

Spectroscopy

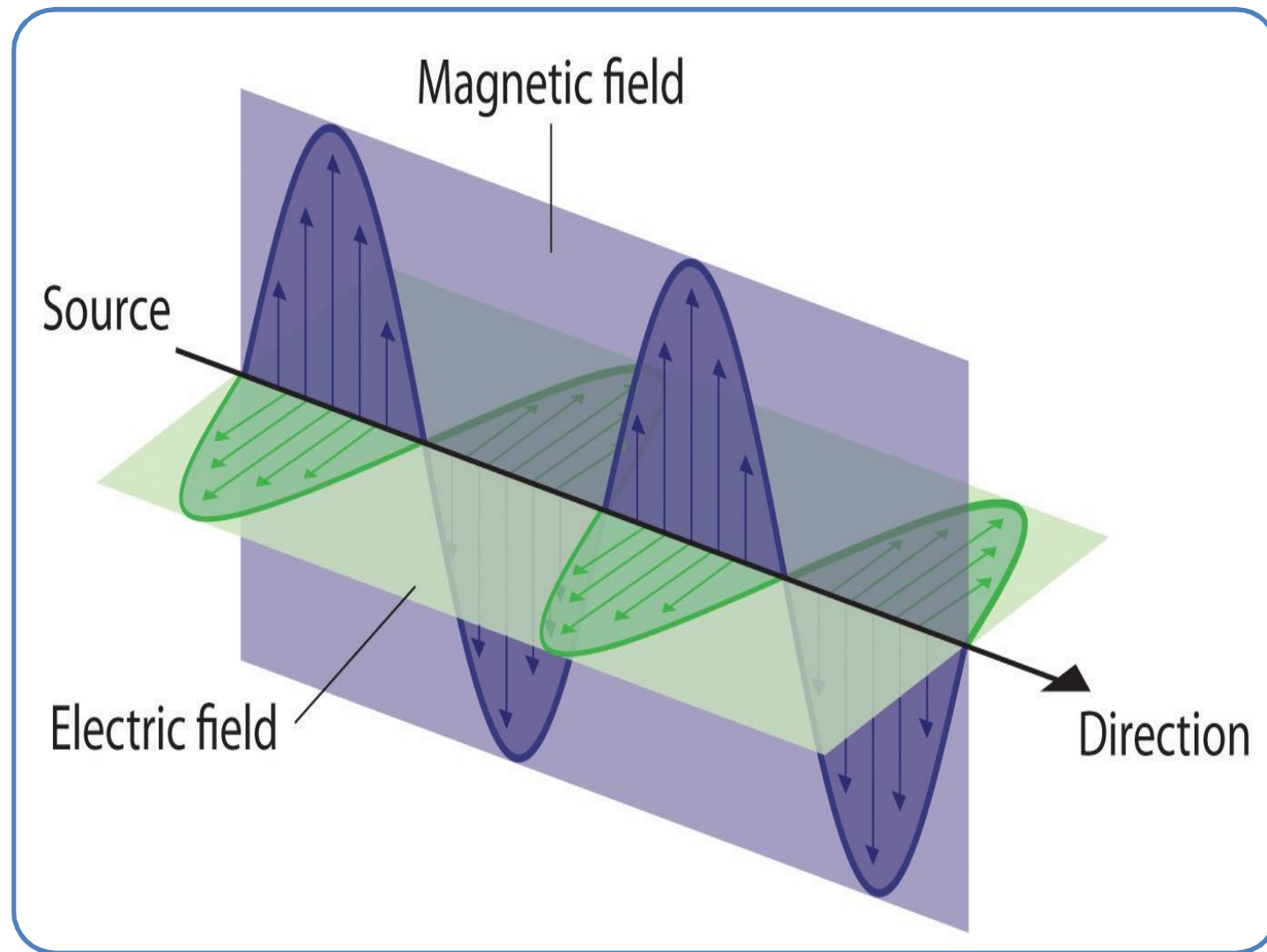
It is the branch of science that deals with the study of interaction of matter with light.

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.

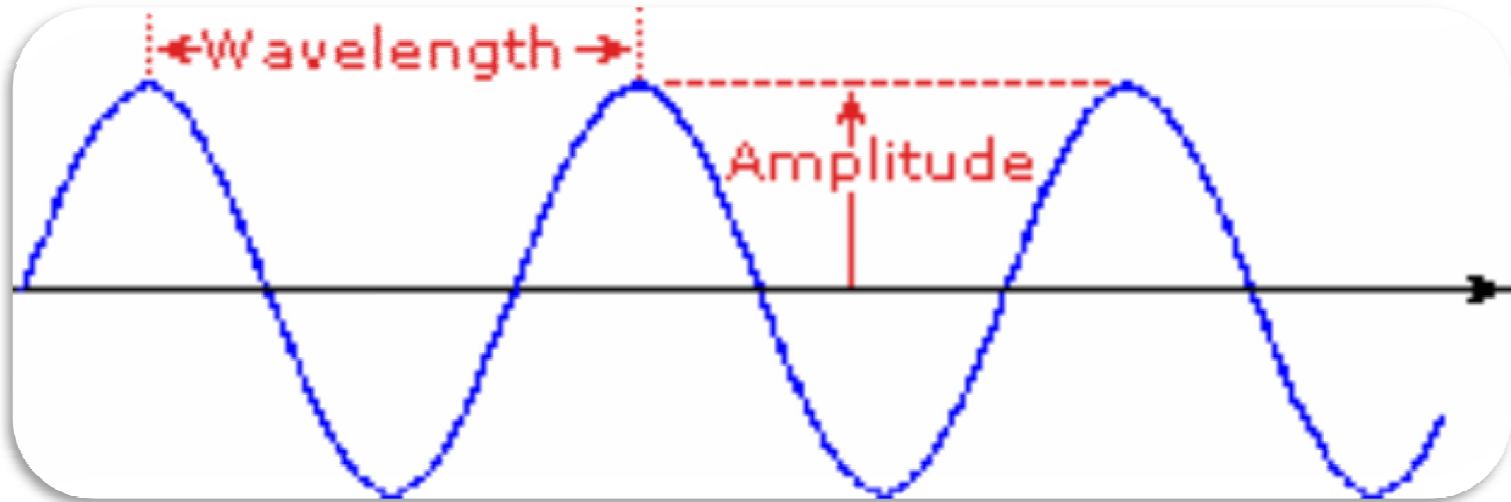


Electromagnetic Radiation

- Frequency (ν):
 - It is defined as the number of times electrical field radiation oscillates in one second.
 - The unit for frequency is Hertz (Hz).

$1 \text{ Hz} = 1 \text{ cycle per second}$
- Wavelength (λ):
 - It is the distance between two nearest parts of the wave in the same phase i.e. distance between two nearest crest or troughs.

Electromagnetic Radiation



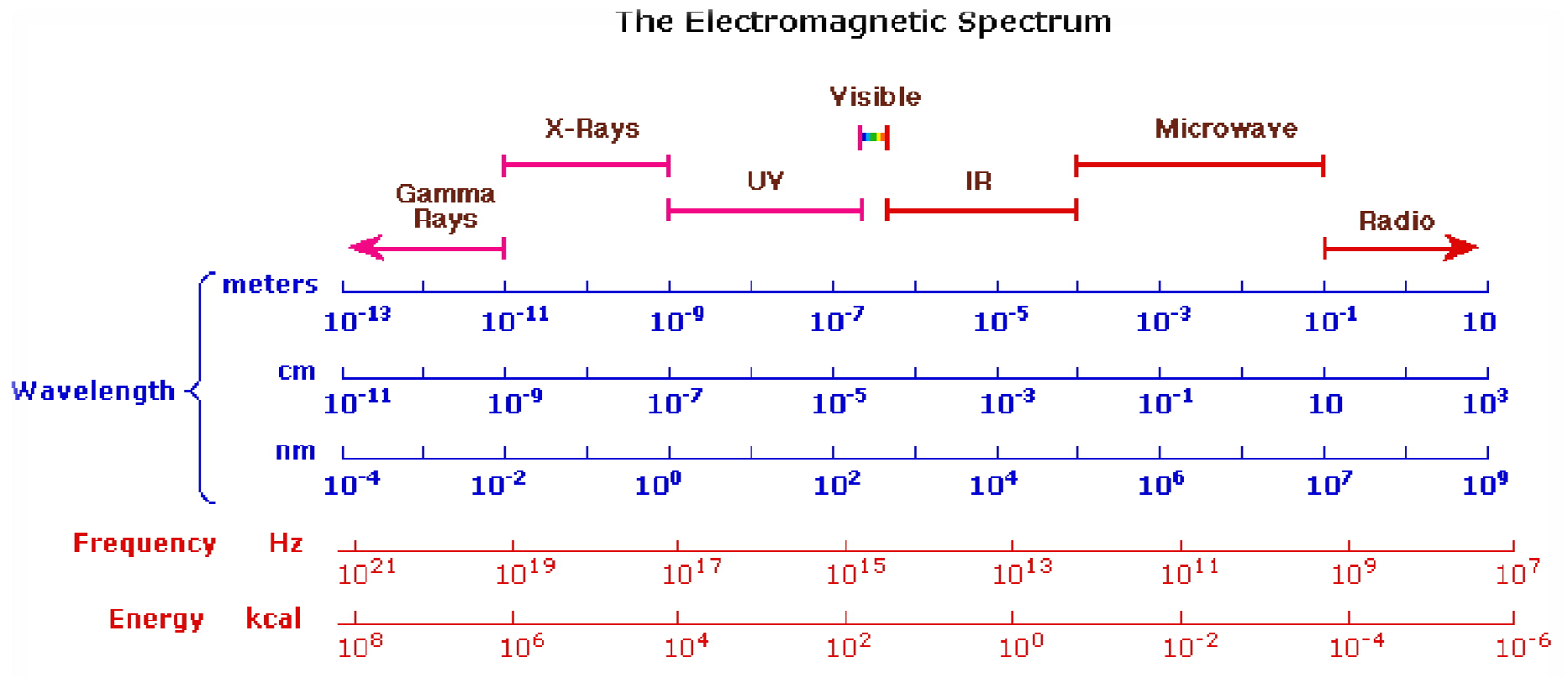
- The relationship between wavelength & frequency can be written as:

$$c = \nu \lambda$$

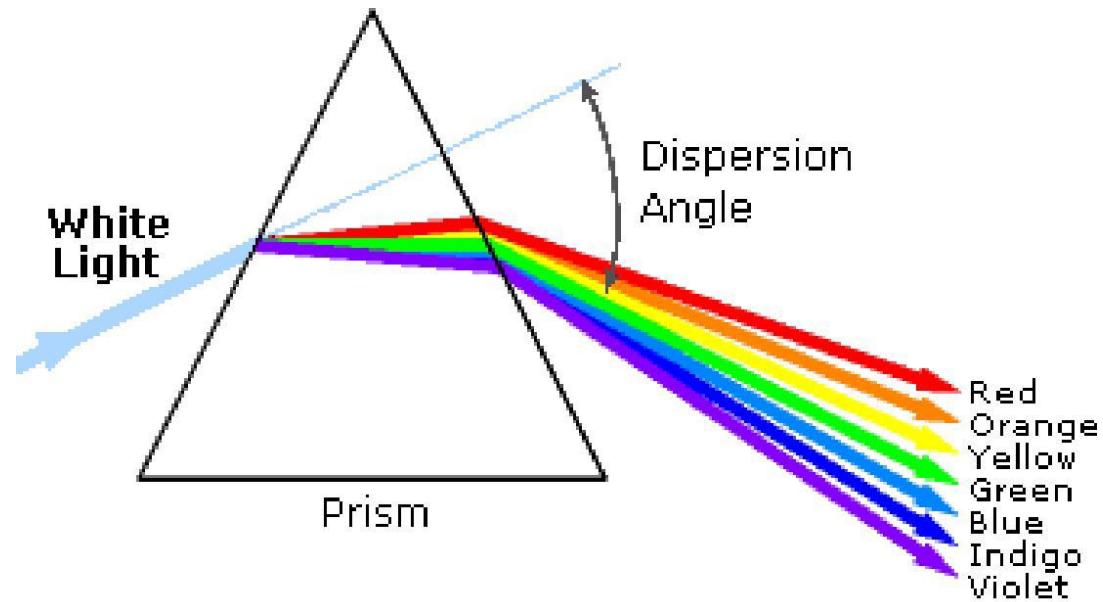
- As photon is subjected to energy, so

$$E = h \nu = h c / \lambda$$

Electromagnetic Radiation



Electromagnetic Radiation



Violet	400 - 420 nm	Yellow	570 - 585 nm
Indigo	420 - 440 nm	Orange	585 - 620 nm
Blue	440 - 490 nm	Red	620 - 780 nm
Green	490 - 570 nm		

Principles of Spectroscopy

Principles of Spectroscopy

- The principle is based on the measurement of spectrum of a sample containing atoms / molecules.
- Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (ν) or wavelength (λ).
- Spectrometer is an instrument design to measure the spectrum of a compound.

Molecular spectroscopy – basic idea

- ❖ In a spectroscopy experiment, electromagnetic radiation of a specified range of wavelengths is allowed to pass through a sample containing a compound of interest.
- ❖ The sample molecules absorb energy from some of the wavelengths, and as a result **jump from a low energy 'ground state' to some higher energy 'excited state'**.
- ❖ Other wavelengths are *not* absorbed by the sample molecule, so they pass on through.
- ❖ A detector on the other side of the sample records which wavelengths were absorbed, and to what extent they were absorbed.

❖ *A given molecule will specifically absorb only those wavelengths which have energies that correspond to the energy difference of the transition that is occurring.*

❖ Thus, if the transition involves the molecule jumping from ground state A to excited state B, with an energy difference of ΔE , the molecule will specifically absorb radiation with wavelength that corresponds to ΔE , while allowing other wavelengths to pass through unabsorbed.

❖ By observing which wavelengths a molecule absorbs, and to what extent it absorbs them, we can gain information about the nature of the energetic transitions that a molecule is able to undergo, and thus information about its structure.

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:

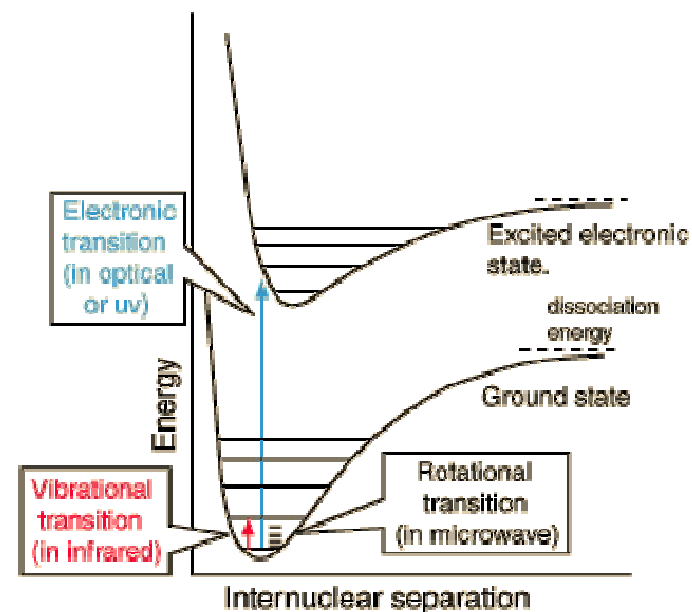
- An analytical technique in which emission (of a particle or radiation) is dispersed according to some property of the emission & the amount of dispersion is measured.
- e.g. Mass Spectroscopy

Interaction of
EMR

with
Matter

Molecular Spectra

- ❖ The most commonly observed molecular spectra involves electronic, vibrational, or rotational transitions.
- ❖ For a diatomic molecule, the electronic states can be represented by plots of potential energy as a function of internuclear distance.
- ❖ Electronic transitions are vertical or almost vertical lines on such a plot since the electronic transition occurs so rapidly that the internuclear distance can't change much in the process.
- ❖ Vibrational transitions occur between different vibrational levels of the same electronic state.
- ❖ Rotational transitions occur mostly between rotational levels of the same vibrational state, although there are many examples of combination vibration-rotation transitions for light molecules



Interaction of EMR with matter

1. Electronic Energy Levels:

- At room temperature the molecules are in the lowest energy levels E_0 .
- When the molecules absorb UV-visible light from EMR, one of the outermost bond / lone pair electron is promoted to higher energy state such as E_1 , E_2 , ... E_n , etc is called as electronic transition and the difference is as:

$$\Delta E = h \nu = E_n - E_0 \quad \text{where } (n = 1, 2, 3, \dots \text{ etc})$$

$$\Delta E = 35 \text{ to } 71 \text{ kcal/mole}$$

Interaction of EMR with matter

2. Vibrational Energy Levels:

- These are less energy level than electronic energy levels.
- The spacing between energy levels are relatively small i.e. 0.01 to 10 kcal/mole.
- e.g. when IR radiation is absorbed, molecules are excited from one vibrational level to another or it vibrates with higher amplitude.

Interaction of EMR with matter

3. Rotational Energy Levels:

- These energy levels are quantized & discrete.
- The spacing between energy levels are even smaller than vibrational energy levels.

$$\Delta E_{\text{rotational}} < \Delta E_{\text{vibrational}} < \Delta E_{\text{electronic}}$$

Lambert's Law

Lambert's Law

- When a monochromatic radiation is passed through a solution, the decrease in the intensity of radiation with thickness of the solution is directly proportional to the intensity of the incident light.
- Let I be the intensity of incident radiation.
 x be the thickness of the solution.
 Then

Lambert's Law

$$2.303 \log \frac{I}{I_0} = -Kl$$

$$\log \frac{I}{I_0} = -\frac{K}{2.303}l$$

Where,

Absorbance

$$\frac{K}{2.303} = E \quad \text{Absorption coefficient}$$

$$A = E.l$$

Lambert's Law

Beer's Law

- When a monochromatic radiation is passed through a solution, the decrease in the intensity of radiation with thickness of the solution is directly proportional to the intensity of the incident light as well as concentration of the solution.
- Let I be the intensity of incident radiation.
 x be the thickness of the solution.
 C be the concentration of the solution.

Then

Beer's Law

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

$$\text{So, } -\frac{dI}{dx} = K' C \cdot I$$

Integrate equation between limit

$I = I_0$ at $x = 0$ and

$I = I$ at $x=l$,

We get,

$$\ln \frac{I}{I_0} = -K' C \cdot l$$

Beer's Law

$$2.303 \log \frac{I_0}{I} = K.C.l$$

$$\log \frac{I_0}{I} = \frac{K}{2.303} C.l$$

Where, $\log \frac{I_0}{I} = A$ Absorbance

$$\frac{K}{2.303} = E$$

Molar extinction coefficient

$$A = E.C.l$$

Beer's Law

Beer's Law

$$A = E.C.l$$

$$T = \frac{I}{I_0} \quad \text{OR} \quad -\log T = \log \frac{I}{I_0} = A$$

From the equation it is seen that the absorbance which is also called as optical density (OD) of a solution in a container of fixed path length is directly proportional to the concentration of a solution.

Principles of UV - Visible Spectroscopy

Principle

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

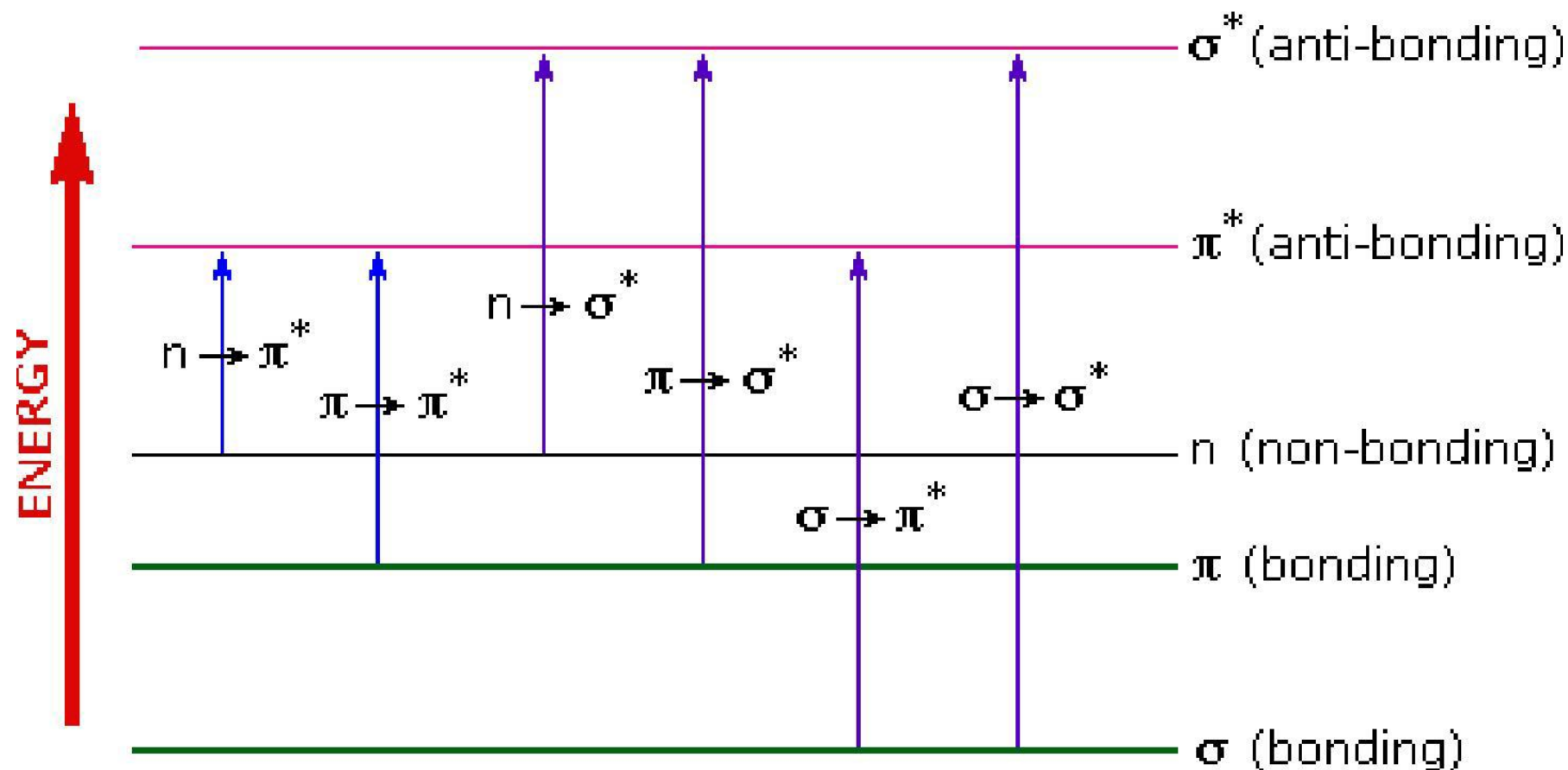
Near UV Region: 200 nm to 400 nm

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 different electronic transitions in UV visible spectroscopy can be graphically shown as



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

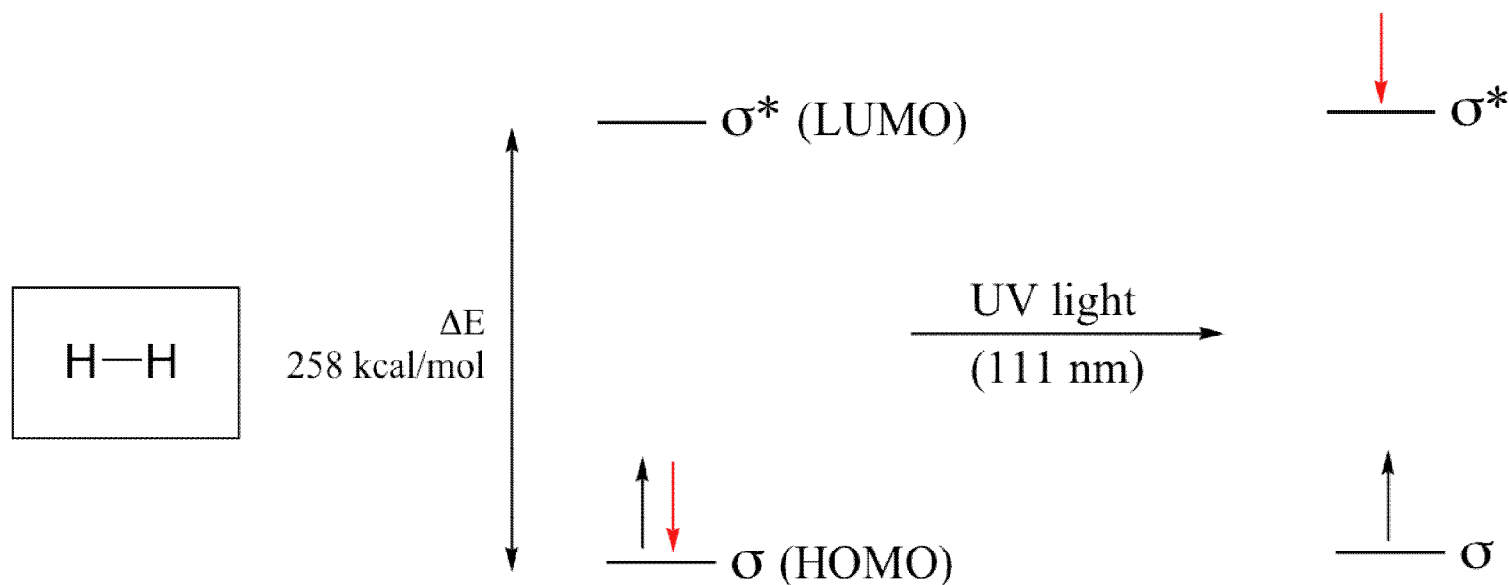
6

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

- $\sigma \rightarrow \sigma^*$ transition

- σ electron from orbital is excited to corresponding anti-bonding orbital σ^* .
- The energy required is large for this transition.
- e.g. Methane (CH_4) has C-H bond only and can undergo $\sigma \rightarrow \sigma^*$ transition and shows absorbance maxima at 125 nm.

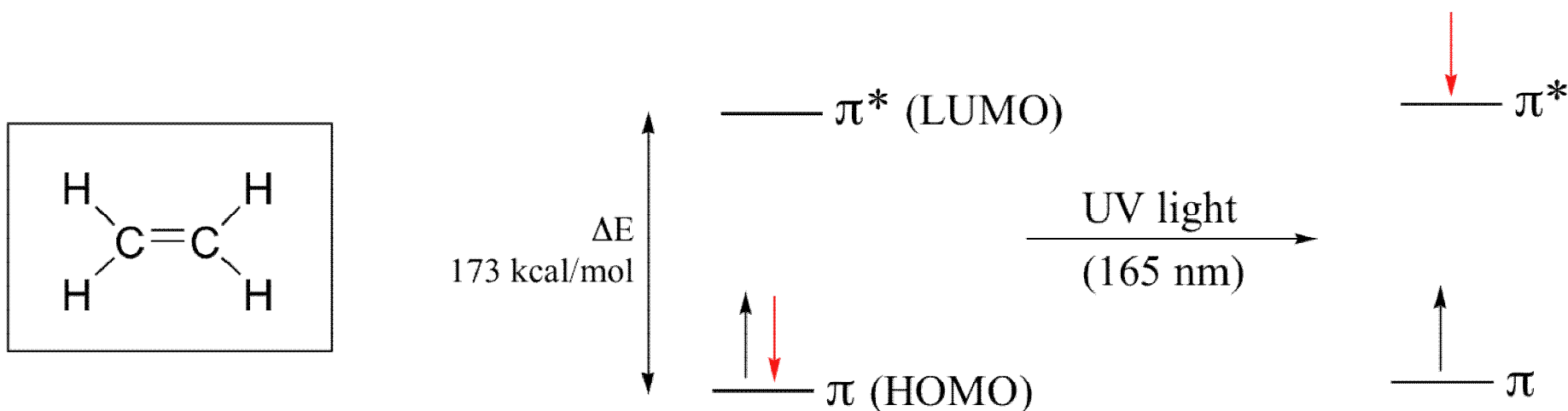
If the molecule is exposed to light of a wavelength with energy equal to ΔE , the HOMO-LUMO energy gap, this wavelength will be absorbed and the energy used to bump one of the electrons from the HOMO to the LUMO – in other words, from the σ to the σ^* orbital. This is referred to as a **$\sigma - \sigma^*$ transition**. ΔE for this electronic transition is 258 kcal/mol, corresponding to light with a wavelength of 111 nm.



- $\pi \rightarrow \pi^*$ 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 $\pi \rightarrow \pi^*$ transitions.
- e.g. Alkenes generally absorb in the region 170 to 205 nm.

When a double-bonded molecule such as ethene (common name ethylene) absorbs light, it undergoes **a $\pi - \pi^*$ transition**. Because $\pi - \pi^*$ energy gaps are narrower than $\sigma - \sigma^*$ gaps, ethene absorbs light at 165 nm - a longer wavelength than molecular hydrogen.



- $n \rightarrow \sigma^*$ 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 π^* orbital.
- Compounds containing double bond involving hetero atoms ($C=O$, $C\equiv N$, $N=O$) 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 chromophores.

OR

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

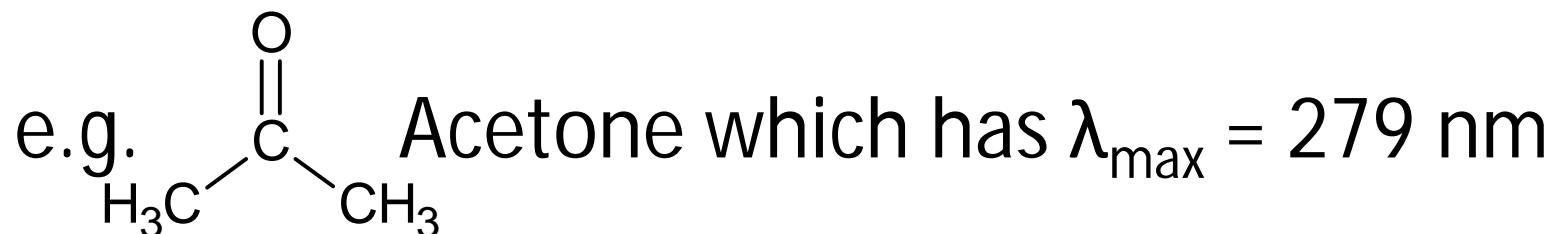
e.g. NO_2 , $\text{N}=\text{O}$, $\text{C}=\text{O}$, $\text{C}=\text{N}$, $\text{C}\equiv\text{N}$, $\text{C}=\text{C}$, $\text{C}=\text{S}$, etc

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



and that cyclohexane has $\lambda_{\text{max}} = 291 \text{ nm}$.

When double bonds are conjugated in a compound λ_{max} is shifted to longer wavelength.

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

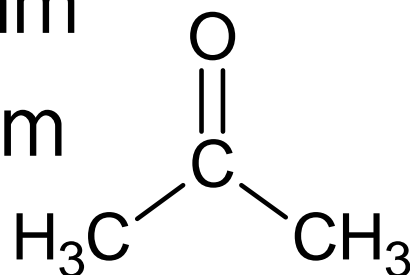
2,4 - hexadiene has $\lambda_{\text{max}} = 227 \text{ nm}$

Chromophore

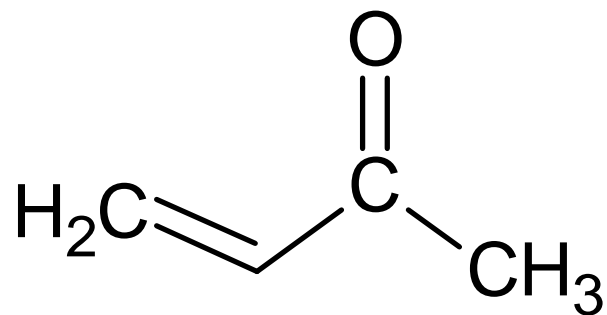
3. Conjugation of C=C and carbonyl group shifts the λ_{max} of both groups to longer wavelength.

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

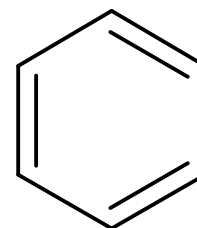
The functional groups attached to a chromophore which modifies the ability of the chromophore to absorb light , altering the wavelength or intensity of absorption.

OR

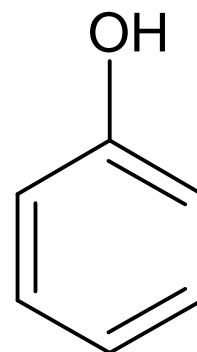
The functional group with non-bonding electrons that does not absorb radiation in near UV region but when attached to a chromophore alters the wavelength & intensity of absorption.

Auxochrome

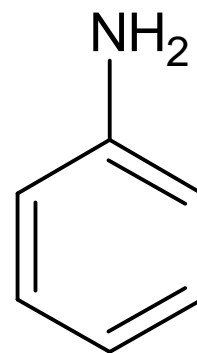
e.g. Benzene $\lambda_{\text{max}} = 255 \text{ nm}$



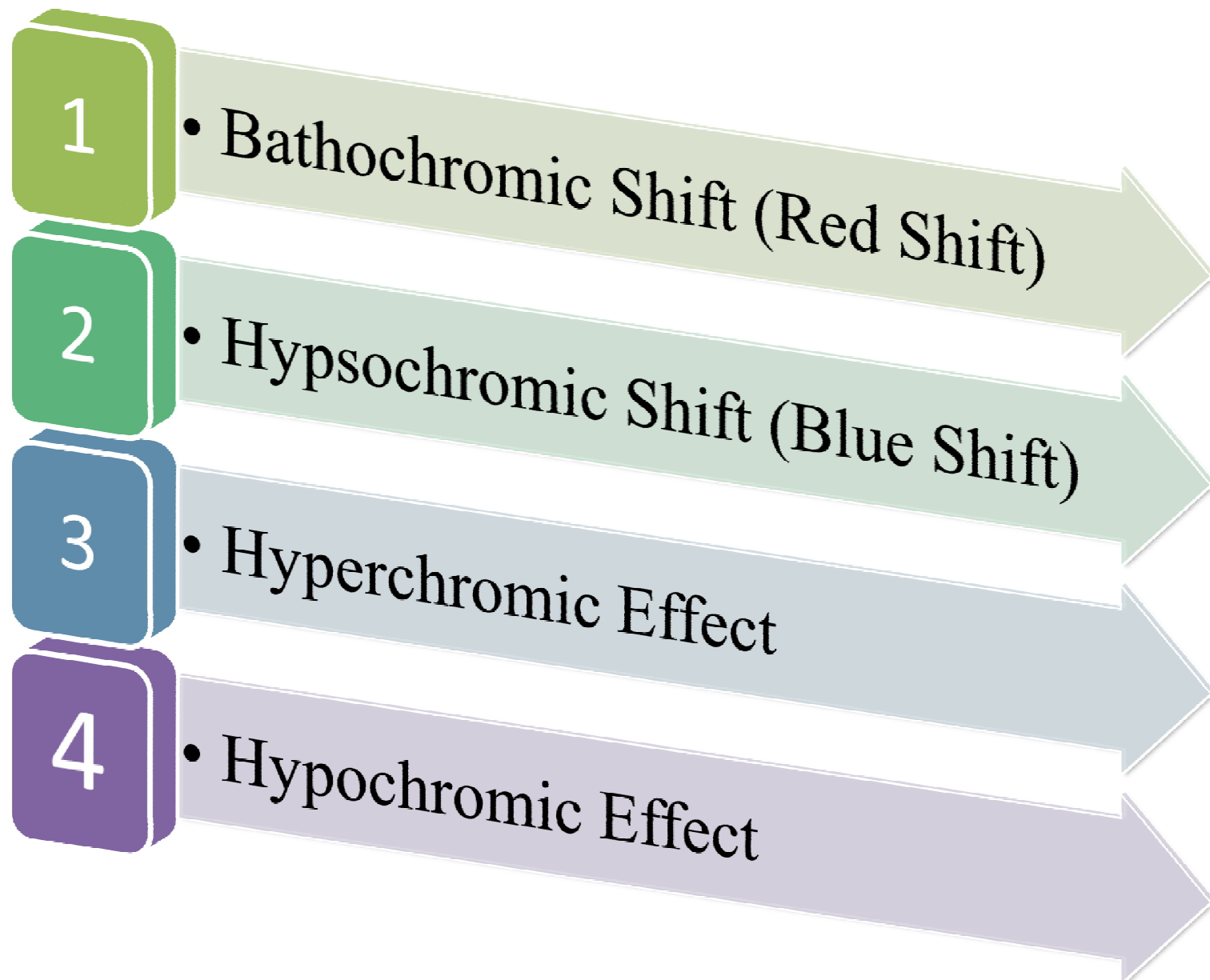
Phenol $\lambda_{\text{max}} = 270 \text{ nm}$



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



Absorption & Intensity Shifts



1

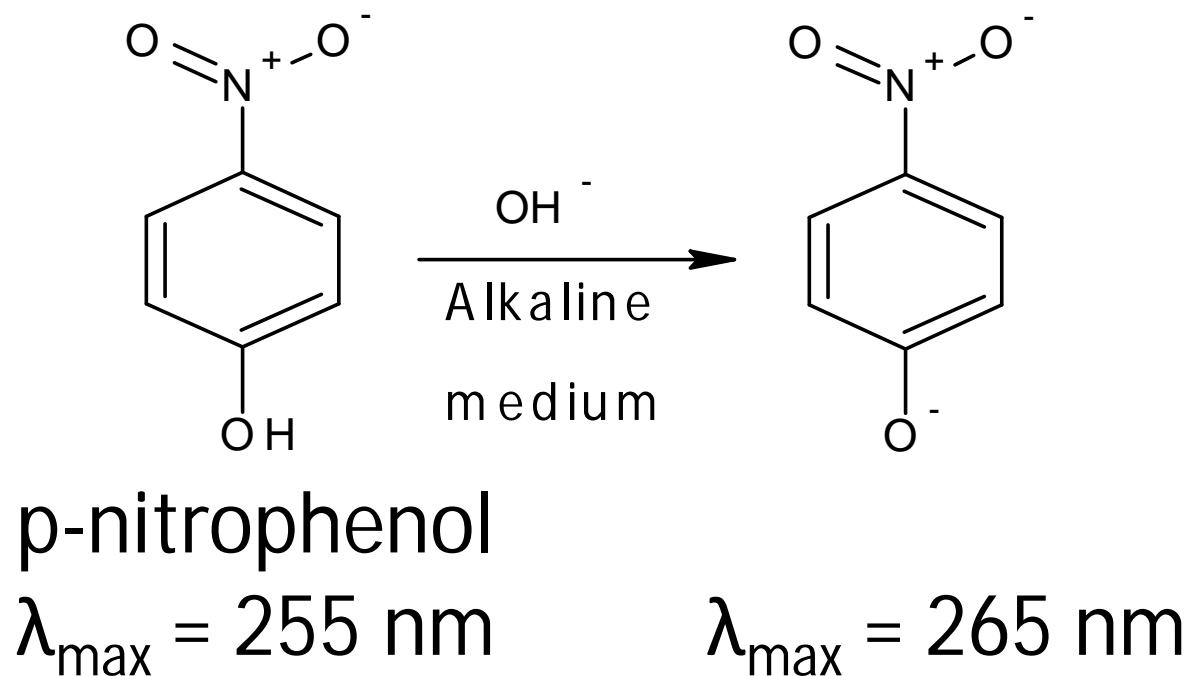
• Bathochromic Shift (Red Shift)

- When absorption maxima (λ_{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.

1

• 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.



2

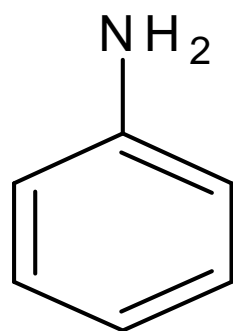
• Hypsochromic Shift (Blue Shift)

- When absorption maxima (λ_{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.

2

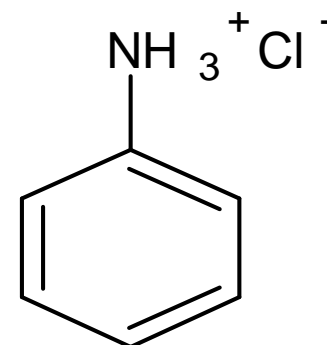
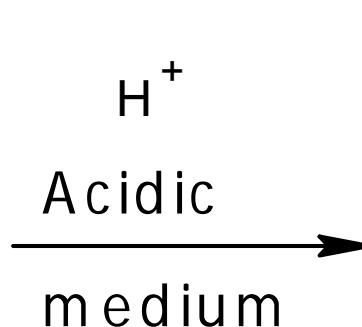
• Hypsochromic Shift (Blue Shift)

- Aniline shows blue shift in acidic medium, it loses conjugation.



Aniline

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

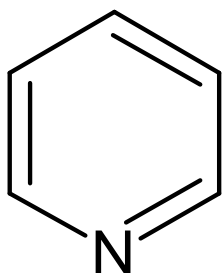


$\lambda_{\text{max}} = 265 \text{ nm}$

3

• Hyperchromic Effect

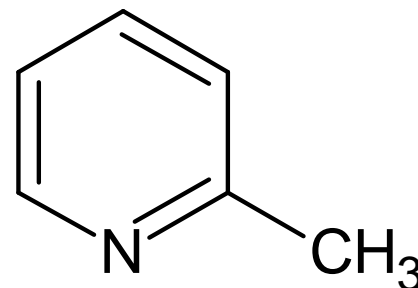
- When absorption intensity (ϵ) of a compound is increased, it is known as hyperchromic shift.
- If auxochrome introduces to the compound, the intensity of absorption increases.



Pyridine

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

$$\epsilon = 2750$$



2-methyl pyridine

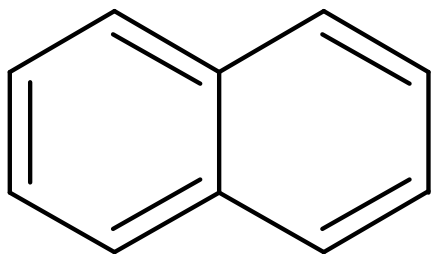
$$\lambda_{\max} = 260 \text{ nm}$$

$$\epsilon = 3560$$

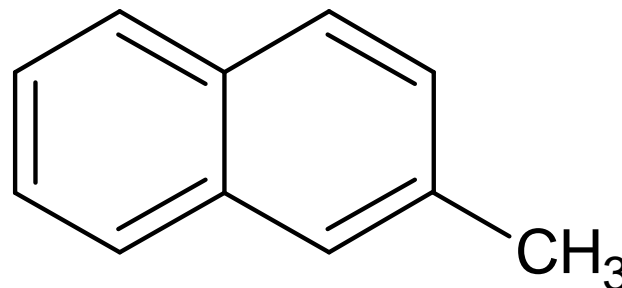
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• Hypochromic Effect

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

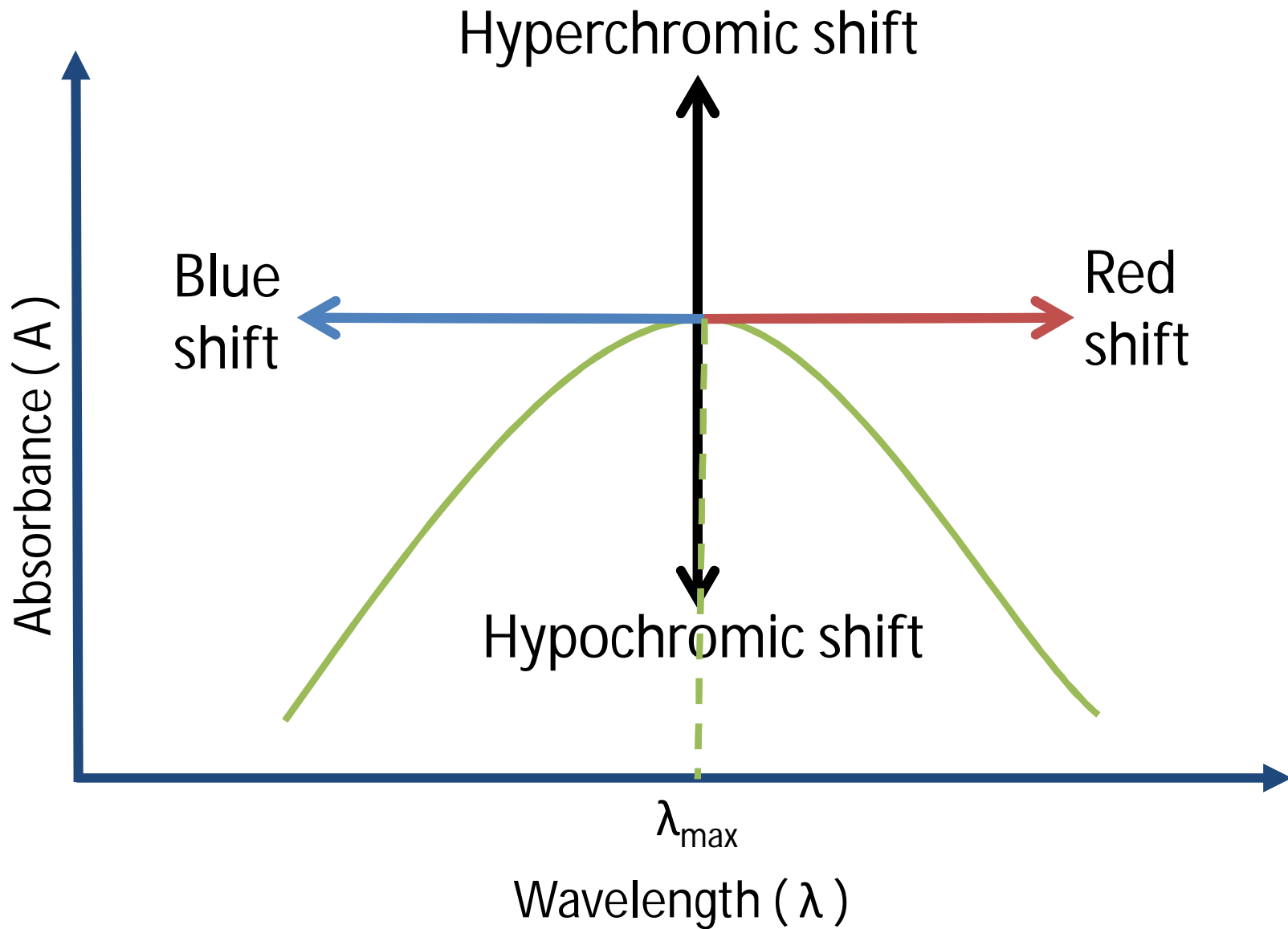


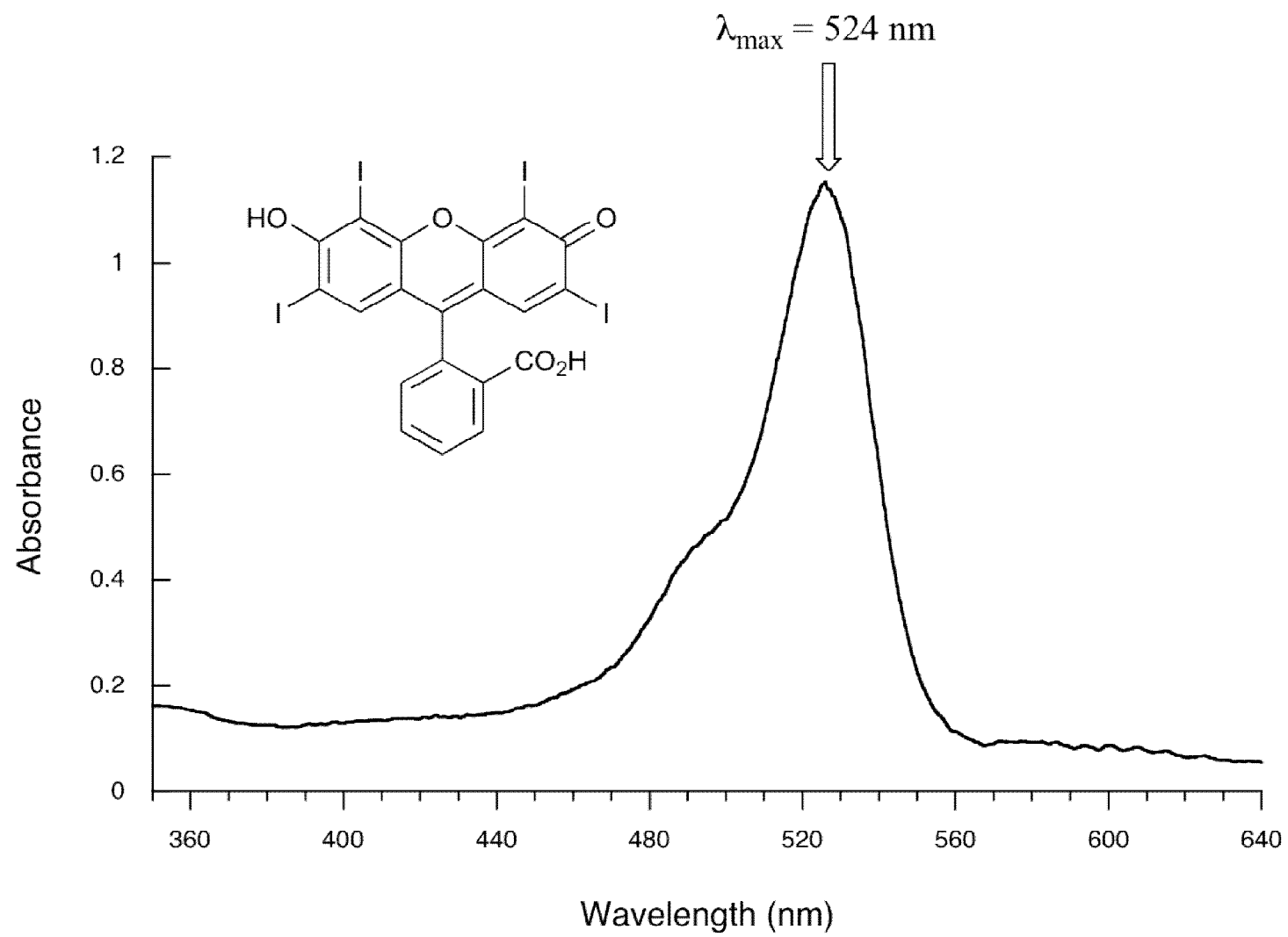
Naphthalene
 $\epsilon = 19000$



2-methyl naphthalene
 $\epsilon = 10250$

Shifts and Effects





Applications of UV-Visible Spectroscopy

- ☐ Quantitative analysis
- ☐ Detection of Impurities
- ☐ Detection of Isomers: Cis or trans
- ☐ Detection of molecular weight
- ☐ Structure determination
- ☐ To study the kinetics of reaction