

# UV-Visible Spectroscopy

By

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# The Beer-Lambert Law

- **The Beer-Lambert law states**

Absorbance is directly proportional to:

1. concentration,  $c$ , of absorbing species in the sample
2. path length of light,  $L$ , through the sample

$$A = E C L \dots\dots\dots (i)$$

Where,  $E$  = Molar absorbance coefficient of the absorber

$C$  = Concentration of absorbing solution, and

$b$  = Path length through the solution (or thickness)

# The Beer-Lambert Law

Absorbance (A):

$$A = E C L \dots\dots\dots (i)$$

Where C, Concentration of the analyte is given in unit mol/L (M)

L, The path length, L, in cm

*and E*, is called the molar absorptivity or molar absorption coefficient

“Absorbance of 1 M solution measured in a cell of 1 cm pathlength”

- *E*, is characteristic for each substance at a particular wavelength,.

# PRINCIPLES OF UV – VISIBLE SPECTROSCOPY

- The UV radiation region extends from 10 nm to 400 nm and the visible radiation region extends from 400 nm to 800 nm.

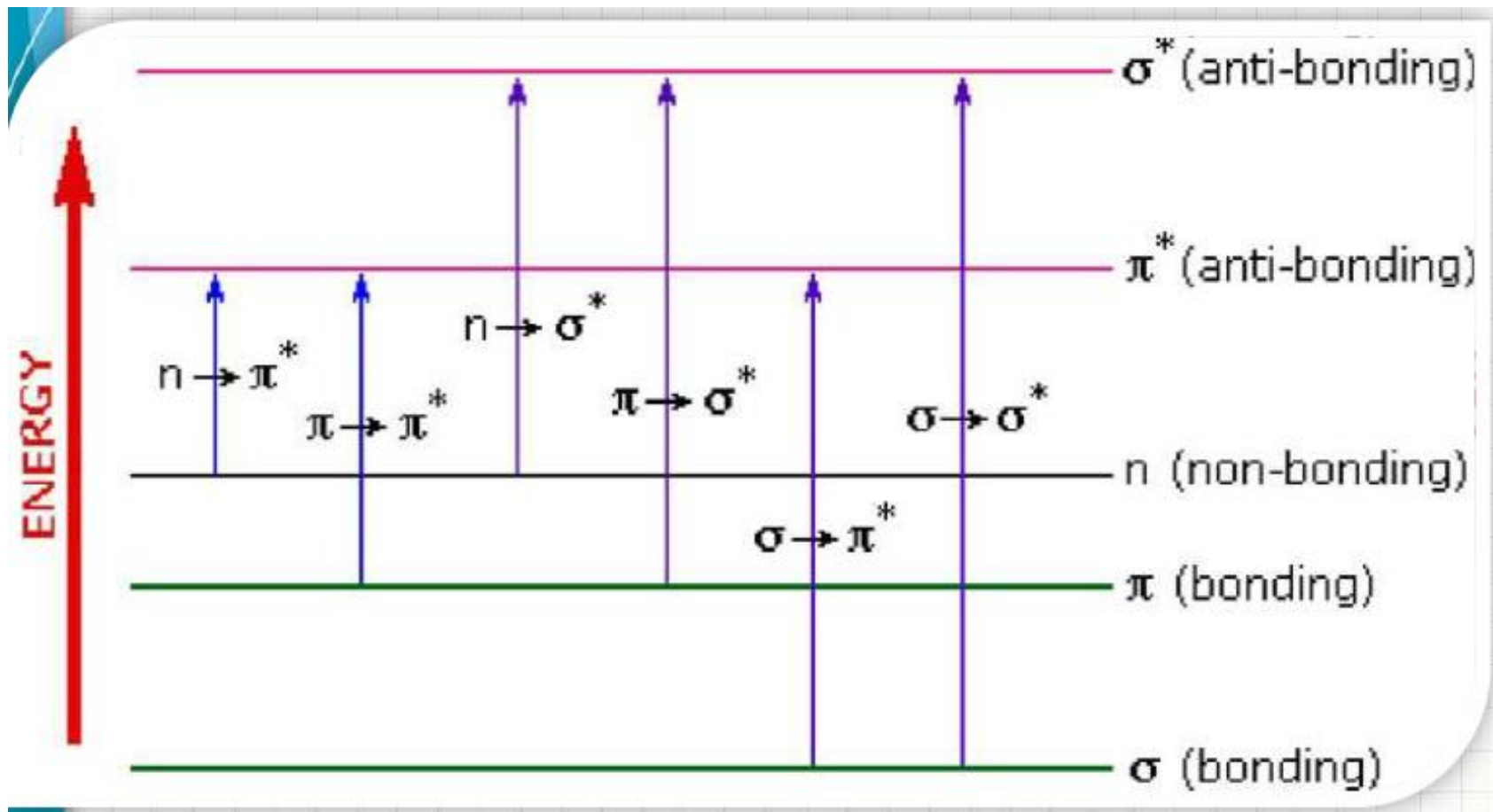
Near UV Region: 200 nm to 400 nm

Far UV Region: below 200 nm

- Far UV spectroscopy is studied under vacuum condition.
- The common solvent used for preparing sample to be analyzed is either ethyl alcohol or hexane.

# Electronic Transitions

- The possible electronic transitions can graphically shown as:



# Electronic Transitions

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

## $\sigma \rightarrow \sigma^*$ transition

- $\sigma$  electron from orbital is excited to corresponding anti-bonding orbital  $\sigma^*$ .
- The energy required is large for this transition.
- e.g. Methane ( $\text{CH}_4$ ) has C-H bond only and can undergo  $\sigma \rightarrow \sigma^*$  transition and shows absorbance maxima at 125 nm.

# $\pi \rightarrow \pi^*$ transition

- $\pi$  electron in a bonding orbital is excited to corresponding anti-bonding orbital  $\pi^*$ .
- Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo  $\pi \rightarrow \pi^*$  transitions.
- e.g. Alkenes generally absorb in the region 170 to 205 nm.



# $n \rightarrow \sigma^*$ transition

- Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of  $n \rightarrow \sigma^*$  transition.
- These transitions usually requires less energy than  $\sigma \rightarrow \sigma^*$  transitions.
- The number of organic functional groups with  $n \rightarrow \sigma^*$  peaks in UV region is small (150 – 250 nm).

# $n \rightarrow \pi^*$ transition

- An electron from non-bonding orbital is promoted to anti-bonding  $\pi^*$  orbital.
- Compounds containing double bond involving hetero atoms ( $\text{C}=\text{O}$ ,  $\text{C}\equiv\text{N}$ ,  $\text{N}=\text{O}$ ) undergo such transitions.
- $n \rightarrow \pi^*$  transitions require minimum energy and show absorption at longer wavelength around 300 nm.

## $\sigma \rightarrow \pi^*$ transition & $\pi \rightarrow \sigma^*$ transition

- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus,  $n \rightarrow \pi^*$  &  $\pi \rightarrow \pi^*$  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.

# Terms used in UV / Visible spectroscopy

- Chromophore

The part of a molecule responsible for imparting color, are called as chromospheres.

OR

The functional groups containing multiple bonds capable of absorbing radiations above 200 nm due to  $n \rightarrow \pi^*$  &  $\pi \rightarrow \pi^*$  transitions.

e.g.  $\text{NO}_2$ ,  $\text{N}=\text{O}$ ,  $\text{C}=\text{O}$ ,  $\text{C}=\text{N}$ ,  $\text{C}\equiv\text{N}$ ,  $\text{C}=\text{C}$ ,  $\text{C}=\text{S}$ , etc.

# Chromophore

To interpretate UV – visible spectrum following points should be noted:

1. Non-conjugated alkenes show an intense absorption below 200 nm & are therefore inaccessible to UV spectrophotometer.
2. Non-conjugated carbonyl group compound give a weak absorption band in the 200 – 300 nm region.

# Chromophore

e.g. Acetone which has  $\lambda_{\text{max}} = 279 \text{ nm}$  and that cyclohexane has  $\lambda_{\text{max}} = 291 \text{ nm}$ .

When double bonds are conjugated in a compound  $\lambda_{\text{max}}$  is shifted to longer wavelength.

e.g. 1,5 - hexadiene has  $\lambda_{\text{max}} = 178 \text{ nm}$  and  
2,4 - hexadiene has  $\lambda_{\text{max}} = 227 \text{ nm}$

# Chromophore

3. Conjugation of C=C and carbonyl group shifts the  $\lambda_{\text{max}}$  of both groups to longer wavelength.

e.g. Ethylene has  $\lambda_{\text{max}} = 171 \text{ nm}$

Acetone has  $\lambda_{\text{max}} = 279 \text{ nm}$

Crotonaldehyde has  $\lambda_{\text{max}} = 290 \text{ nm}$

# 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.

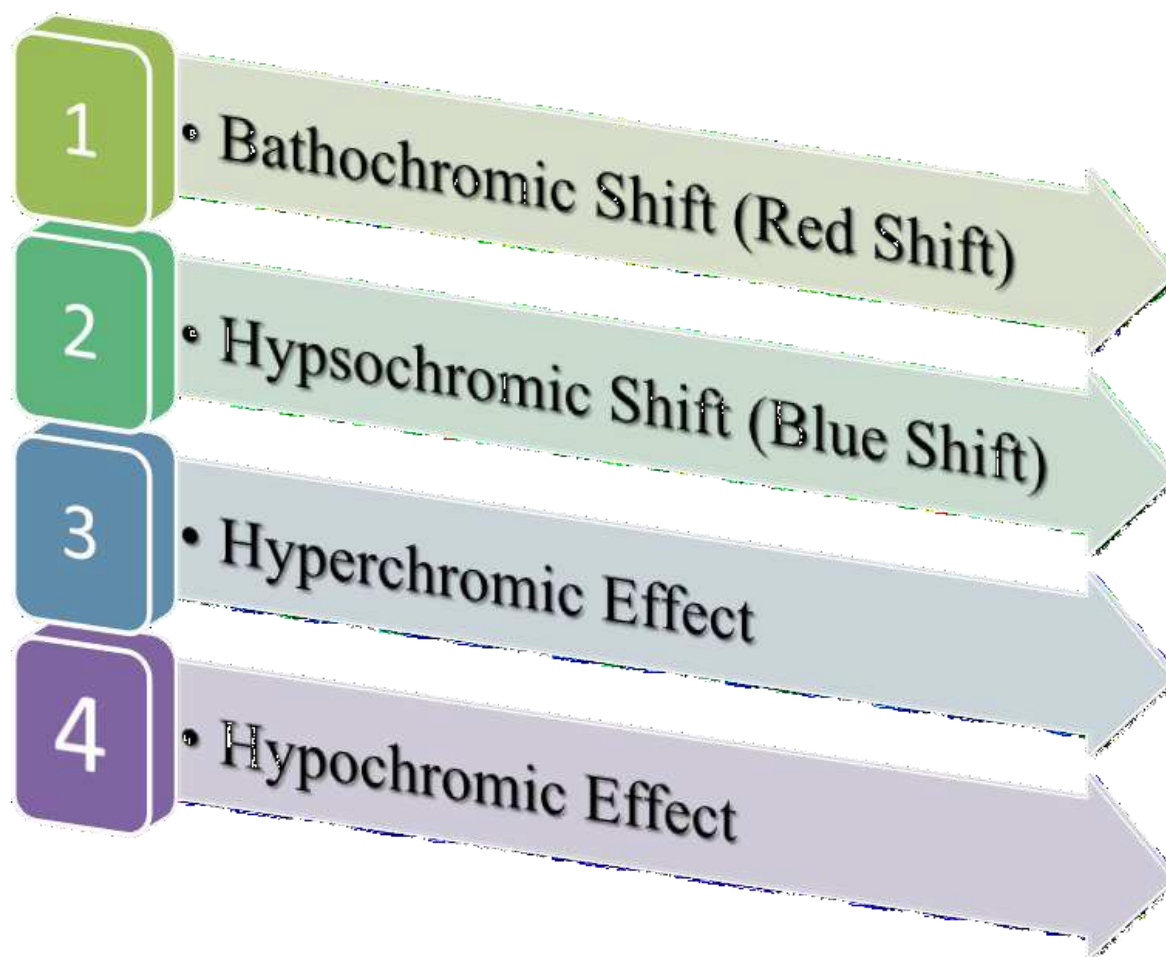
e.g. Benzene  $\lambda_{\text{max}} = 255 \text{ nm}$

Phenol  $\lambda_{\text{max}} = 270 \text{ nm}$

Aniline  $\lambda_{\text{max}} = 280 \text{ nm}$



# Absorption & Intensity Shifts



# Bathochromic Shift (Red Shift)

- When absorption maxima ( $\lambda_{\text{max}}$ ) 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  $-\text{OH}$ ,  $-\text{OCH}_3$  causes absorption of compound at longer wavelength.

# Bathochromic Shift (Red Shift)

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

p-nitrophenol  $\lambda_{\text{max}} = 255 \text{ nm}$  shifted to  $\lambda_{\text{max}} = 265 \text{ nm}$

# Hypsochromic Shift (Blue Shift)

- When absorption maxima ( $\lambda_{\text{max}}$ ) 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.

Aniline  $\lambda_{\text{max}}$  = 280 nm shifted to  $\lambda_{\text{max}}$  = 265 nm

# Hyperchromic Effect

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

Pyridine

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

2-methyl pyridine

$\lambda_{\text{max}} = 260 \text{ nm}$

# Hypochromic Effect

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

Naphthalene

$$\epsilon = 19000$$

2-methyl naphthalene

$$\epsilon = 10250$$

# APPLICATIONS OF UV / VISIBLE SPECTROSCOPY

- 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.