and or by changing solvent.

Aniline (280 nm) Anilinium (204 nm) in acidic solution



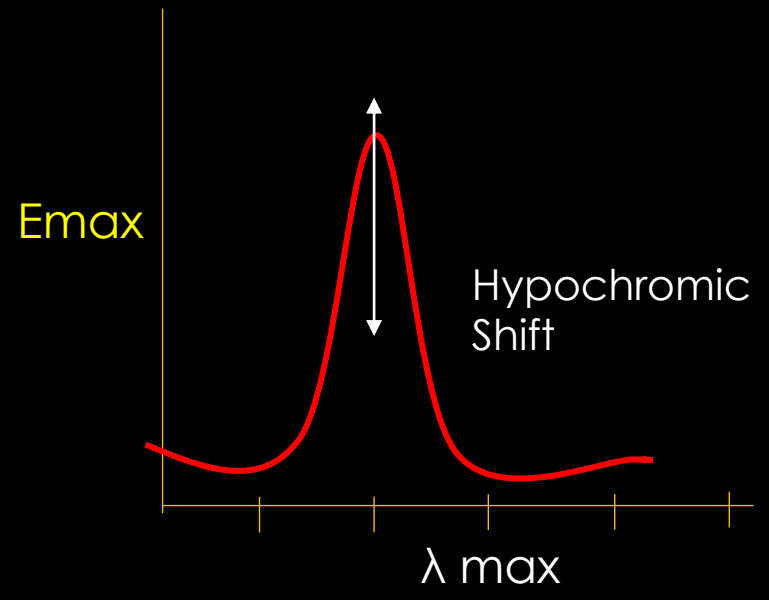
Absorption and Intensity Shift

- **Hyperchromic Shift:** It refers to increase the intensity of absorption maxima (Emax).
- The introduction an auxochrome usually increase the Emax
- E.g., the B-Band for Pyridine at 257 nm (Emax 2750) is shifted to 262 nm (Emax 3560) for 2-methyl pyridine



Absorption and Intensity Shift

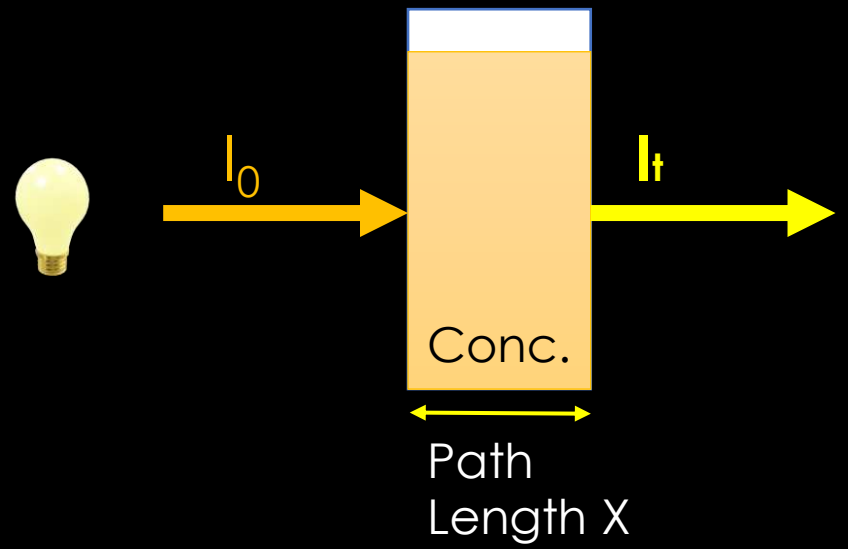
- **Hypochromic Shift:** It refers to decrease the intensity of absorption maxima (Emax).
- The introduction of a group, which destroy or twist the geometry of molecule cause hypochromic



Biphenyl (250 nm, Emax 19000 2-methyl biphenyl (237 nm, Emax 10250)

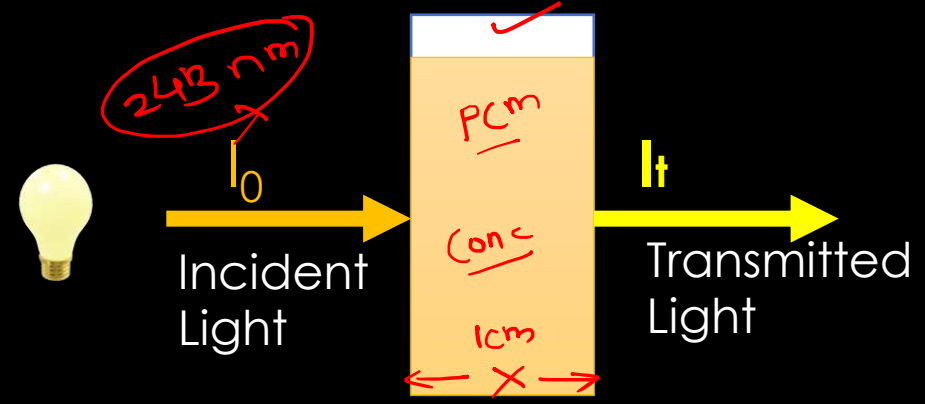
UV-Visible Spectroscopy (Part 4)

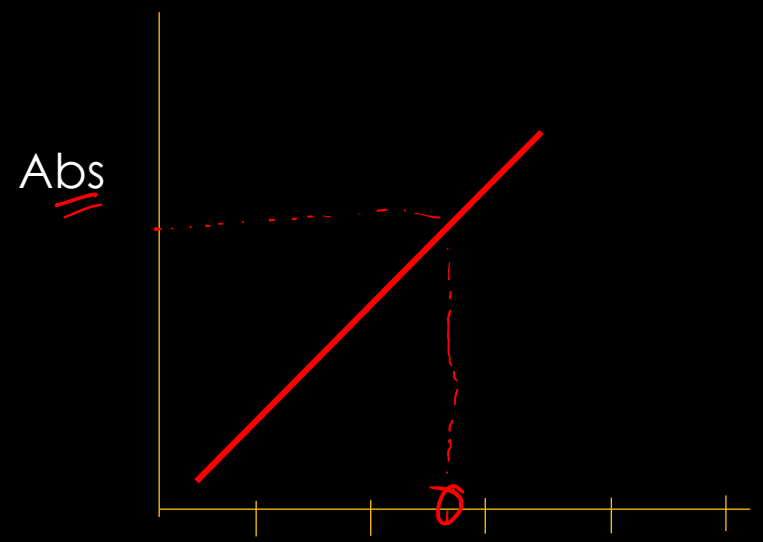
- ✓ Absorption Law
- ✓ Beer's Law
- ✓ Lambert' Law
- ✓ Beer's-Lambert's Law
- ✓ Limitation

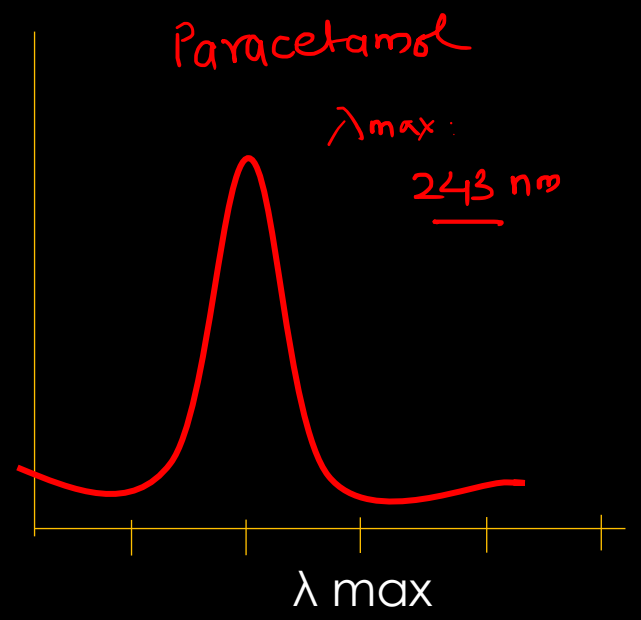


Spectroscopy
Instrumental Analysis

UV-VISIBLE SPECTROSCOPY







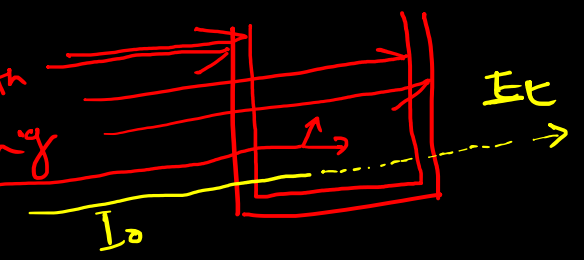
Case 1.

$I_0 = I_t$ $I_0 = \frac{I_0}{I_0} \times 100$

Intensity of incident light (I_0) = 100

$\frac{I_t}{I_0} \times 100$

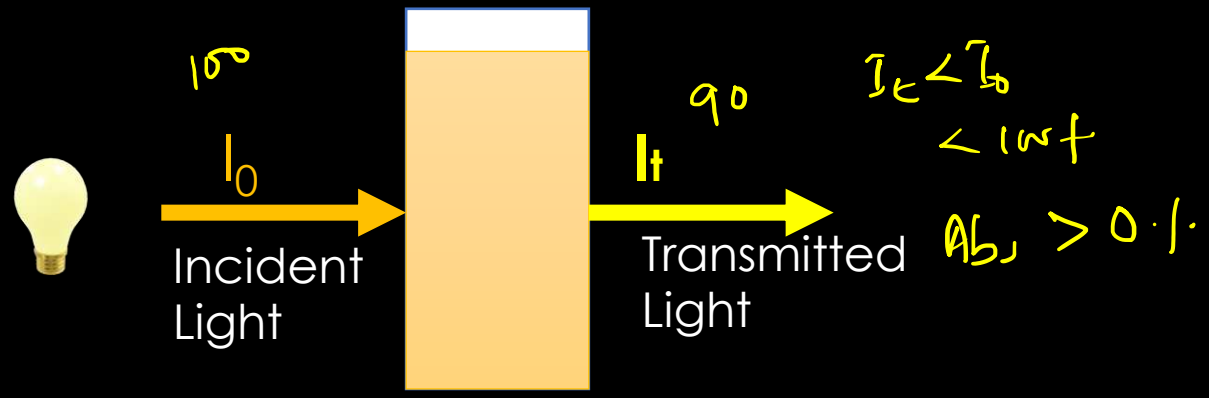
Intensity of transmitted light (I_t) = 100



Absorption % = [(I_0 – I_t) / I_0] x 100

= 0 %

UV-VISIBLE SPECTROSCOPY



Case 2.

Intensity of incident light (I_0) = 100

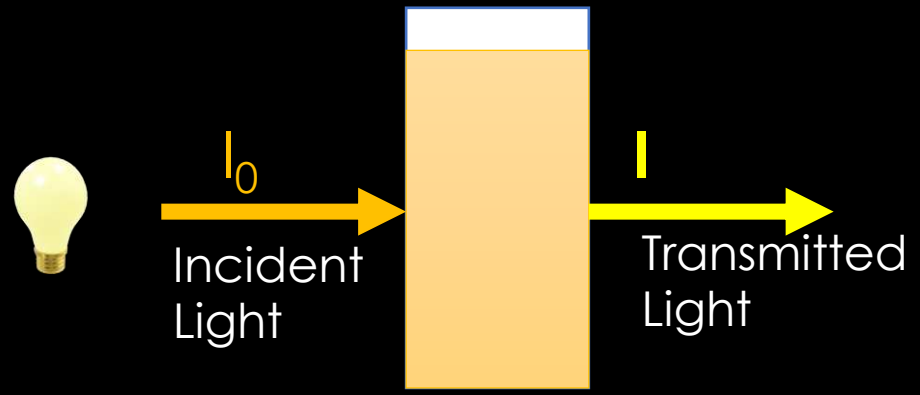
Intensity of transmitted light (I_t) = 90

Absorption % = [(I_o – I_t) / I_o] x 100

= [(100 – 90) / 100] X 100

= 10 %

UV-VISIBLE SPECTROSCOPY



Relation between Absorption (A) & Transmittance (T)

$T = I_t/I_o$

$A = \log (1/T)$
$= \log (I_o/I_t)$

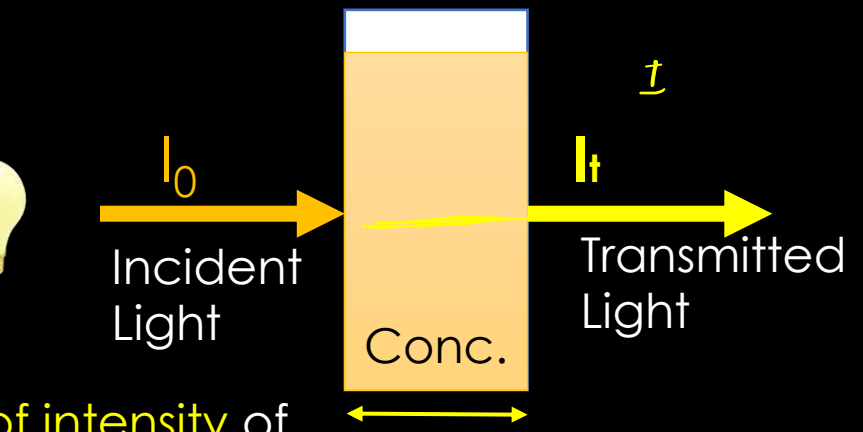
$\log \frac{1}{T}$

$\log \frac{1}{I_t} \cdot I_o$

UV-VISIBLE SPECTROSCOPY

Lambert's Law:

- "when a beam of monochromatic radiation passes through a homogenous absorbing medium, the rate of decrease of intensity of radiation with thickness (path length) of the absorbing solution is proportional to the intensity of incident radiation."



$-\frac{dI}{dx}$ rate of decrease of intensity of Incident light with thickness (x) of absorbing media

k = Proportionally constant or absorption coefficient

$$-\frac{dI}{dx} \propto I$$

$$-\frac{dI}{dx} = kI$$

$$-\frac{dI}{I} = k\,dx$$

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

1) when $x = 0$, $I = I_0$

2) when $x = x$, $I = I_t$

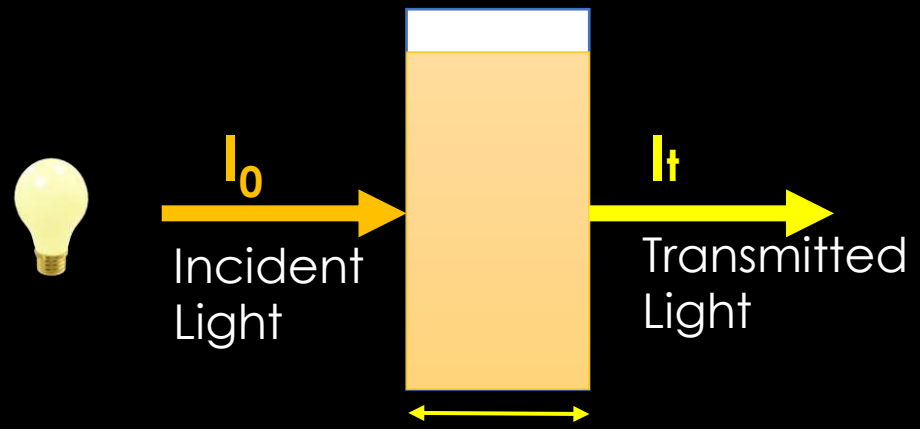
$$\ln \frac{I_0}{I_t} = kx$$

$$\log \frac{I_0}{I_t} = \frac{k}{2.303} x$$

$$\log \frac{I_0}{I_t} = \log \frac{I}{I} = A = \epsilon x$$

$$\epsilon = \frac{k}{2.303}$$

UV-VISIBLE SPECTROSCOPY



Lambert's Law: Abs depends on path length

$$A = \log (I_o/I_t) \propto X$$

$$A = \varepsilon X$$

ε = molar extinction coefficient or molar absorptivity (M^{-1} cm^{-1})

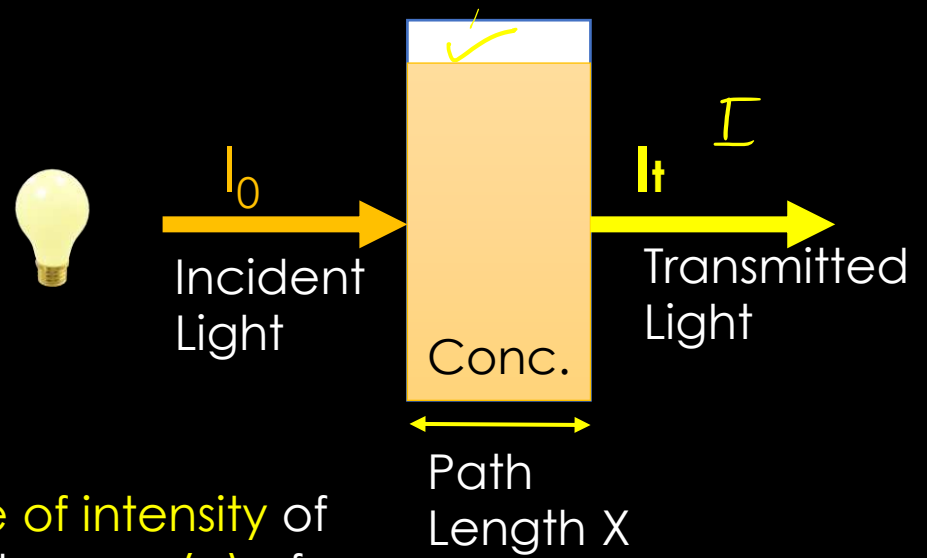
X = path length (cm)

$L\ mol^{-1}\ cm^{-1}$

UV-VISIBLE SPECTROSCOPY

Beer's Law:

- when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with concentration of the absorbing solution is proportional to the intensity of incident radiation"



$$-\frac{dI}{dc} \propto I$$

$$-\frac{dI}{dc} = kI$$

$$-\frac{dI}{I} = k\,dc$$

$$-\int_{I_0}^{I_t} \frac{dI}{I} = \int k\,dc$$

$$\ln \frac{I_0}{I_t} = kc$$

$$\log \frac{I_0}{I_t} = \frac{kc}{2.303}$$

① when $c = 0$, $I = I_0$

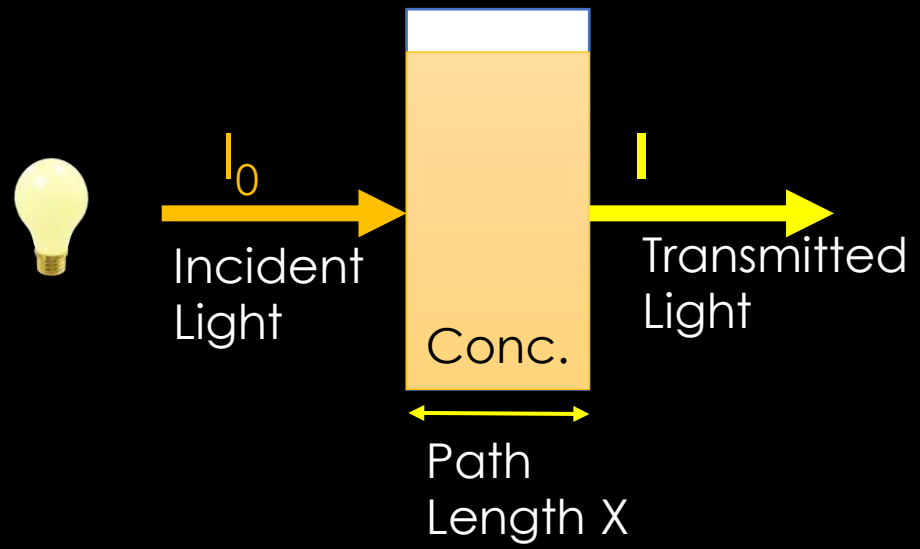
② when $c = c$, $I = I_t$

$-\frac{dI}{dc}$ rate of decrease of intensity of Incident light with conc (c) of absorbing media

k Proportionally constant or molar absorption coefficient

$$\log \frac{I_0}{I_t} = \log \frac{1}{T} = A = \epsilon C$$

UV-VISIBLE SPECTROSCOPY



Beer's Law: Abs depends on concentration

$$A = \log (I_0/I) \propto C$$

$$A = \varepsilon C$$

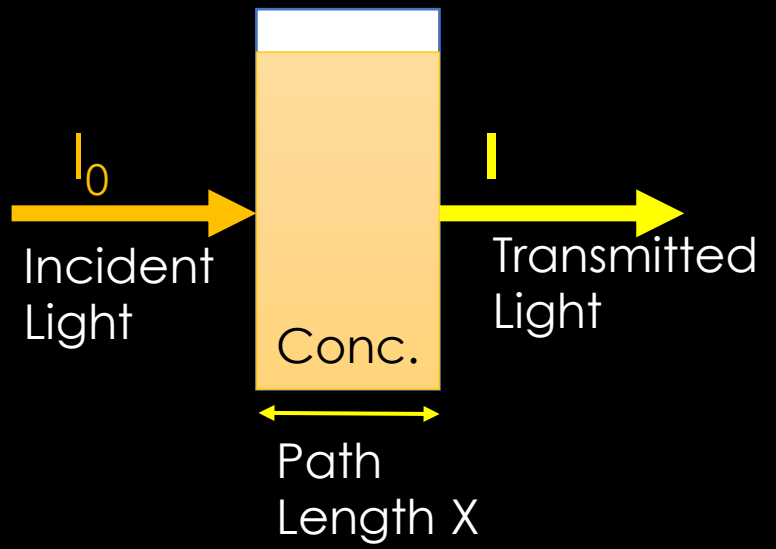
ε = molar extinction coefficient or molar absorptivity ($M^{-1} cm^{-1}$)

C = conc. (M)

UV-VISIBLE SPECTROSCOPY

Beer's-Lambert's Law:

- when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the intensity of incident radiation as well as the concentration of the solution."



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

$$-\frac{dI}{dx} = kIc$$

$$-\frac{dI}{I} = kc\,dx$$

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

$$\ln \frac{I_0}{I_t} = kcx$$

$$\log \frac{I_0}{I_t} = \frac{k}{2.303} \cdot cx = A = \epsilon cx$$

$$= \epsilon . b c$$

Beer's-Lambert's Law: Abs depends on concentration and pathlength both

$$A = \log (I_0/I)\ a\ C\ X$$

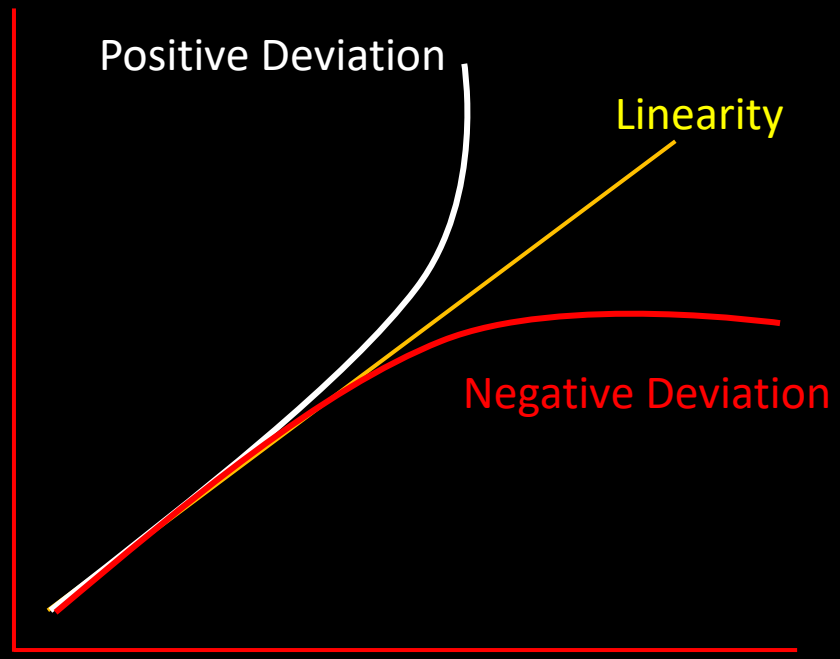
$$A = \varepsilon\ C\ X$$

UV-VISIBLE SPECTROSCOPY

Beer's-Lambert's Law Limitation:

- When different forms of the absorbing molecules are in equilibrium as in keto-enol tautomer's.
- Presence of fluorescent compound
- When solute and solvent form complexes through some sort of association

UV-Visible Spectroscopy (Part 5)

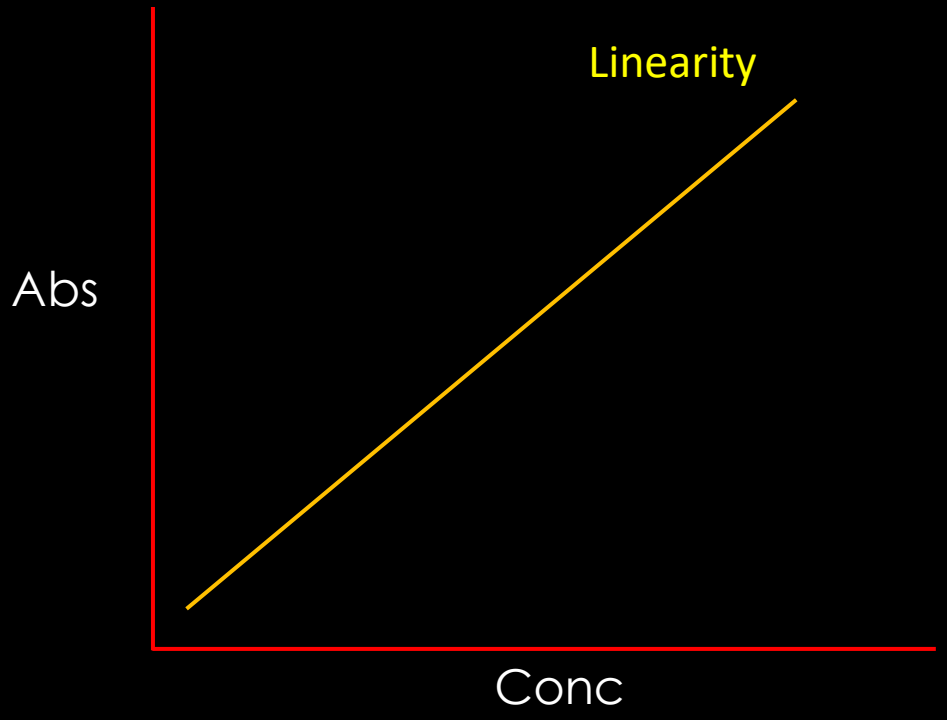


- ✓ Deviation of Beer's-Lambert's Law
- ✓ Solvent Effects on spectra
- ✓ Isosbestic Point

Spectroscopy
Instrumental Analysis

Deviation of Beer's-Lambert's

- A system is said to obey Beer's law, when a plot of absorbance Vs concentration gives a straight line.
- The straight line is obtained by using line of best fit



$Abs = \epsilon C X$

ϵ = Exc. coff = Const.

C = Conc.

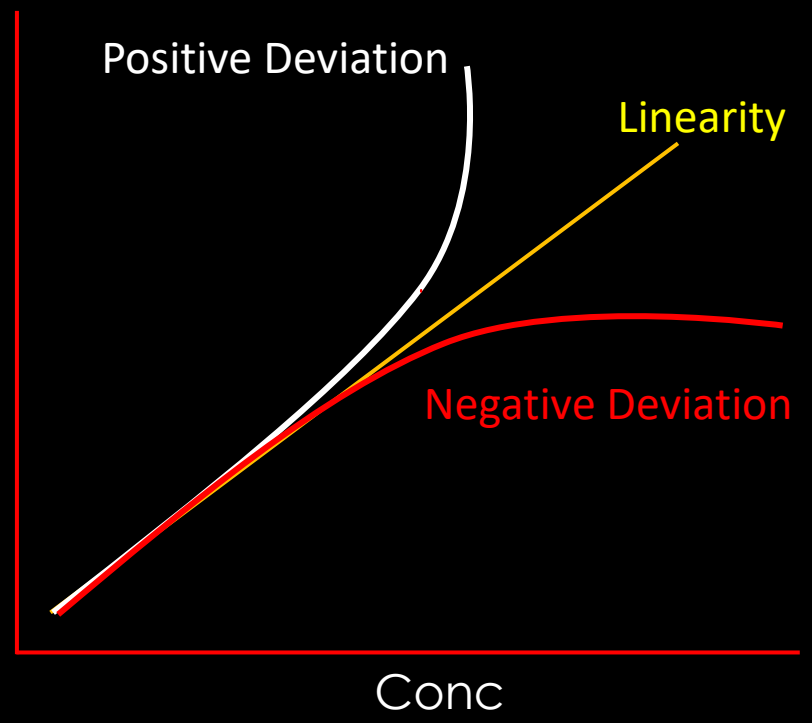
X = path length = 1cm

1cm

$Abs \propto Conc$

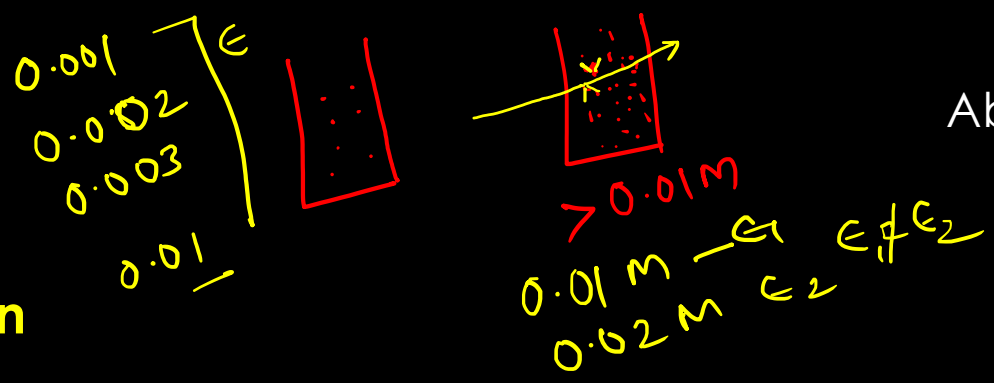
Deviation of Beer's-Lambert's

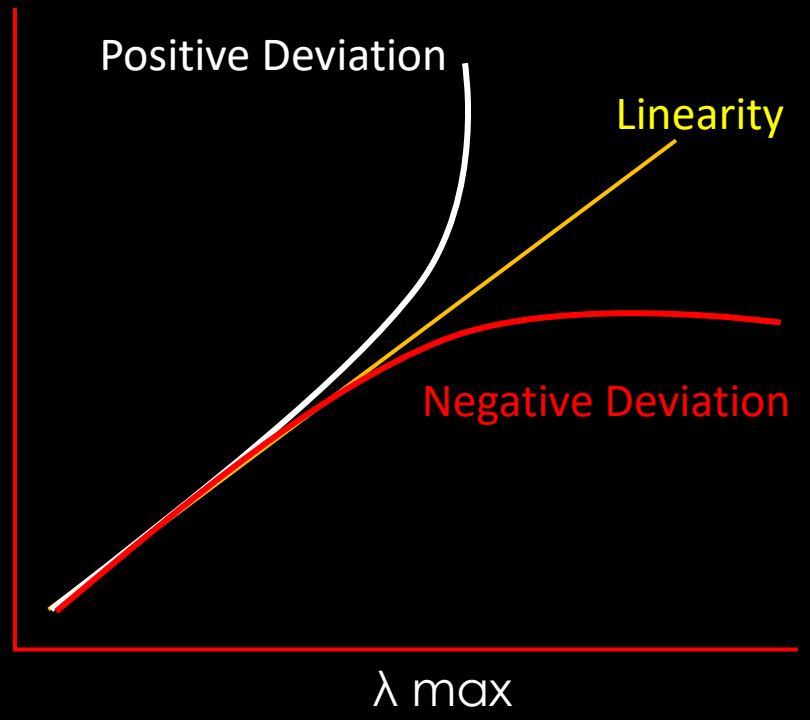
- When a straight line is not obtained i.e. non-linear curve is obtained in a plot of concentration Vs absorbance i.e. called as Beer's deviation; that may be positive or negative deviation
- Positive deviation- when a small change in concentration produces greater change in absorbance.
- Negative deviation when a large change in concentration produces small change in absorbance



Deviation of Beer's-Lambert's

- Deviation from linearity are divided into 3 categories:
- Fundamental
- Chemical, &
- Instrumental





1. Fundamental Deviation

- Beer's-Lambert's law is valid for low conc. (< 0.01M). At the high conc. (>0.01M) the individual particles of analyte doesn't behave independently, the interaction between particles may alter the molar absorptivity (ε). Since absorptivity (a) and molar absorptivity (ε) depends on a sample refractive index.

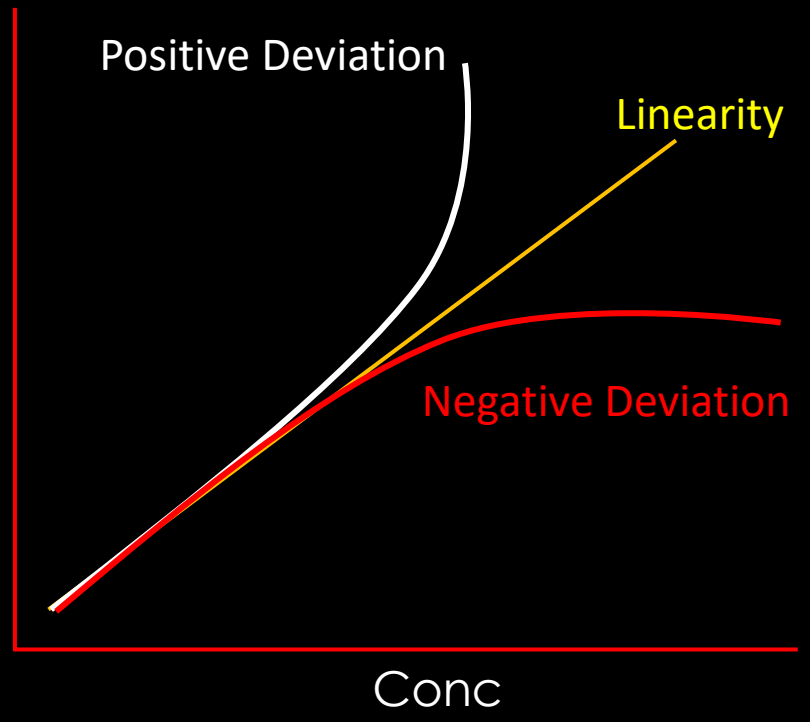
$A = \varepsilon c x$

Deviation of Beer's-Lambert's

- Deviation from linearity are divided into 3 categories:

2. Chemical Deviation

- Chemical deviation arise when analyte undergoes chemical changes like dissociation, association, complex formation, and polymerization.
- 4 $C_6H_5CH_2OH$ →$CHCl_3$→ $(C_6H_5CH_2OH)_4$ Polymer formation
- (275 nm) (300 nm)
  (+ve Deviation) (-ve Deviation)

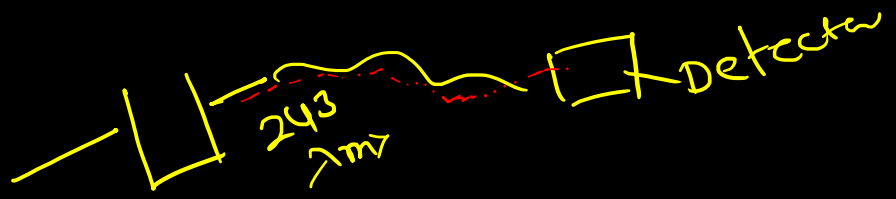


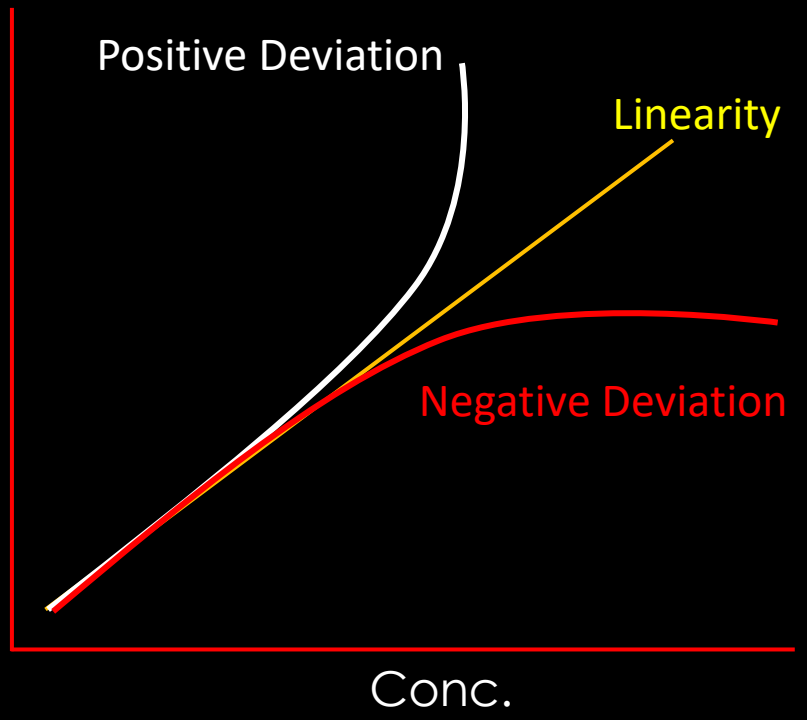
Deviation of Beer's-Lambert's

- Deviation from linearity are divided into 3 categories:

3. Instrmental Deviation

- In determination instrumental variation may show deviation which may be due to stray light, polychromatic light, mismatch cell, slit width, etc.
- **Stray light**: any other radiation (except specific wavelength; monochromator) reaching to the detector. It arise from scattering and refraction inside the monochromator, mainly due to imperfection on optical surface.



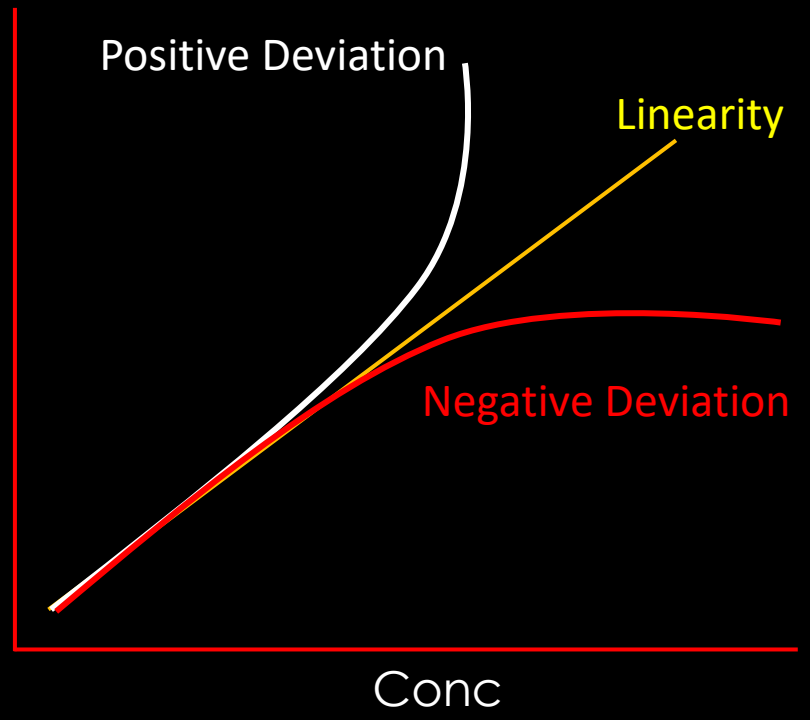


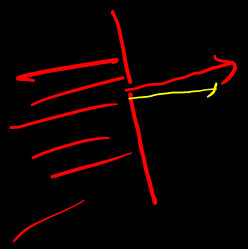
Deviation of Beer's-Lambert's

- Deviation from linearity are divided into 3 categories:

3. Instrmental Deviation

- **Stray light**: any other radiation (except specific wavelength; monochromator) reaching to the detector. It arise from scattering and refraction inside the monochromator, mainly due to imperfection on optical surface.
- If **slit width** is not proper, it allows undesirable radiation to fall on the sample.
- Improper handling of **sampling cuvette**

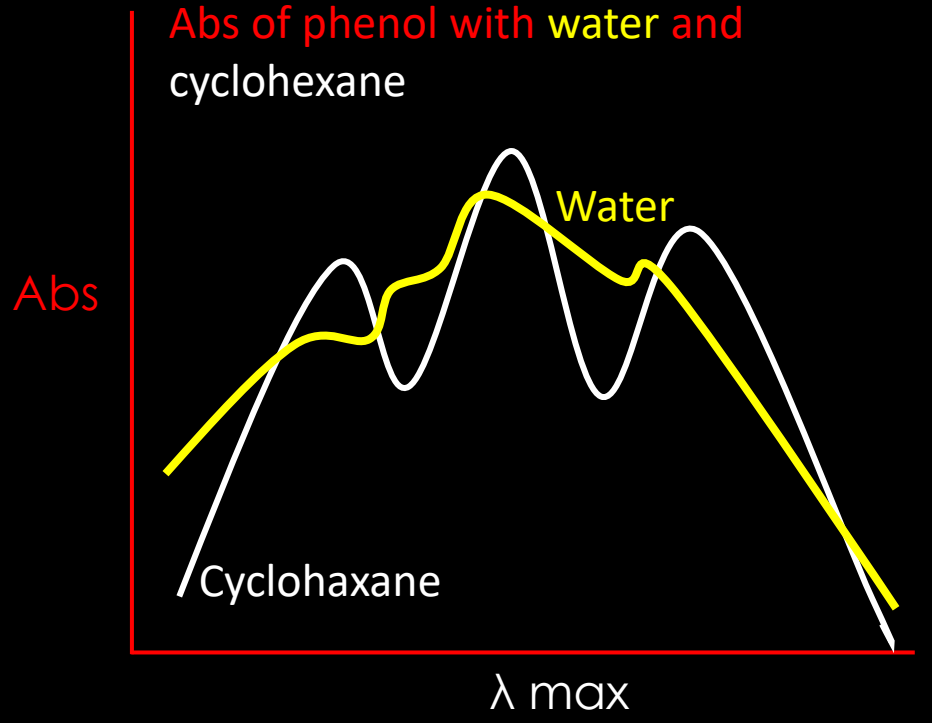




UV-VISIBLE SPECTROSCOPY

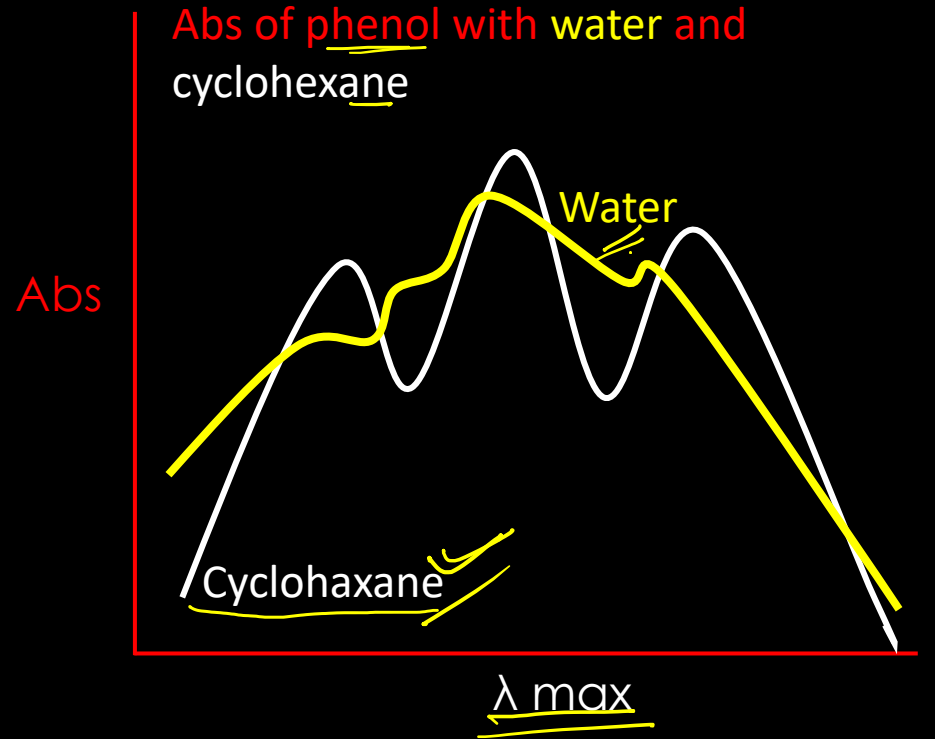
Solvent Effects on absorption spectra

- The choice of solvents is very important in the UV-Visible spectroscopy.
- The absorption spectrum of pharmaceutical substance depends practically upon the solvent that has been employed to solubilize the substance
- First criteria: It should not absorb uv-visible radiation in the same region as the analyte (solute) absorbe
- Usually unconjugated solvent system is suitable for this purpose.
- Slovents: distilled water (190 nm), 95% ethanol (205 nm), n-hexane (201 nm), methanol (205 nm), chloroform (240 nm), cyclohexane (195 nm)



Solvent Effects on absorption spectra

- Second criteria for the good solvent is its effect on the fine structure of an absorption band
- In the figure we observe that phenol has little interaction with non polar solvent (cyclohexane) while with polar solvent (water) it shows strong interactions (salvation, H-bonding)
- A drug may absorb a maximum radiation energy at particular wavelength in one solvent but shall absorb partially at the same wavelength in another
- **Polarity plays an important role in the *position and intensity of absorption maximum of a*** particular chromophore

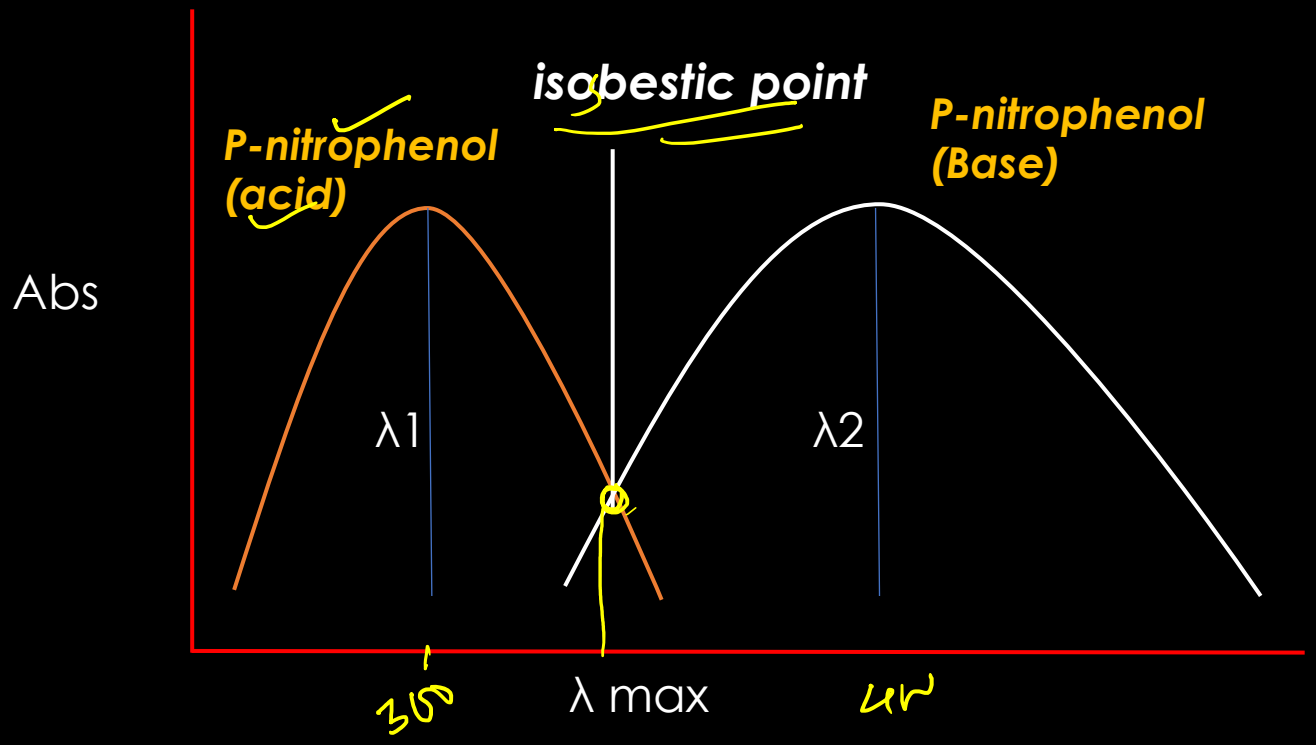


Solvent Effects on absorption spectra

- E.g. In case of non-polar solvents e.g. Iodine solution (purple color; 518 nm same as iodine vapor), whereas in case of polar solvents, a brownish color appear (Shorter wavelength)
- By increasing the polarity of the solvent, compounds like dienes & conjugated hydrocarbons do not experience any appreciable shift
- If the chromophore involved in the transition is more polar in its ground state than in its excited state, then the ground state is more stabilized than the excited state by a more polar solvent due to solvation. Chromophores with $n \rightarrow \pi^*$ or $n \rightarrow \sigma^*$ transitions exhibit such behavior.
- ***H-bonding shifts uv aborption to longer wavelength (Red shift) by increasing the polarity of solvent*** ($\pi \rightarrow \pi^*$, Carbonyl compound)
- ***H-bonding shifts uv aborption to shorter wavelength (Blue shift) by increasing the polarity of solvent*** ($n \rightarrow \sigma^*$, Alcohol, Amines)

Isoabsorptive points or Isosbestic point

- The wavelength of equal absorptivity of the two species (A & B), or same substance in two different mediums, that wavelength is known as ***isosbestic point***.



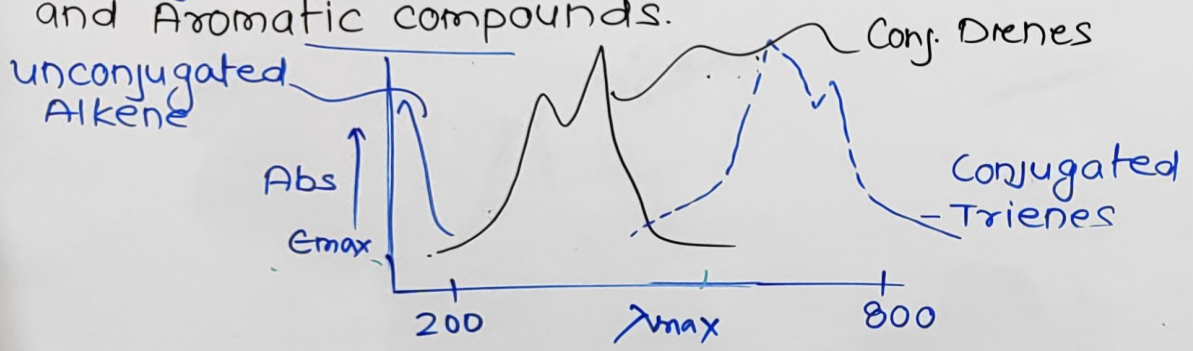
UV-Visible Spectroscopy (Part 6)

Woodward Fieser Rules

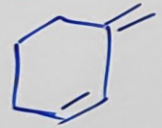
Spectroscopy
Instrumental Analysis

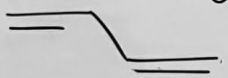
WOODWARD-FIESER RULES

↳ Rules for the calculating Absorptn maxima in Conjugated Dienes, α,β unsaturated carbonyl and Aromatic compounds.



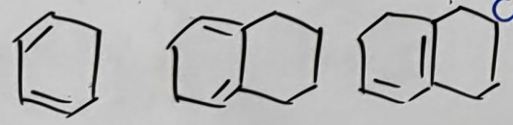
↳ Conjugation System

A)  A Cyclic Diene.

 open chain "Butadienes"

→ 217 nm

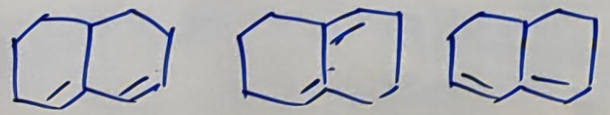
B) Homoannular Dienes / Homodienes



conj. tnt in same ring

→ 253 nm

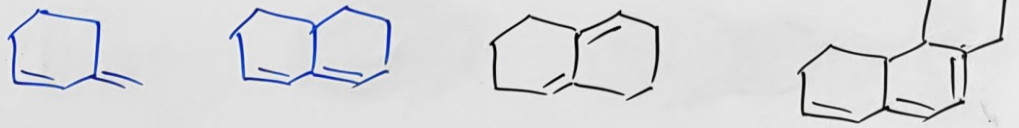
C) Hetero annular conj. dienes



conj. tnt in different ring

→ 215 nm

D) Exocyclic double bond System → (=) attached outside the ring = +5 nm



↳ λmax Depends on → Alkyl / Ring residues, no. of (=) involve in conj., & tnc of polar gp (-X, -OR, -SR, -NR_2)

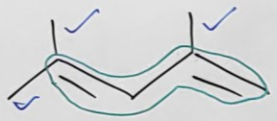
λmax ; Solvent - Ethanol, Transition - π-π*

→ Buta diene / a cyclic conj. system → 217 nm
→ Homo dienes - → 253 nm
→ Hetero annular system → 215 nm

* INCREMENT IN λmax

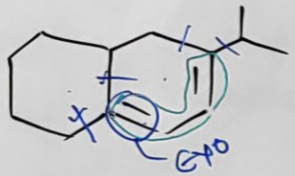
↳ Alkyl / Ring Residue	+ 5 nm
↳ Exocyclic (=) bond	+ 5 nm
↳ (=) Extending conjugation	+ 30 nm
↳ -OR	+ 6 nm
↳ -SR	+ 30 nm
↳ $-NR_2$	+ 60 nm
↳ -X (Cl, Br)	+ 5 nm
↳ $-OCOCH_3$	+ 0 nm

WOODWARD-FIESER RULES

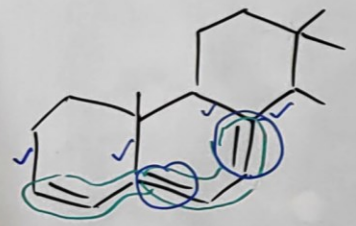
1) 

- Butadiene = 217
- 3 Alkyl = 15
= 232 nm

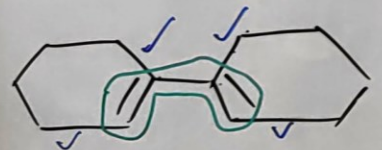
observed = 234 nm

2) 

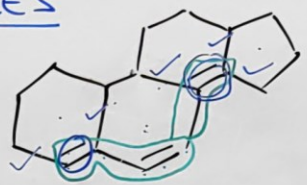
Homodiene = 253
4 Ring residue = 20
1 Exocyclic (=) = 5
= 278 nm

3) 

Homoannular = 253
1 (=) Extending conjugation = 30
4 Ring residue = 20
2 Exo. (=) = 10
= 313 nm

4 

Base = 215
4 Ring residue 20
= 235 nm

5 

Hetero — 215
(=) conj. — 30
3 exo (=) — 15
5 Ring res. — 25
= 285

6) 

Base = 217
2 EXO. (=) = 10
2 Ring r. = 10
1 bicyclo = 15
= 252 nm

λmax ; Solvent - Ethanol, Transition - π-π*

→ Butadiene / acyclic conj. system → 217 nm
→ Homodienes - → 253 nm
→ Heteroannular system → 215 nm
Base value

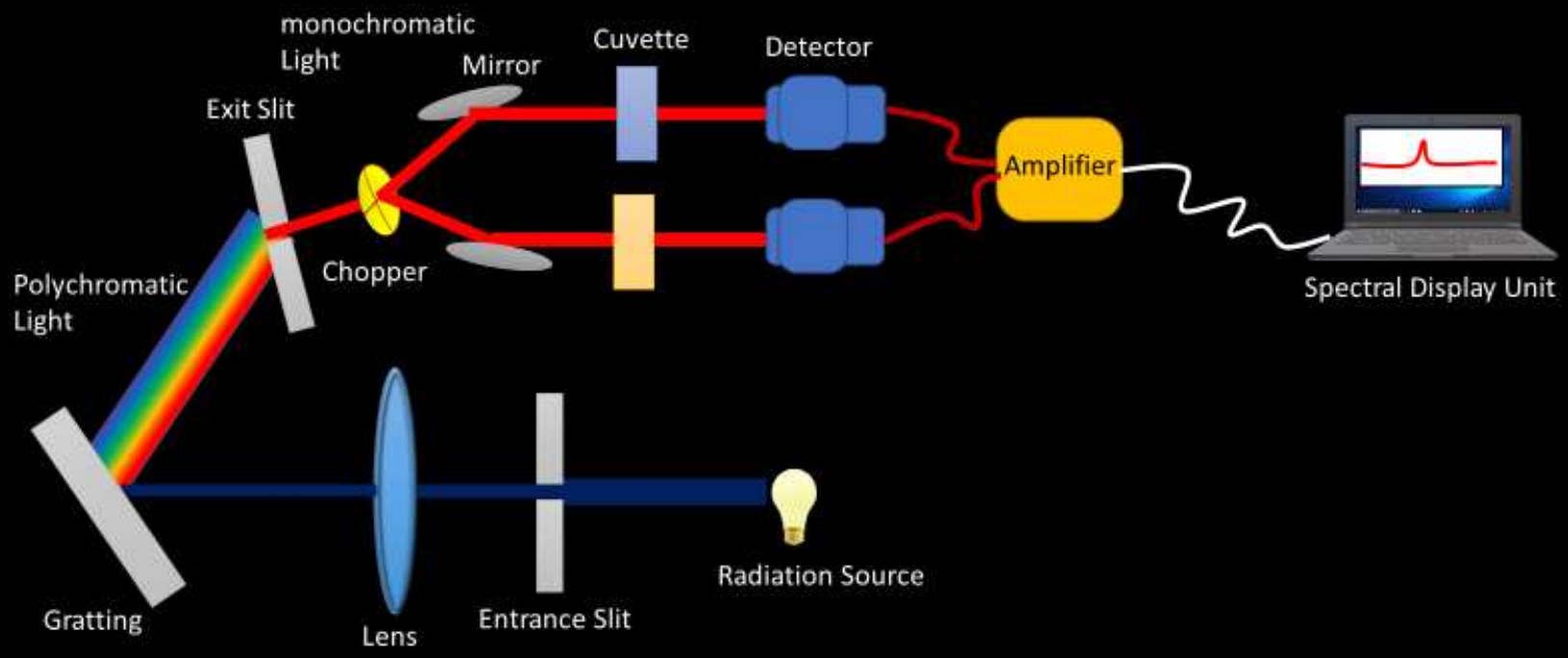
* INCREMENT IN λmax

↳ Alkyl / Ring Residue	+ 5 nm
↳ Exocyclic (=) bond	+ 5 nm
↳ (=) Extending conjugation	+ 30 nm
↳ -OR	+ 6 nm
↳ -SR	+ 30 nm
↳ $-NR_2$	+ 60 nm
↳ -X (Cl, Br)	+ 5 nm
↳ $-OCOCH_3$	+ 0 nm

217 + 30 = 247

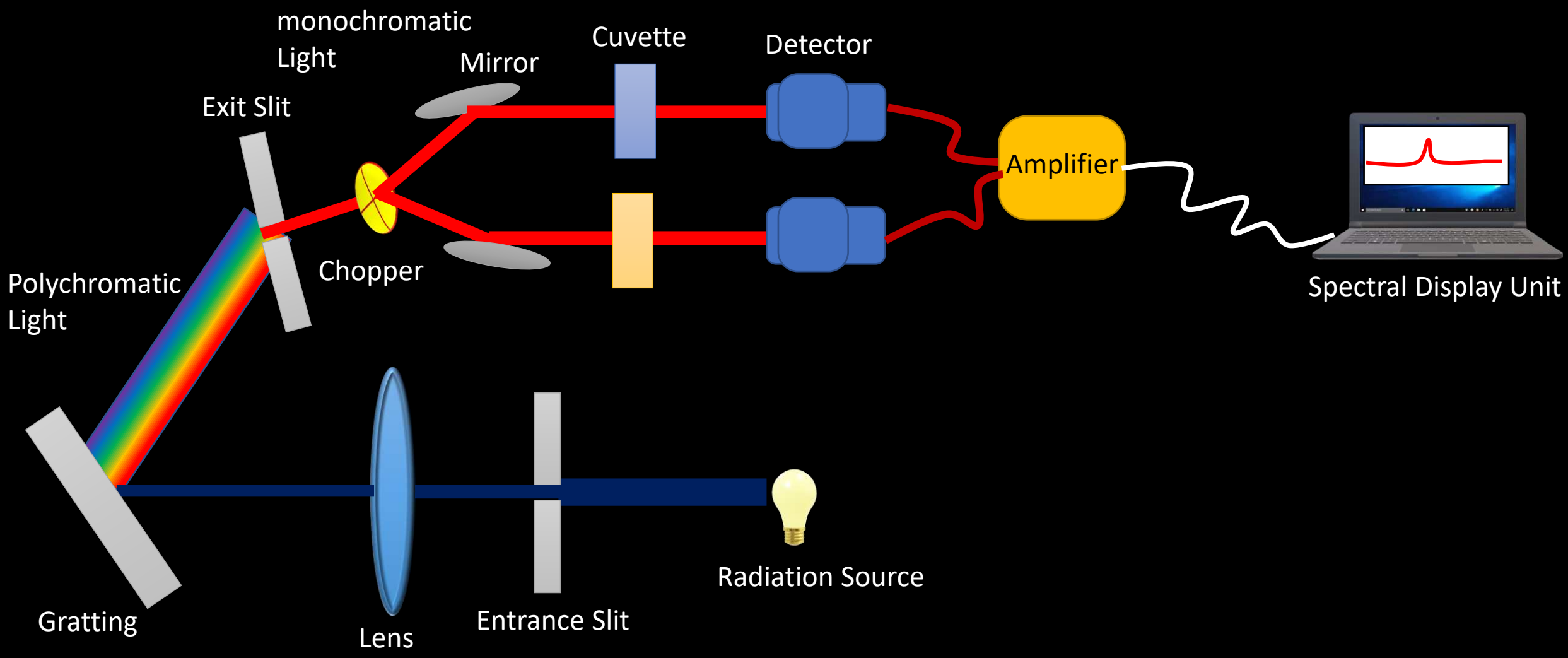
UV-Visible Spectroscopy (Part 7)

Instrumentation



Spectroscopy
Instrumental Analysis

UV-VISIBLE SPECTROSCOPY



UV-VISIBLE SPECTROSCOPY

1. Light Source

- Light source should be stable, intensity should be adequate abd not be fluctuate.
- Tungsten Filament Lamp: widely used in visible spectroscopy, tungsten filament lamp is particularly rich in red radiation (radiation with 375 nm). it consist Tungsten Filament in a vacuum glass envelope. This type of lamp is used in the wavelength range of 350 - 2500 nm. Life span is limited due to evaporation of the tungsten and decrease the intensity over the time because of the lamp darkness from inside the envelope.
- Tungsten Halogen lamp: more expensive and better than Tungsten Filament Lamp, it consist quartz envelop filled with halogen gas, which prevent the evaporation of tungsten and increase the life span.
- Xenon-arc lamp: provide intense radiation by passage of current through a atmosphere of xenon.

UV-VISIBLE SPECTROSCOPY

1. Light Source

- Hydrogen-Deuterium lamps: widely used in uv spectroscopy, it cover the range below 375 nm. The intensity of this lamp falls above 360 nm

Other source can also used like

- Carbon arc lamp
- Mercury arc lamp
- Light emitting diodes (LEDs)

2. Filters and Monochromators

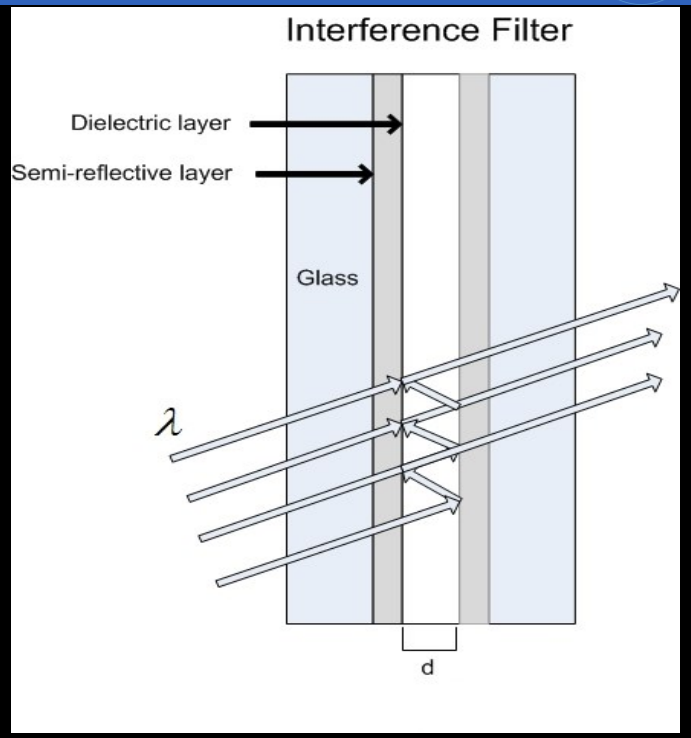
A. Filters: they allow transmission of only limited wavelength regions while absorbing most of the radiation. There are two types:

- Absorption Filters (Glass or Gelatin Filter)
  - Glass Filters: made up of colored glass, it produced by incorporating of metals like Cr, Mn, Fe, Ni, Co, Cu, etc
  - Gelatin Filter: made up of colored gelatin sheets sandwiched between a pair of clear glass

2. Filters and Monochromators

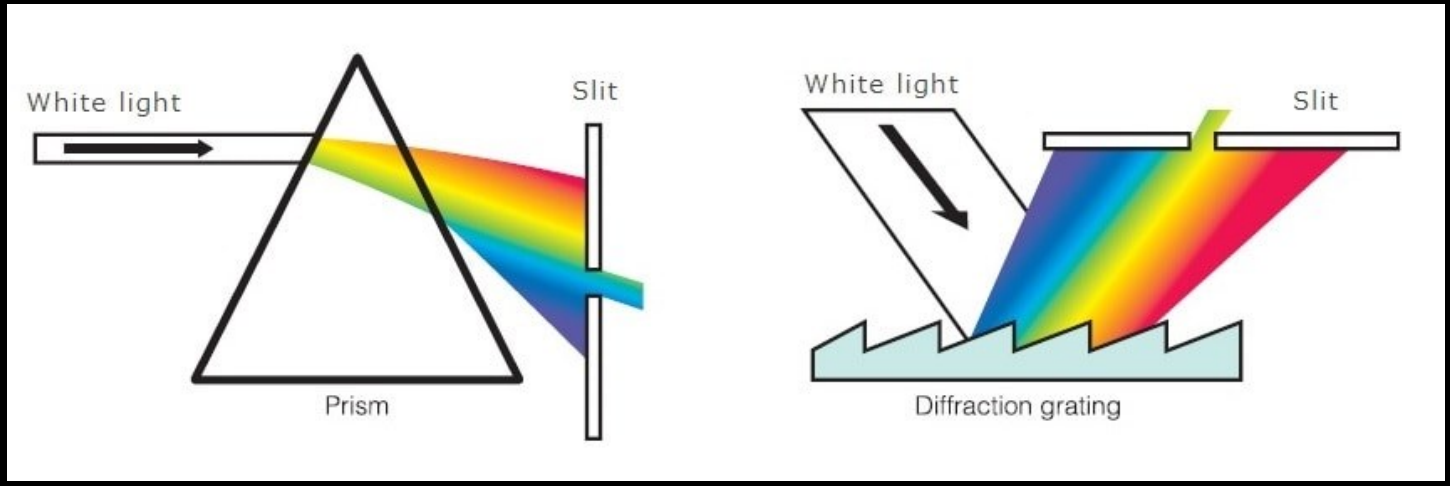
A. Filters:

- Absorption Filters (Glass or Gelatin Filter)
- Interference Filters or Fabry-Perot Filter is constructed by using two parallel glass plates that are silvered internally and separated by a thin film of a transparent dielectric spacer of a low refractive index.



B. Monochromators

- Prism (Refractive type and Reflective type)
- Grating (Diffraction grating and Transmission Grating)



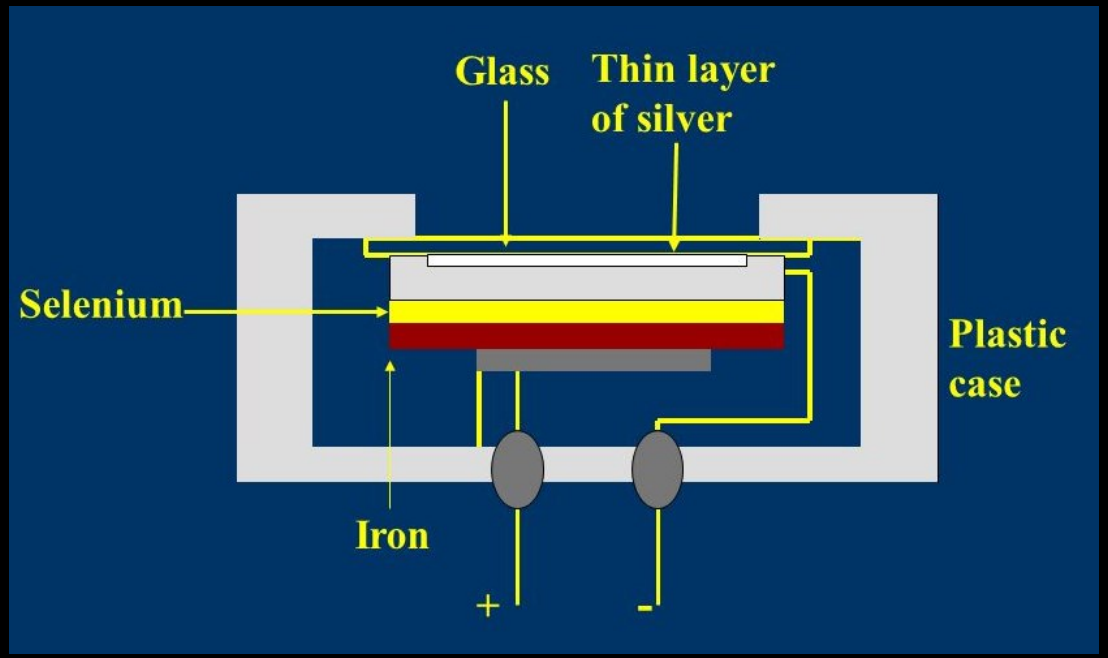
UV-VISIBLE SPECTROSCOPY

3. Sample Tube

Detectors: https://lab-training.com/characteristics-of-uv-vis-spectrophotometric-detectors/

4. Detectors

- Photovoltaic cell or Barrier layer cells



34. Detectors

Phototubes/Photoemissive cells

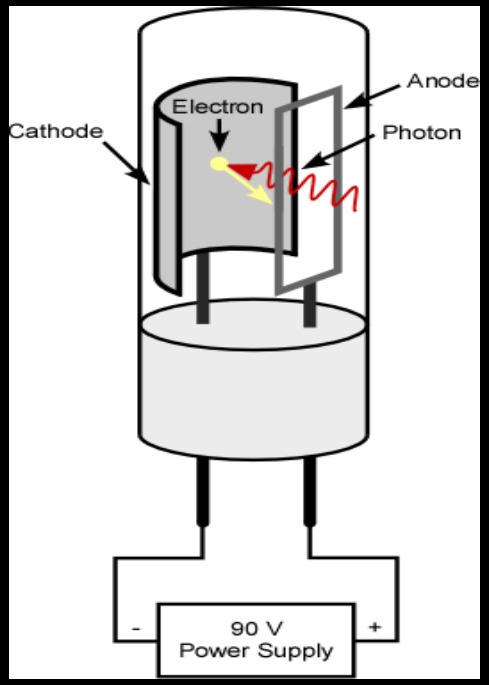
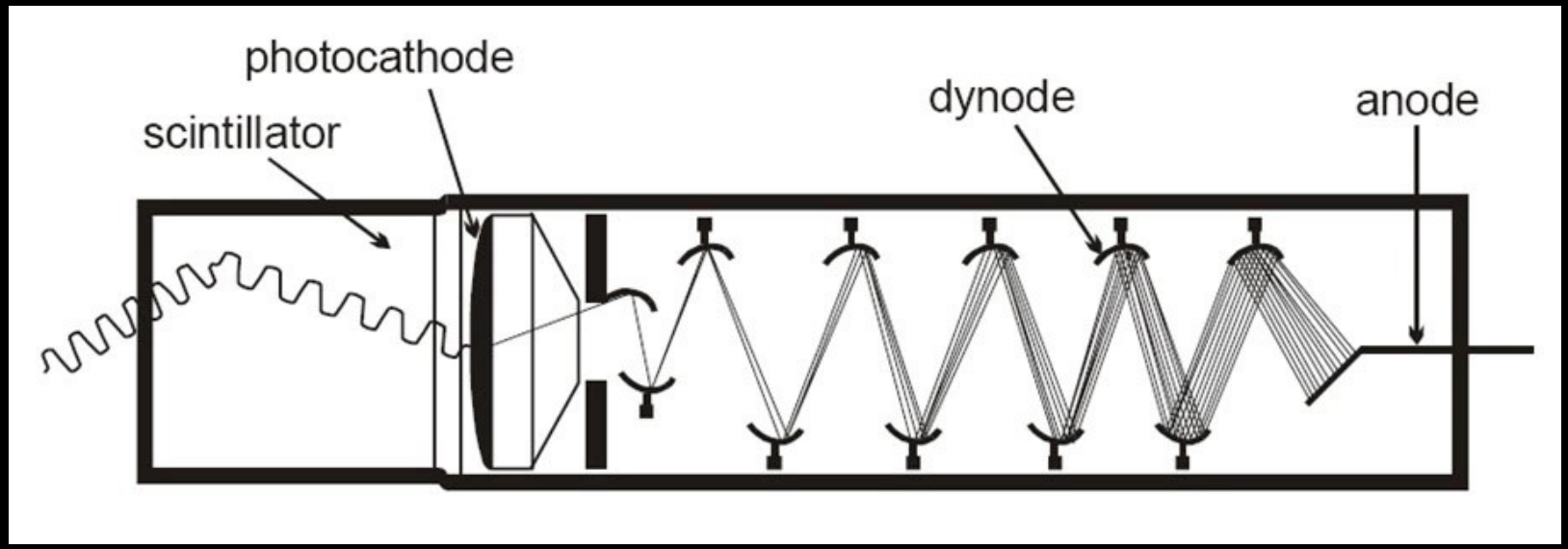
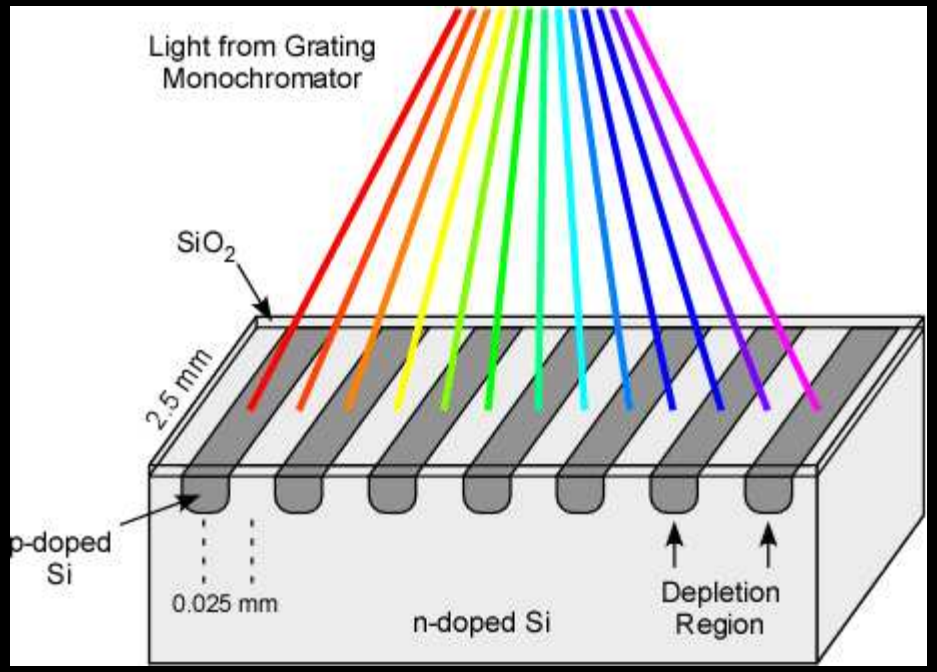


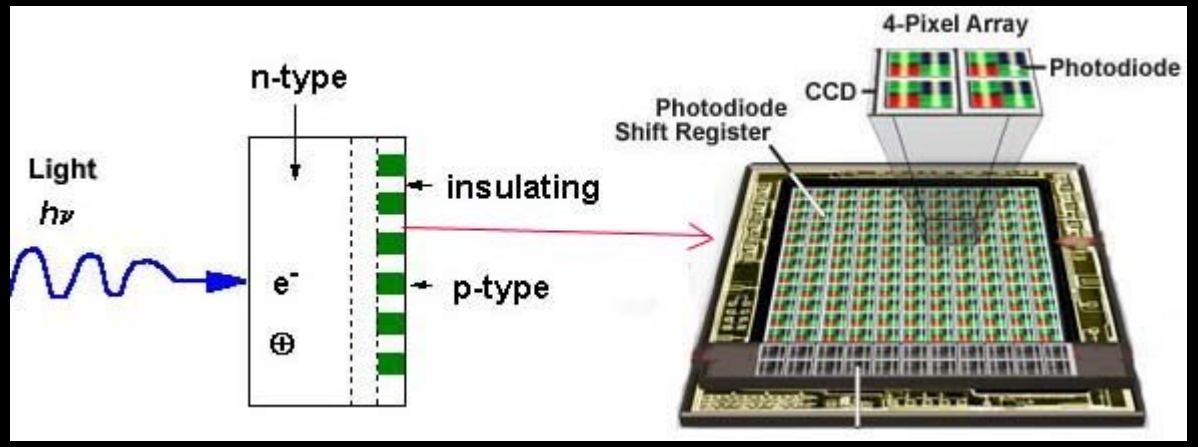
Photo multiplier Tubes (PMT)



34. Detectors

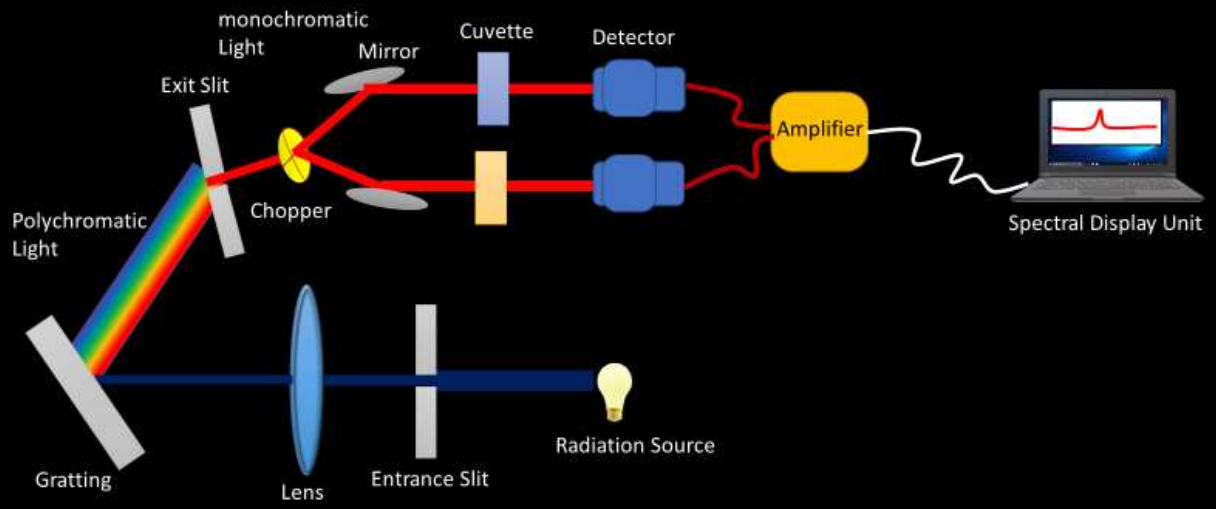
Photodiode array detectors





UV-Visible Spectroscopy (Part 8)

Applications



Spectroscopy
Instrumental Analysis

Application of UV-Visible Spectroscopy
Qualitative Analysis

1. Detection of functional groups & Conjugated System:

- An unknown compound can be identified by comparing its spectrum with the known spectra
- To detect the presence or absence of chromophore present on the sample.
- If the spectrum is transparent in the range between 200-800 nm, it shows the absence of
  - (i) conjugation,
  - (ii) a carbonyl group (aldehyde & ketones) or Chromophores
  - iii) benzene or aromatic compounds,
  - (iv) bromo or iodo atoms.

Application of UV-Visible Spectroscopy
Qualitative Analysis

2. Detection of Purity or decomposition of compounds :

- It is used as a tool to identify if the analyte is pure and did not undergo decomposition.
- For example, this technique is used for quality control of incoming raw material, and for the purity check of biologically relevant compounds such as the nucleic acids, DNA and RNA.

3. In food & beverage industry:

- To monitor and improve product quality and consistency.
- The influence of packing material and stabilizers as well as chemical deterioration and degradation processes can also be observed with this method..

Application of UV-Visible Spectroscopy
Qualitative Analysis

3. In food & beverage industry:

- E.g., check for the purity of olive oil, which enables the product to be classified as "Extra Virgin", "Virgin", or simply "Olive Oil
- Standards are in place for the evaluation of olive oil based on the absorbance characteristics of certain molecules in the UV/VIS spectrum. Olive oil contains about 98% triglycerides. Unsaturated fatty acids in the oil are susceptible to breakdown and oxidation.
- Beside other parameters, this effect is evaluated by the conjugated di-enes and tri-enes of unsaturated fatty acids (conjugated C=C double bonds) which absorb in the range of 230 to 270 nm.

Application of UV-Visible Spectroscopy
Qualitative Analysis

4. In Chemical industry:

- For the determination of the purity of organic solutions. Additional peaks appearing at specific wavelengths can be observed due to impurities in the sample
- Alcohol can be contaminated by benzene, which absorbs light at 280 nm, whereas alcohol absorbs at 210 nm

Application of UV-Visible Spectroscopy
Qualitative Analysis

5. Determination of pKa value of Indicators:

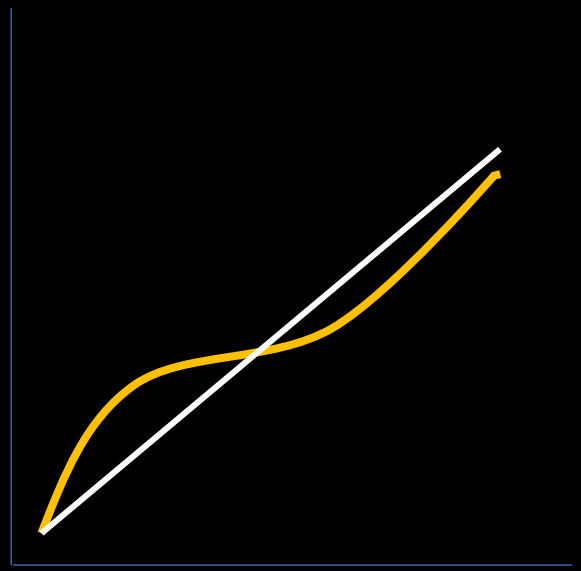
$$pKa = pH - [\log (ionized/unionized)]$$

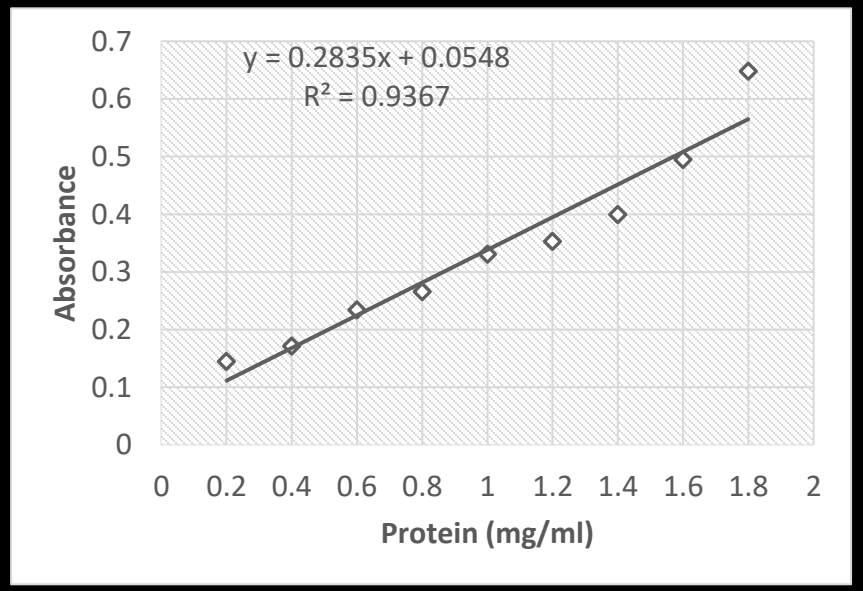
- The value of log (ionized/unionized) can be determined spectrophotometrically i.e. concentration Vs. absorbance at different pH & from the equation pKa can be calculated.

Application of UV-Visible Spectroscopy
Quantitative Analysis

1. Determination of concentration:

- Beer's-Lambert's Law helps to determine the concentration by using Absorbance vs Conc. graph.



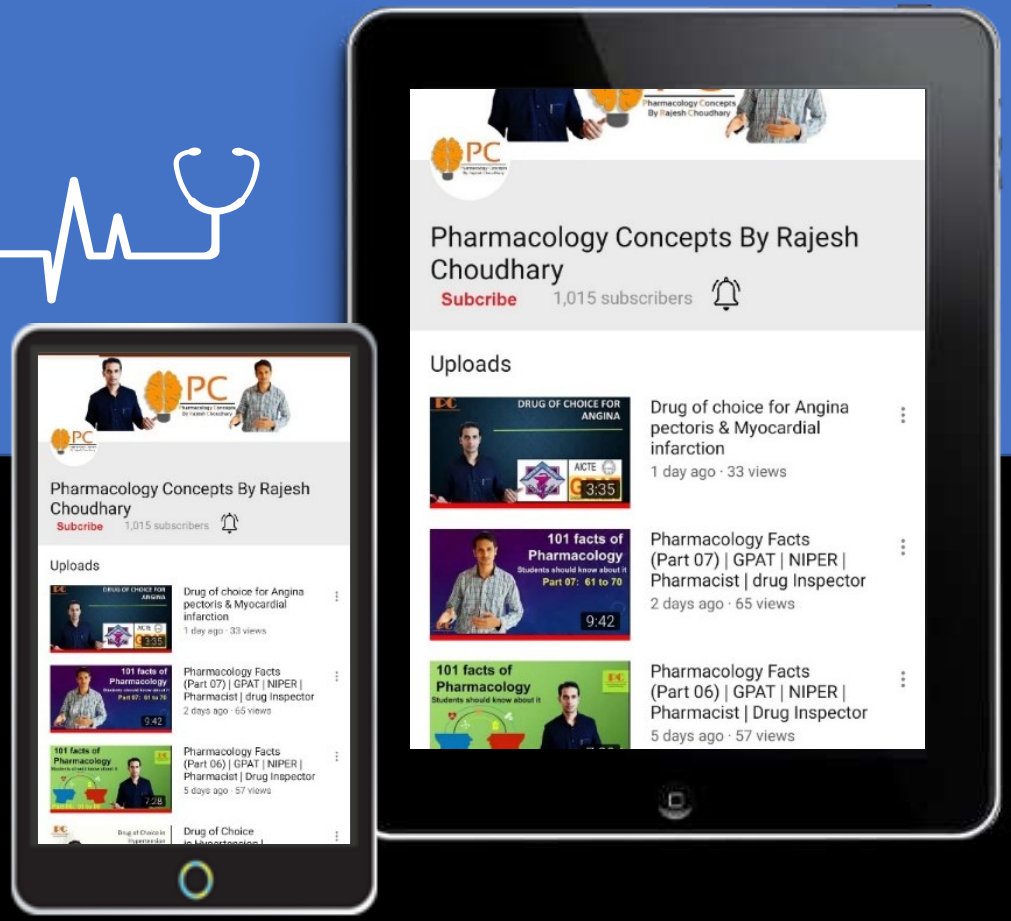


Application of UV-Visible Spectroscopy
Quantitative Analysis

1. *Determination of concentration:*
2. *Chemical kinetics:* Zero order, 1st order, 2nd order reaction
3. *Assay of pharmaceutical substances*

PC
Pharmacology Concepts
By Rajesh Choudhary

Thanks for Watching



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