Advanced Pharmaceutical Analysis

5th Stage

Lab 1

Spectroscopy

- It is the branch of science that deals with the study of interaction of matter with light.

OR

- It is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.

Electromagnetic Radiation

- Electromagnetic radiation consist of discrete packages of energy which are called as photons.

- A photon consists of an oscillating electric field (E) & an oscillating magnetic field (M) which are perpendicular to each other.

Principles of Spectroscopy

1. Absorption Spectroscopy:
   - An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.

- e.g. UV (185 - 400 nm) / Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 µm)

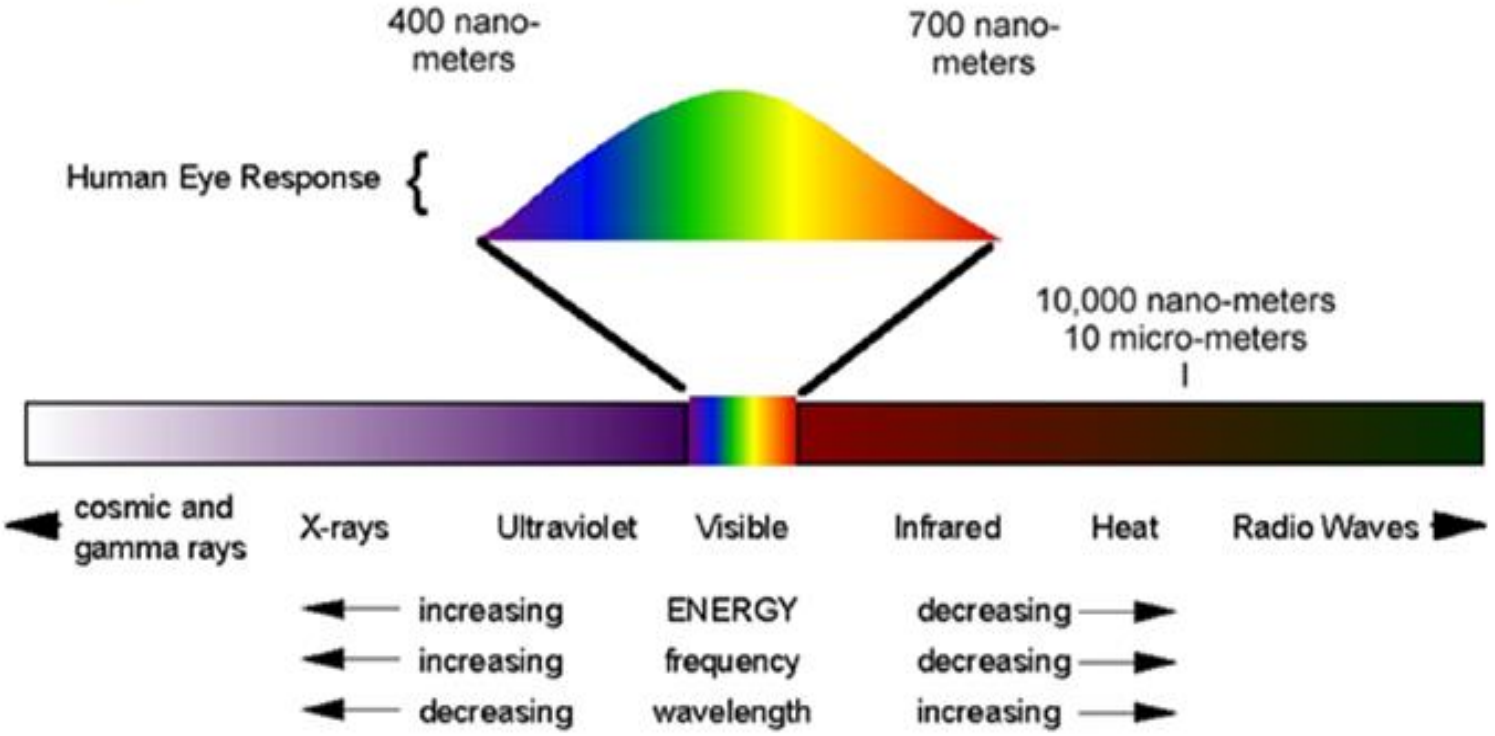
Principles of Spectroscopy

2. Emission Spectroscopy:
   - An analytical technique in which emission (of a particle or radiation) is dispersed according to some property of the emission & the amount of dispersion is measured.
   - e.g. Mass Spectroscopy

Analytical spectroscopy

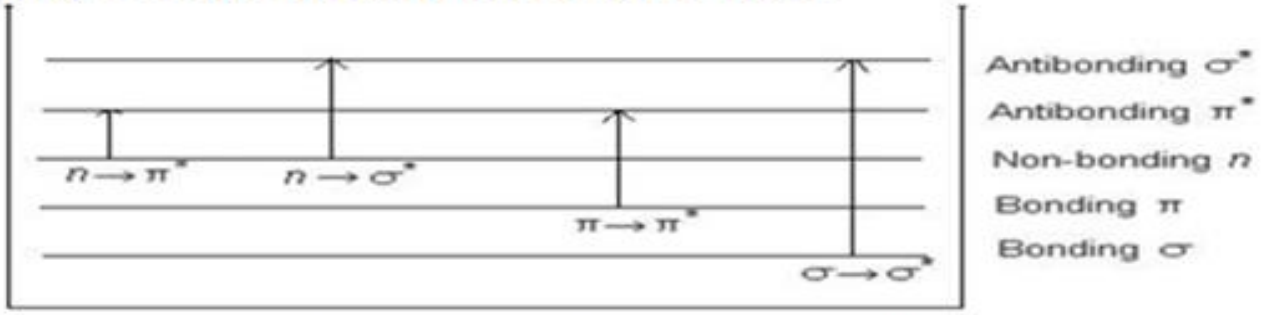
Analytical spectroscopy is the measurement of intensity of the electromagnetic radiation at specific wavelength which is absorbed or emitted by the atoms or molecules & lead to moving from one energy state to another.

Different types of spectroscopy are available, depending on the type or wavelength of electromagnetic radiation absorbed or emitted by the atom or molecule.

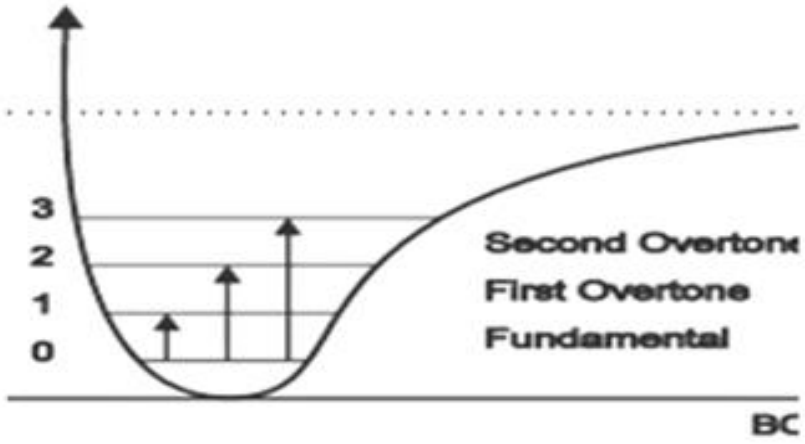


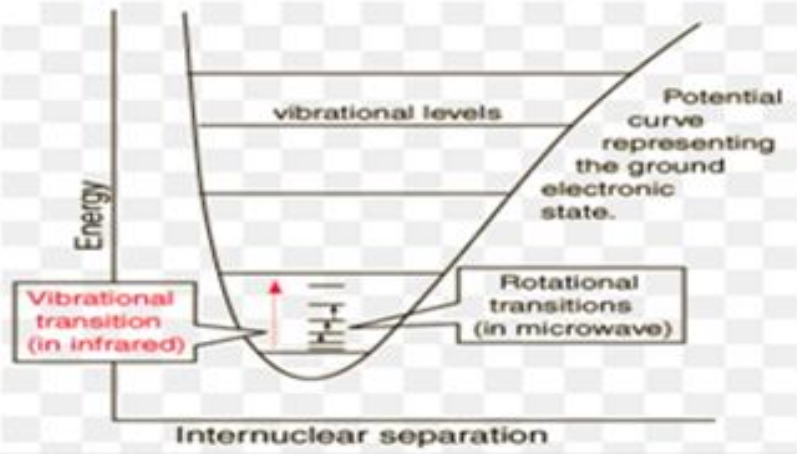
The energy can act in three ways:

1- The energy acts to excite an electron from a bonding orbital to a higher energy antibonding orbital, a so-called electronic transition

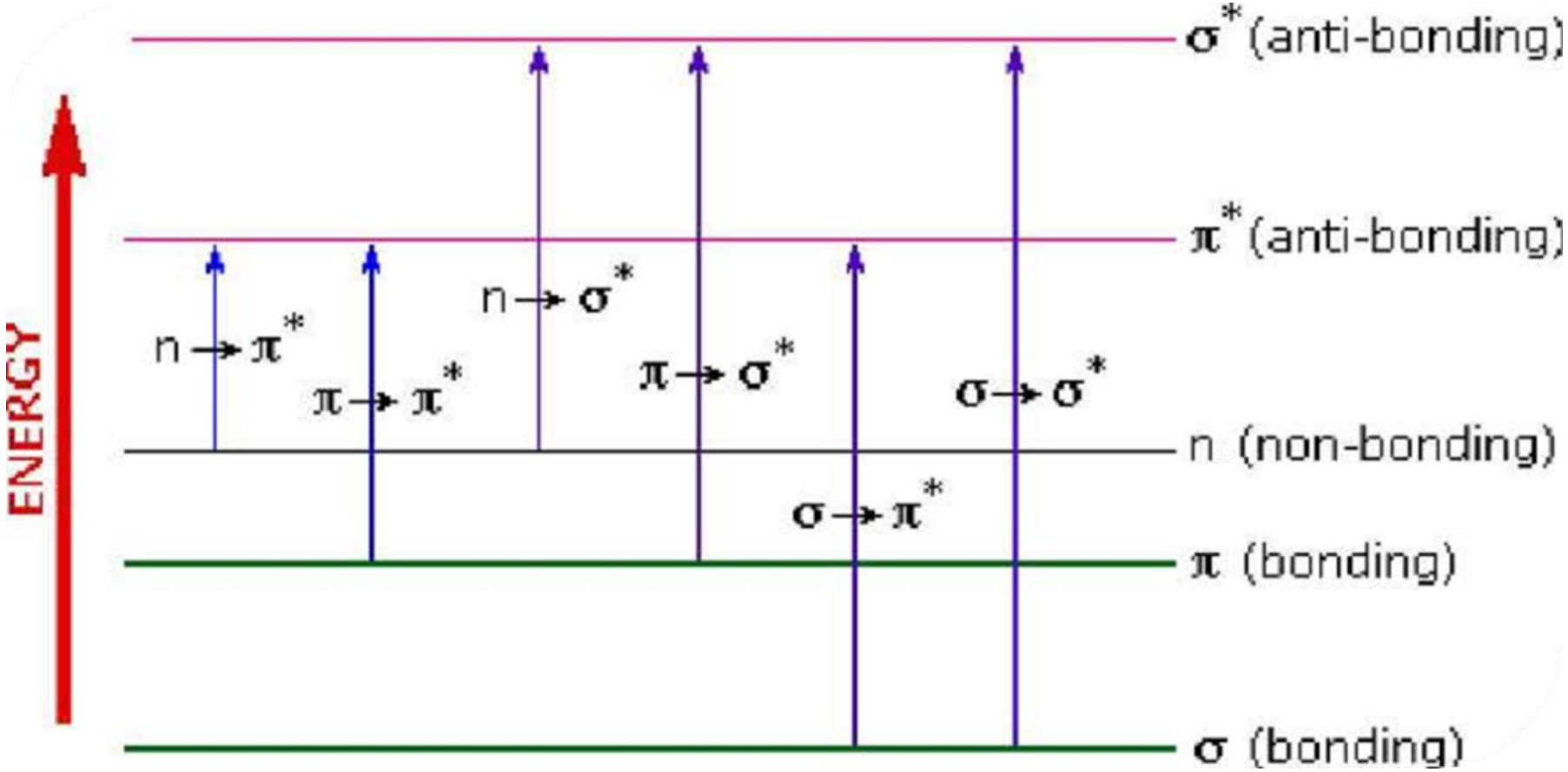


2- The energy can acts to increase the vibration, or oscillation, of atoms around a chemical bond. This is termed a vibrational transition.





3- The energy acts to increase the rotation of atoms around the chemical bond, which is a rotational transition.


ENERGY
σ* (anti-bonding)
π* (anti-bonding)
n → σ*
n → π*
π → π*
π → σ*
σ → σ*
n (non-bonding)
σ → π*
π (bonding)
σ (bonding)

The possible electronic transitions are

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

1 • $\sigma \rightarrow \sigma^*$ transition

σ electron from orbital is excited to corresponding anti-bonding orbital σ*.

The energy required is large for this transition.

• e.g. Methane (CH4) has C-H bond only and can undergo σ → σ* transition and shows absorbance maxima at 125 nm.

2 • $\pi \rightarrow \pi^*$ transition

π electron in a bonding orbital is excited to corresponding anti-bonding orbital π*.

• Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo π → π* transitions.

• e.g. Alkenes generally absorb in the region 170 to 205 nm.

3 • $n \rightarrow \sigma^*$ transition

• Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of n → σ* transition.

• These transitions usually requires less energy than σ → σ* transitions.

• The number of organic functional groups with n → σ* peaks in UV region is small (150 – 250 nm).

4 • $n \rightarrow \pi^*$ transition

- An electron from non-bonding orbital is promoted to anti-bonding π* orbital.
- Compounds containing double bond involving hetero atoms (C=O, C≡N, N=O) undergo such transitions.
- n → π* transitions require minimum energy and show absorption at longer wavelength around 300 nm.

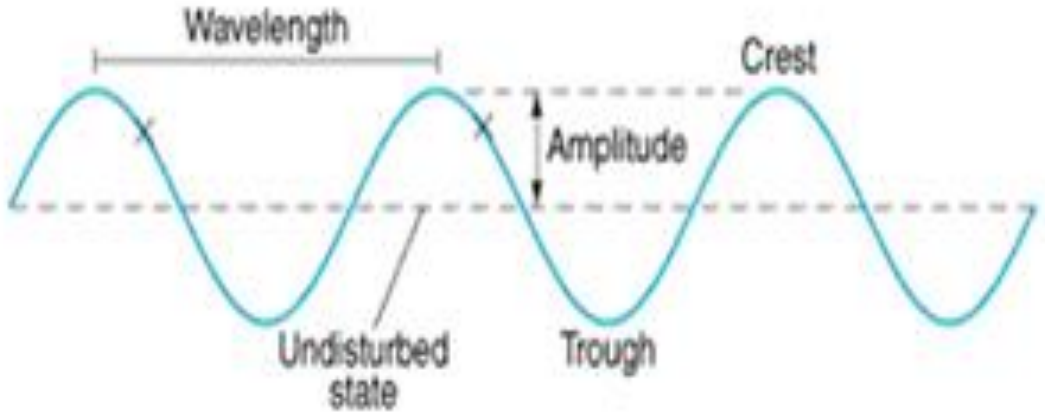
5 • $\sigma \rightarrow \pi^*$ transition

& • $\pi \rightarrow \sigma^*$ transition 6

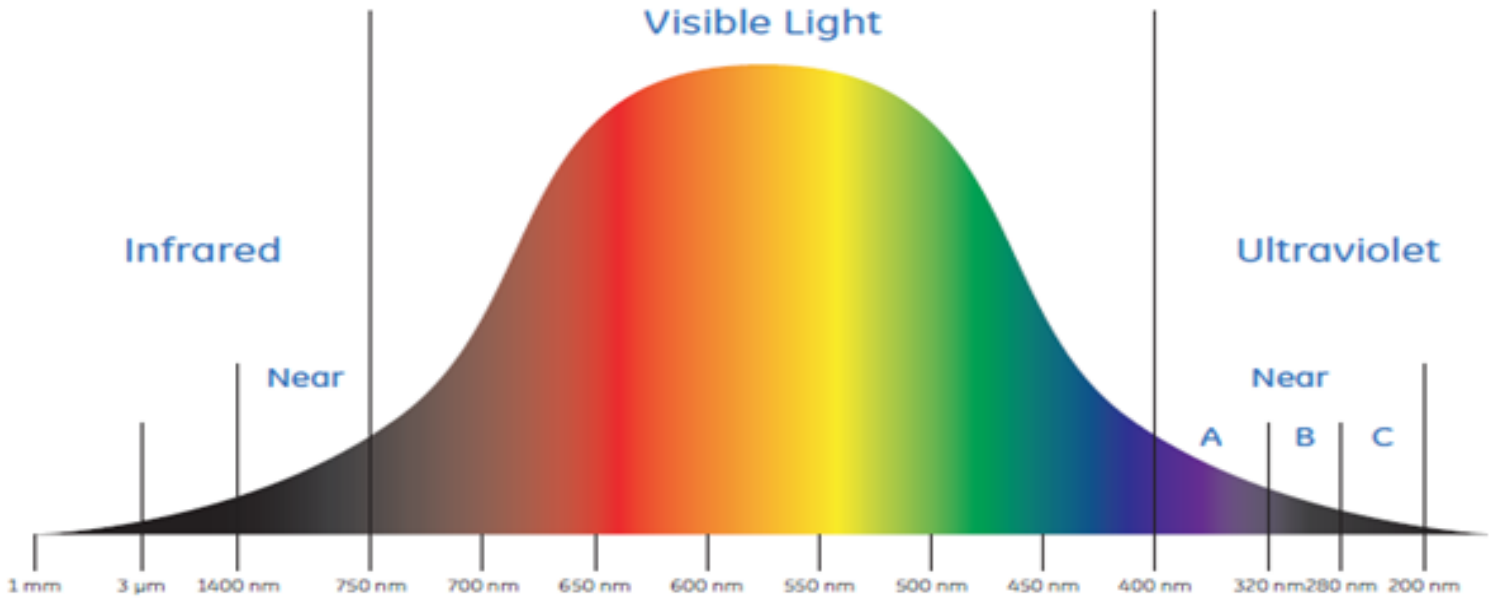
- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus, n → π* & π → π* electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.
- The UV spectrum is of only a few broad of absorption.

The terms which are used extensively in spectroscopy :

1- The wave number: is the number of waves per unit of length (distance).
2- The frequency: is the number of waves per second; the unit of frequency is the hertz (Hz).
3- The wavelength: is distance between two adjacent crests (peaks). (nm).



Spectrophotometry is the science of measuring the light-absorbing and light-transmitting characteristics of a substance. Many substances absorb light and transmit light of specific wavelengths within the ultraviolet region (200 - 400 nm), visible region (400 -700 nm) and near-infrared (700 - 1100 nm) regions of the electromagnetic spectrum. These light-absorbing / light-transmitting characteristics of a substance are useful in determining the presence and concentration of that substance in a sample. **The Spectrophotometer** is the instrument used to measure the amount (intensity) of light of a specific wavelength absorbed or transmitted by a substance. The part of the molecule that is responsible for the absorption of light is called the **chromophore.**



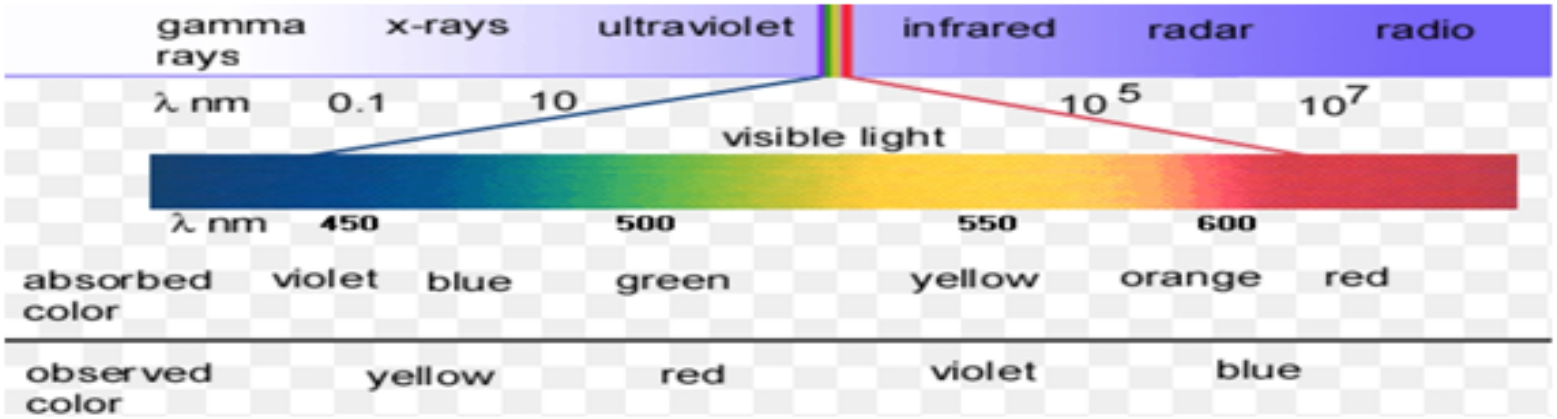
UV - Visible Spectrophotometer

It includes the absorption:

1- within UV-Region (190-400)nm

2- within Visible -Region (380-750)nm : coloured compounds

Wavelength, nm	Color	Complementary color
400-430	Violet	Yellow-green
430-480	Blue	Yellow
480-490	Green-blue	Orange
490-500	Blue-green	Red
500-550	Green	Purple
550-575	Yellow-green	Violet
575-590	Yellow	Blue
590-625	Orange	Green-blue
630-700	Red	Blue-green



Auxochrome

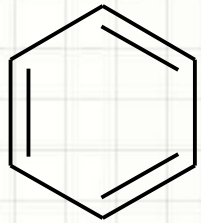
The functional groups attached to a chromophore which modifies the ability of the chromophore to absorb light , altering the wavelength or intensity of absorption.

OR

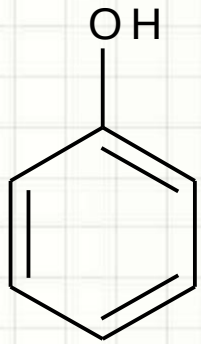
The functional group with non-bonding electrons that does not absorb radiation in near UV region but when attached to a chromophore alters the wavelength & intensity of absorption.

Auxochrome

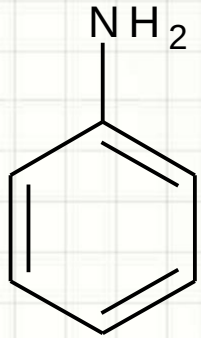
e.g. Benzene λ_{max} = 255 nm



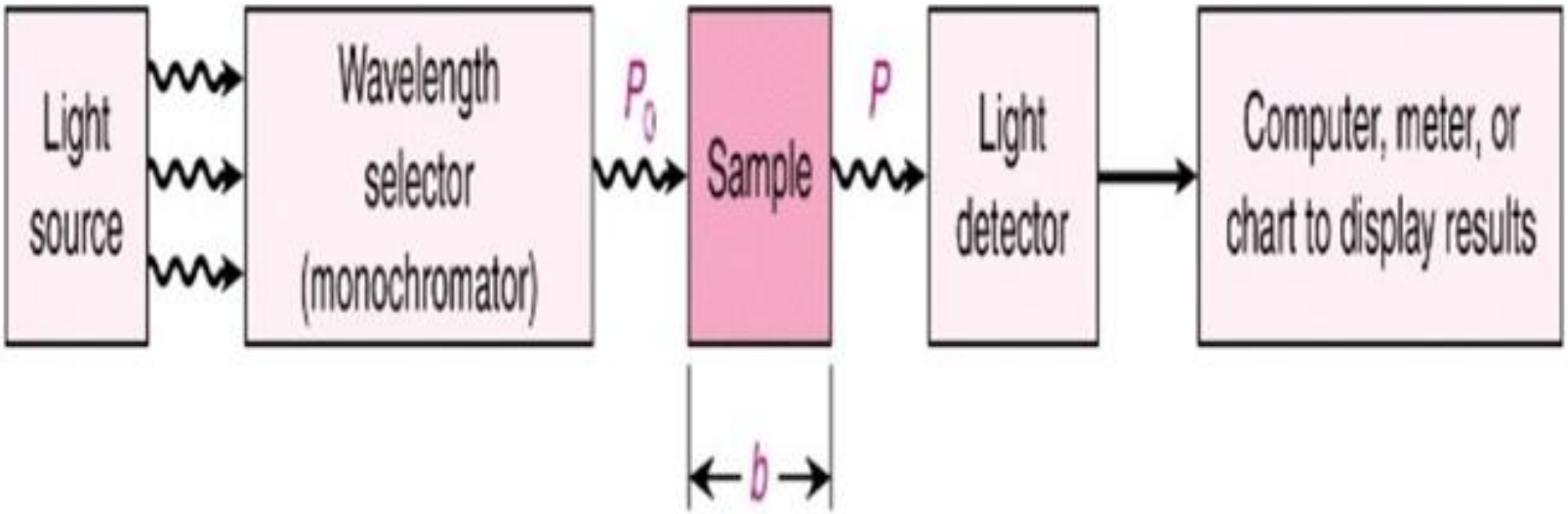
Phenol λ_{max} = 270 nm



Aniline λ_{max} = 280 nm



Parts Of The UV-Visible Spectrophotometer:



1- **Light Source:**

Deuterium Lamp: for the ultraviolet region (190–400 nm),

Tungsten Lamp : for the visible region (370–750 nm).

2- **Monochromator (Quartz Prism , Diffraction Grating):**

filter the light and select the light of a single wavelength reaches the detector.

3- **Cuvette (sample cell or holder):**

Quartz: for the ultraviolet region,

Glass : for the visible region.

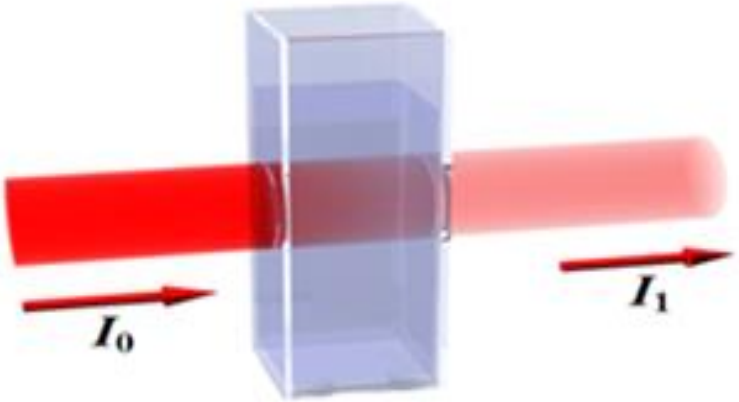
4- **Detector (photomultiplier tube):** convert the absorbed light signal to electrical signal.

5- **Display (data output):** show the results (Absorbance or %T).

Principle Of The UV-Visible Spectrophotometer:

- Electronic Transition: Molecules containing non-bonding electrons (n-electrons) or π-electrons can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding orbitals. The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions ($\pi-\pi^*$, $n-\pi^*$, $\sigma-\sigma^*$, and $n-\sigma^*$), and they can be ordered as follows:
  $\sigma-\sigma^* > n-\sigma^* > \pi-\pi^* > n-\pi^*$.
- Colored compounds are measured within "Visible –Region"
- The spectrophotometer determines the intensity of the light entering the sample & the intensity of the light leaving the sample, then calculates the amount of light transmitted & light absorbed by the substance.

"Transmittance & Absorbance"



Transmittance (T) is the ratio of the intensity of the transmitted light (I_1) to the intensity of the incident light (I_0)

$$\mathbf{T = I_1 / I_0}$$

Because the intensity of the transmitted light (I_1) is never greater than the intensity of the incident light (I_0), transmittance (T) is always less than or equal 1.

Percent Transmittance *(%T)*, which ranges from 0 to 100%

$$\mathbf{\%T = T * 100}$$

If the T of a sample is 0.40, the %T of the sample is 40%. This means that 40% of the photons in the incident light emerge from the sample as transmitted light and reach the photo-detector. If 40% of the photons are transmitted, 60% of the photons were absorbed by the sample.

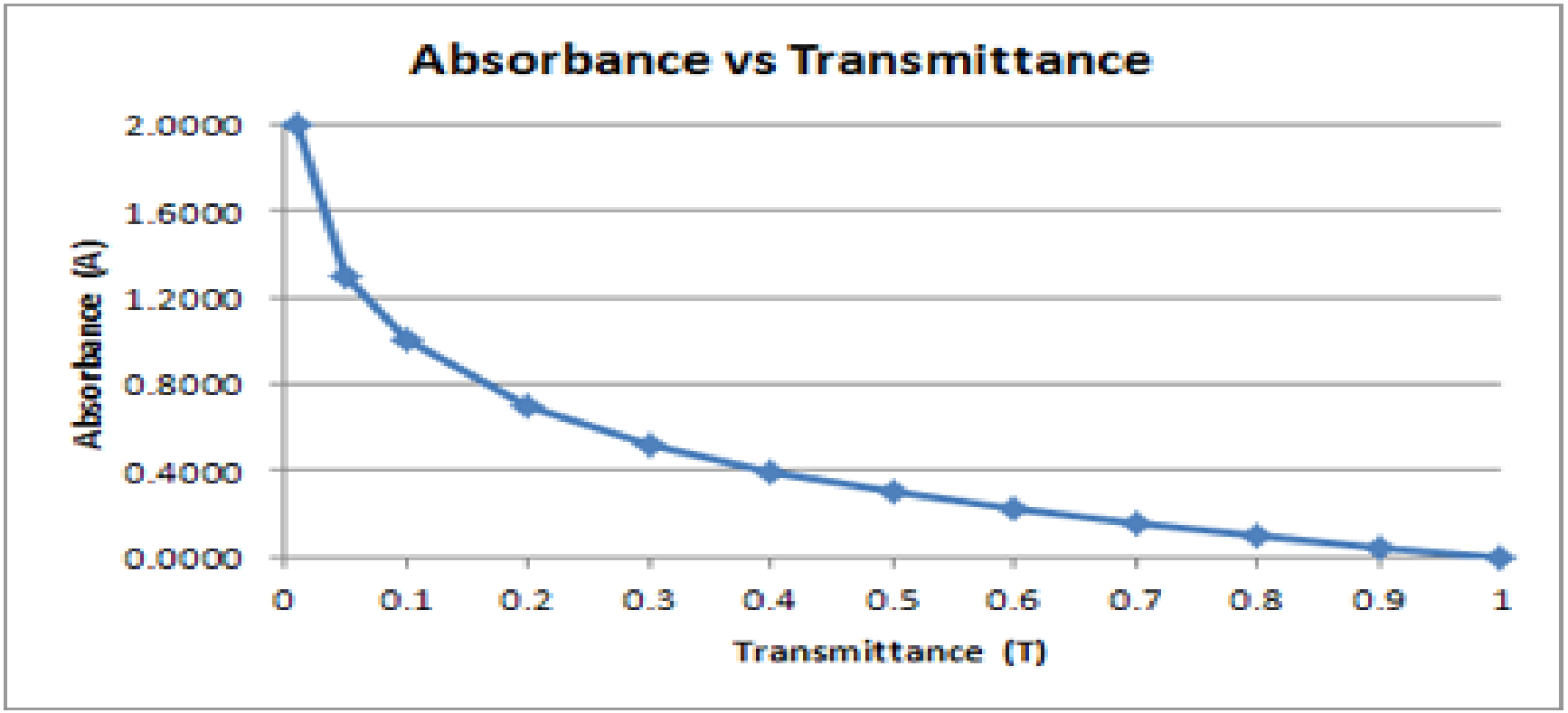
Absorbance (A) is the amount of light absorbed by a sample. It is calculated from T or %T using the following equations:

$$A = -\log T \text{ or } A = \log(1/T),$$

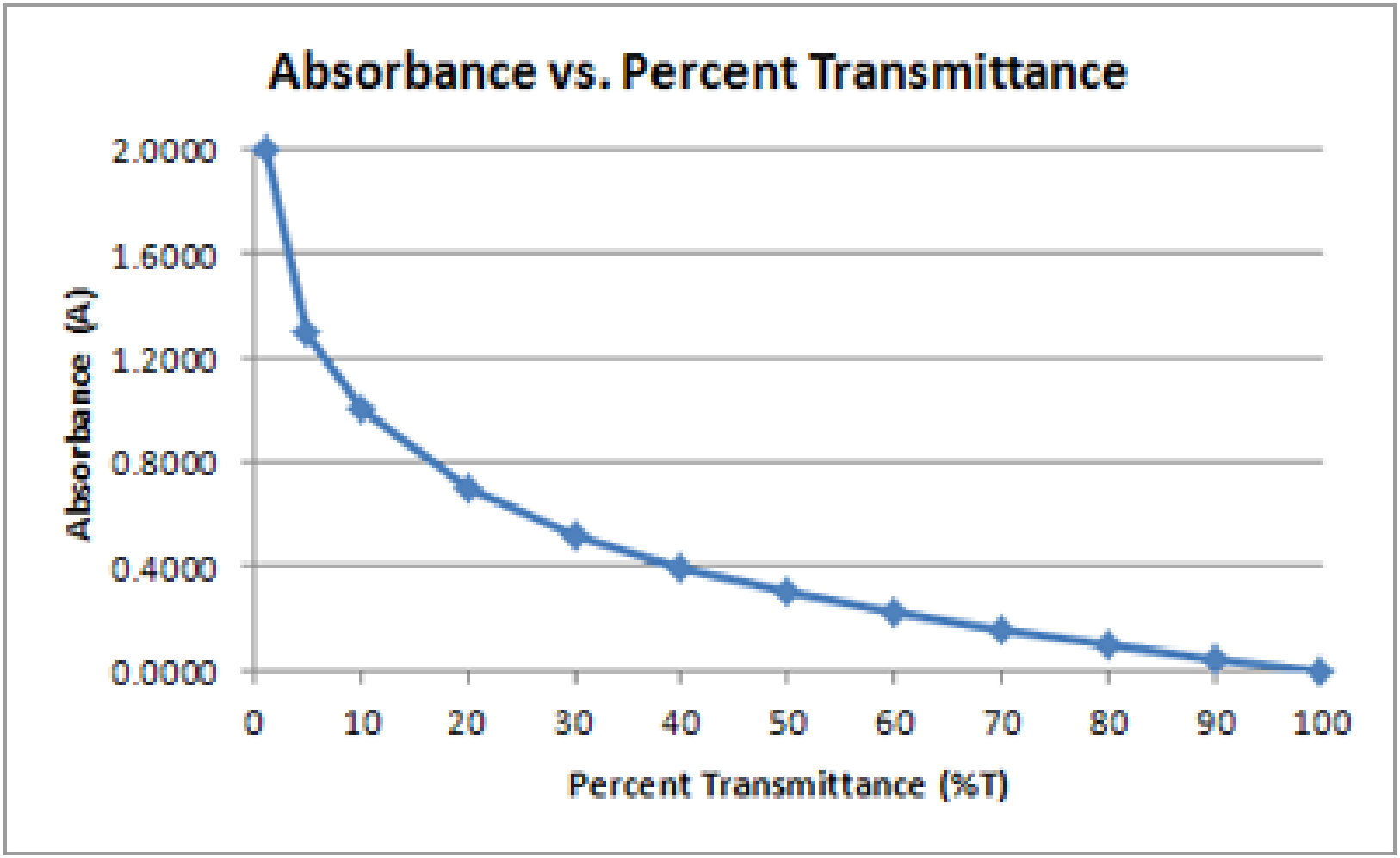
$$A = 2 - \log \%T$$

If %T = 100% , then (A) = 0, If %T = 0 % , then (A) = 2

The inverse logarithmic relationship between absorbance and transmittance and between absorbance and %T are clearly shown in the graphs below. In these graphs, transmittance and %T increase from 0 to 1.0 and 0% to 100%, respectively, absorbance decreases logarithmically from 2.0 to 0.

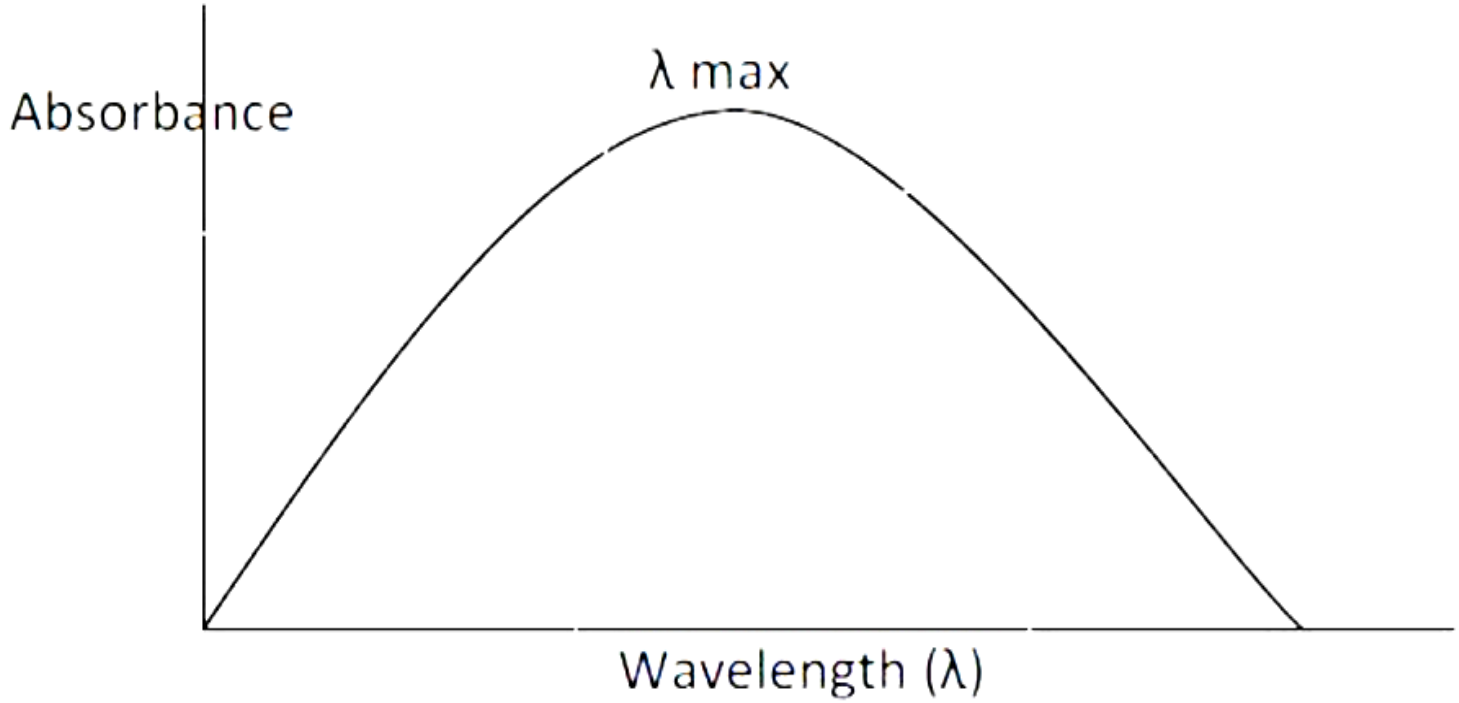


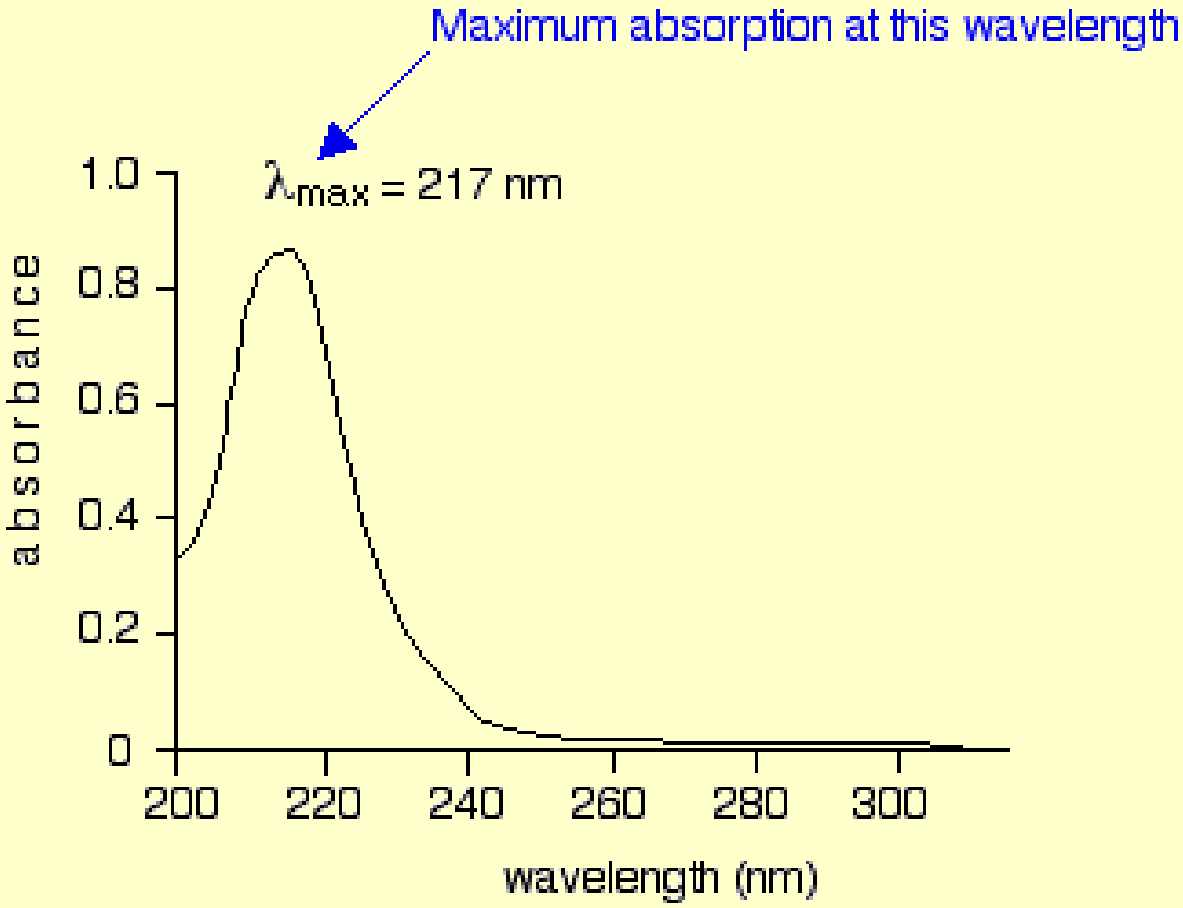
a) Inverse logarithmic relationship between transmittance and absorbance.



b) Inverse logarithmic relationship between percent transmittance and absorbance.

- λ max : refers to the wavelength in the absorption spectrum where the absorbance is maximum and %T is minimum
- λ min : refers to the wavelength in the absorption spectrum where the absorbance is minimum and %T is maximum

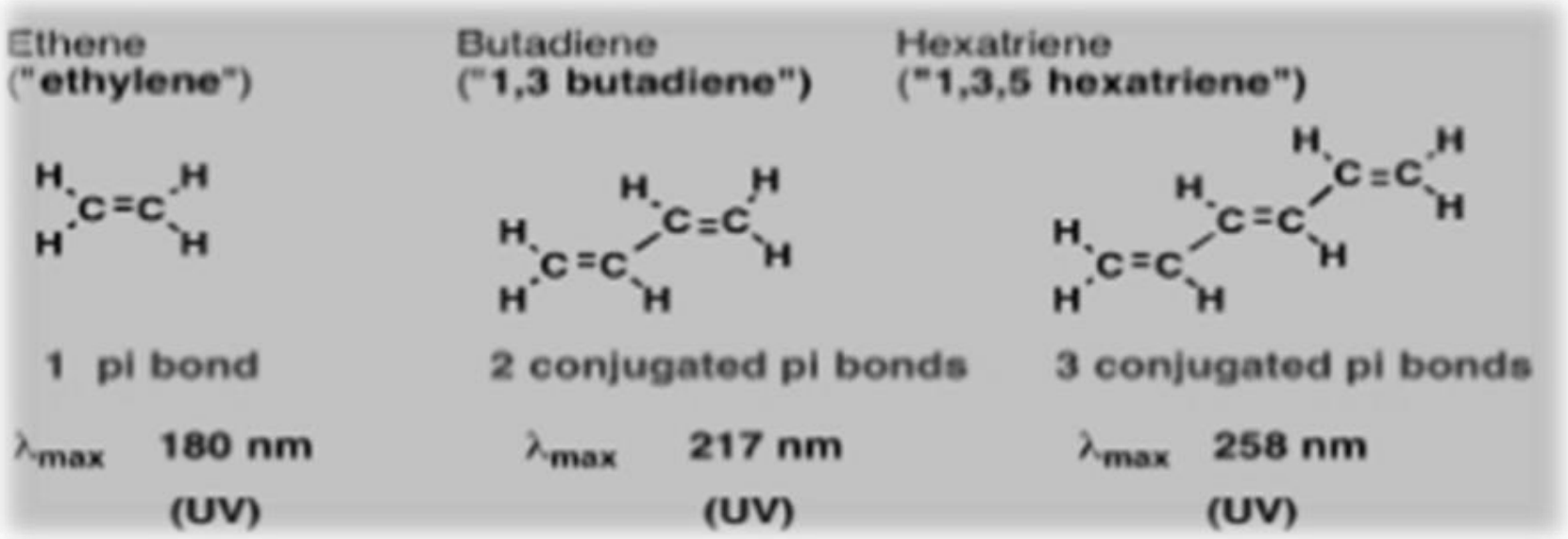


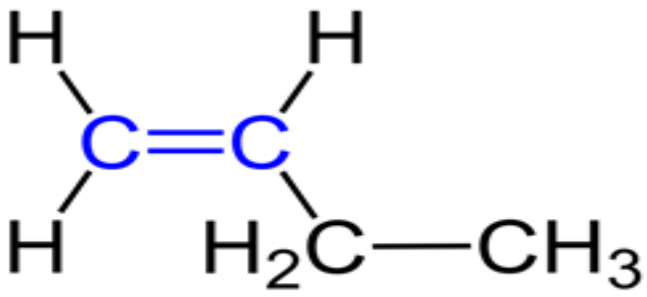


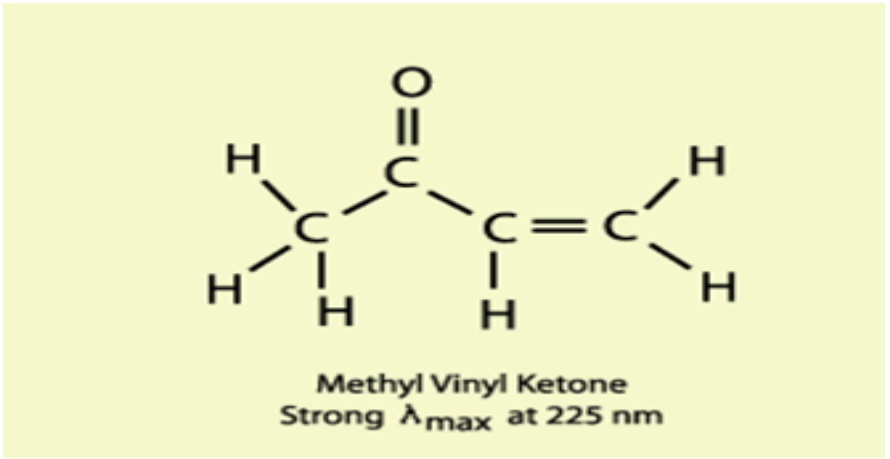
You will see that absorption peaks at a value of 217 nm. This is in the ultra-violet and so there would be no visible sign of any light being absorbed - buta-1,3-diene is colourless. You read the symbol on the graph as "lambda-max".

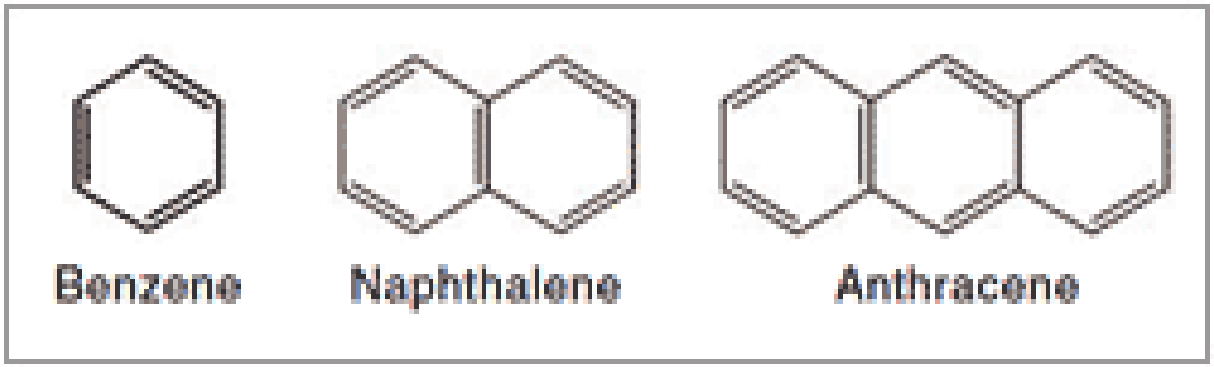
What are the factors affect the absorption peaks (λ max) ?

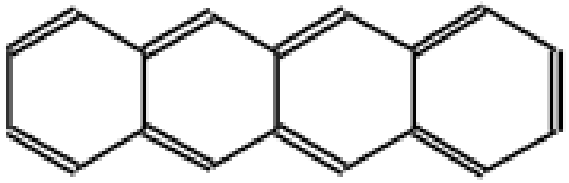
1- Conjugated Systems:











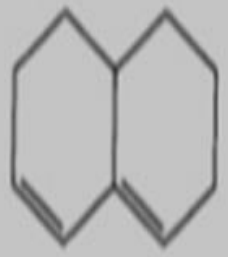
Naphthacene

Benzene: **λ max** **255nm**
Naphthalene **λ max** **286nm**
Anthracene **λ max** **375nm**
Naphthacene **λ max** **477nm**

Homo-annular conjugated double bonds are the conjugated double bonds present in the same ring. It is also called Homodiene. Examples are mentioned below:

Hetero-annular conjugated double bonds are the conjugated double bonds which are not present in the same ring. Examples are as follows:

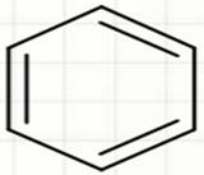
Homoannular diene (cisoid or *s-cis*)
Less intense, ε = 5,000–15,000
λ longer (273 nm)



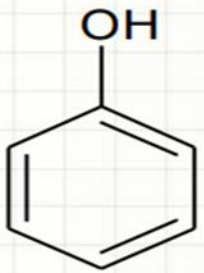
Heteroannular diene (transoid or *s-trans*)
More intense, ε = 12,000–28,000
λ shorter (234 nm)

2-The Influence of Functional Groups :

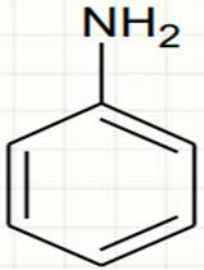
Benzene λ_{max} = 255 nm



Phenol λ_{max} = 270 nm



Aniline λ_{max} = 280 nm



3- Change of solvent

1. Bathochromic Shift (Red Shift)
2. Hypsochromic Shift (Blue Shift)
3. Hyperchromic Effect
4. Hypochromic Effect

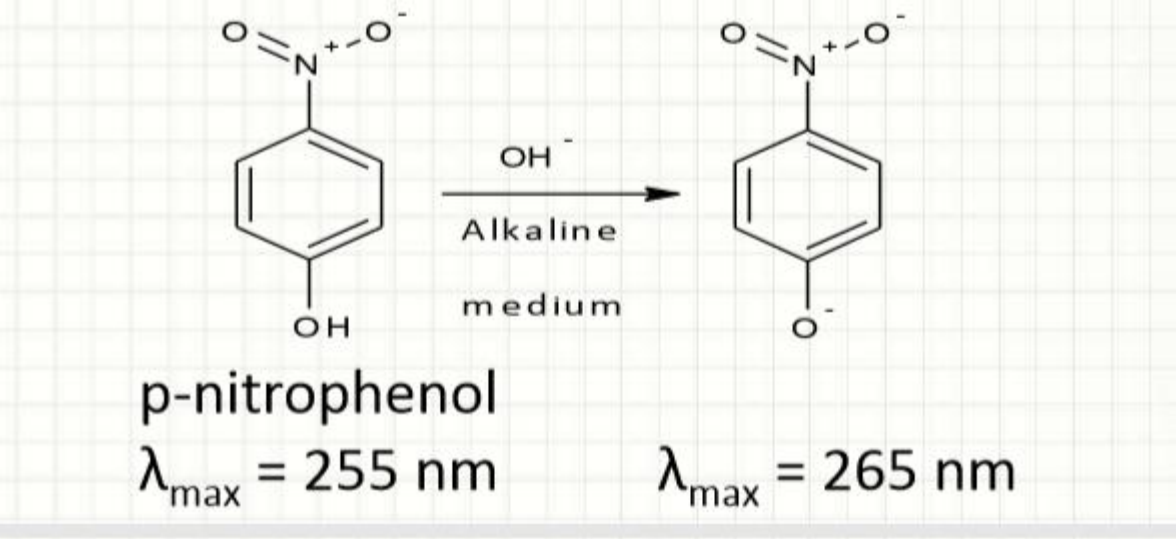
1 • Bathochromic Shift (Red Shift)

• When absorption maxima (λmax) of a compound shifts to longer wavelength, it is known as bathochromic shift or red shift.

• The effect is due to presence of an auxochrome or by the change of solvent.

• e.g. An auxochrome group like –OH, -OCH3 causes absorption of compound at longer wavelength.

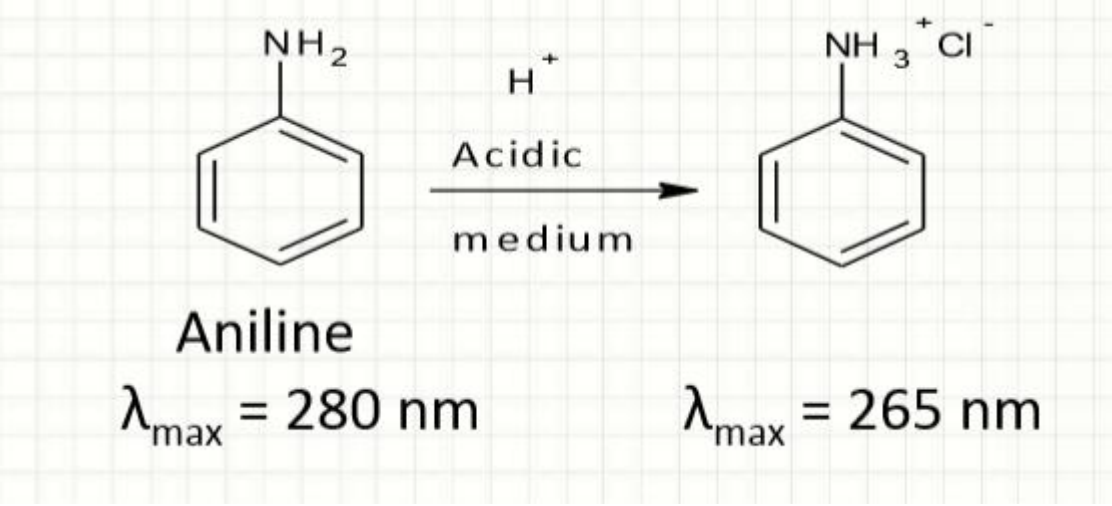
In alkaline medium, p-nitrophenol shows red shift. Because negatively charged oxygen delocalizes more effectively than the unshared pair of electron.



2 • Hypsochromic Shift (Blue Shift)

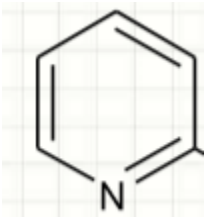
- When absorption maxima (λmax) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.
- The effect is due to presence of an group causes removal of conjugation or by the change of solvent.

Aniline shows blue shift in acidic medium, it loses conjugation

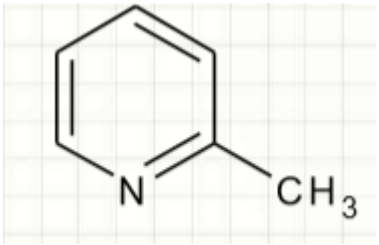


3 • Hyperchromic Effect

- When absorption intensity (ε) of a compound is increased, it is known as hyperchromic shift.
  - If auxochrome introduces to the compound, the intensity of absorption increases.



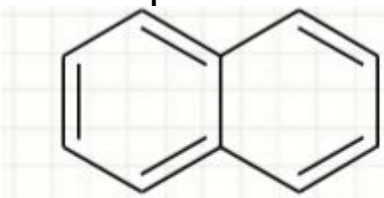
Pyridine
λmax = 257 nm



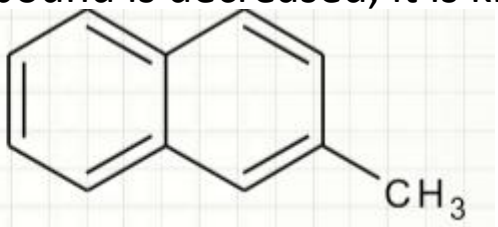
2-methyl pyridine
λmax = 260 nm

4 • Hypochromic Effect

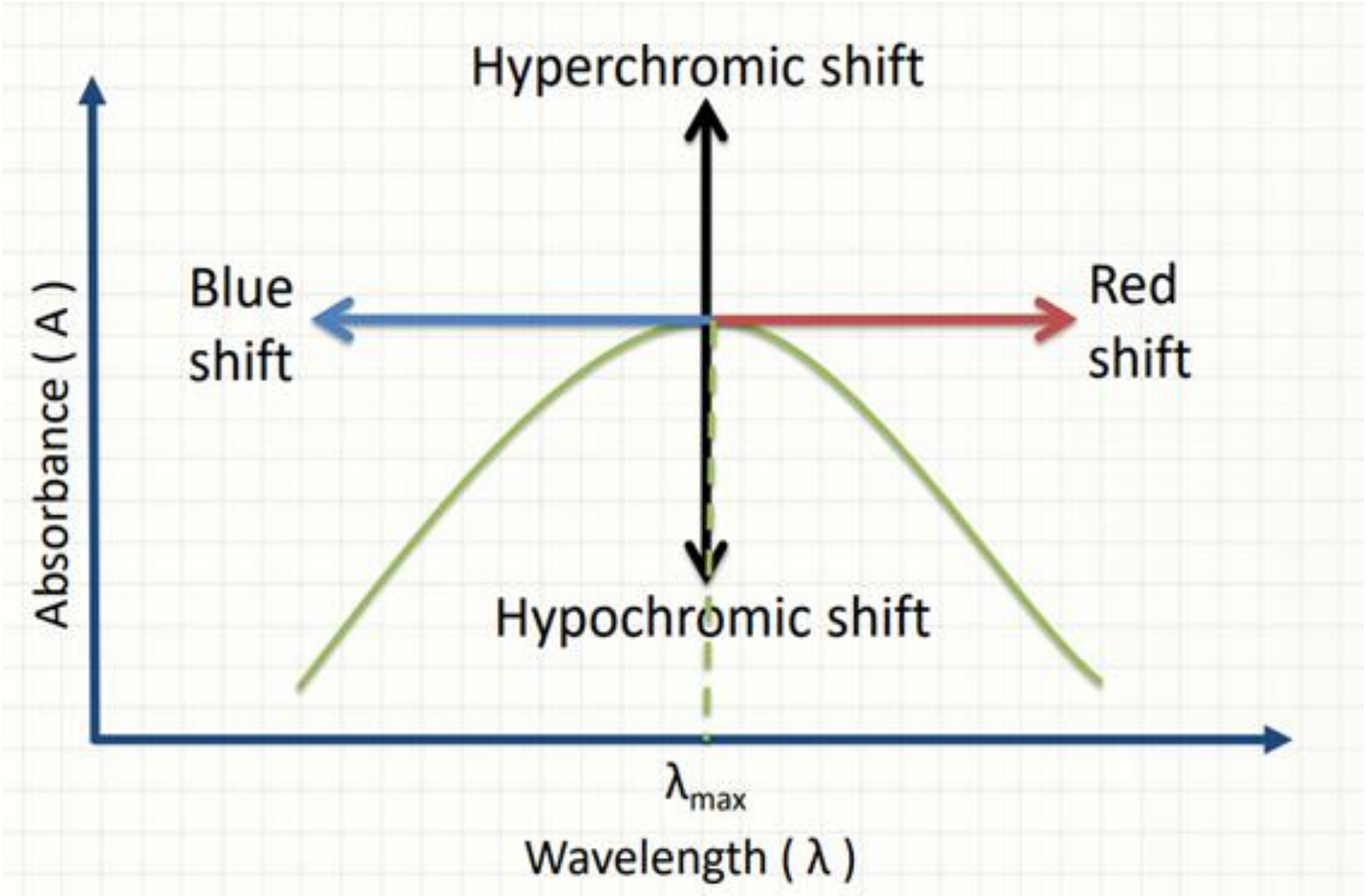
When absorption intensity (ε) of a compound is decreased, it is known as hypochromic shift.



Naphthalene
ε = 19000



2-methyl naphthalene
ε = 10250


Hyperchromic shift
Blue shift
Red shift
Hypochromic shift
Absorbance (A)
λmax
Wavelength (λ)

Applications

- Qualitative & Quantitative Analysis:
  - It is used for characterizing aromatic compounds and conjugated olefins.
  - It can be used to find out molar concentration of the solute under study.
- Detection of impurities:
  - It is one of the important method to detect impurities in organic solvents.
- Detection of isomers are possible.
- Determination of molecular weight using Beer's law.

Practical Part Of Lab

I- $Cr(NO3)_3$: at Visible Region (colored compound)

1- Prepare 0.02 M $Cr(NO3)_3$ solution as standard

2- Use D.W. as blank.

3- Read (%T) at the following wavelengths (nm):(zero the blank at each reading)

370, 385, 400, 415, 430, 450, 470, 480, 500, 520, 545, 565, 585, 600, 620, 650, 700

4- Calculate (A) at each wavelength

5- Plot ,on graph paper, (A) Vs wavelength

6- Determine λ max and λ min

II- Metronidazole : at UV Region

1- Prepare 0.01 mg/ml Metronidazole solution as standard

2- Use 0.01 M HCl as blank.

3- Read (%T) at the following wavelengths (nm):
190, 210, 225, 240, 255 , 275, 290, 310, 330, 350, 375, 400

4- Calculate (A) at each wavelength

5- Plot ,on graph paper, (A) Vs wavelength

6- Determine λ max and λ min