UV / Visible Spectroscopy

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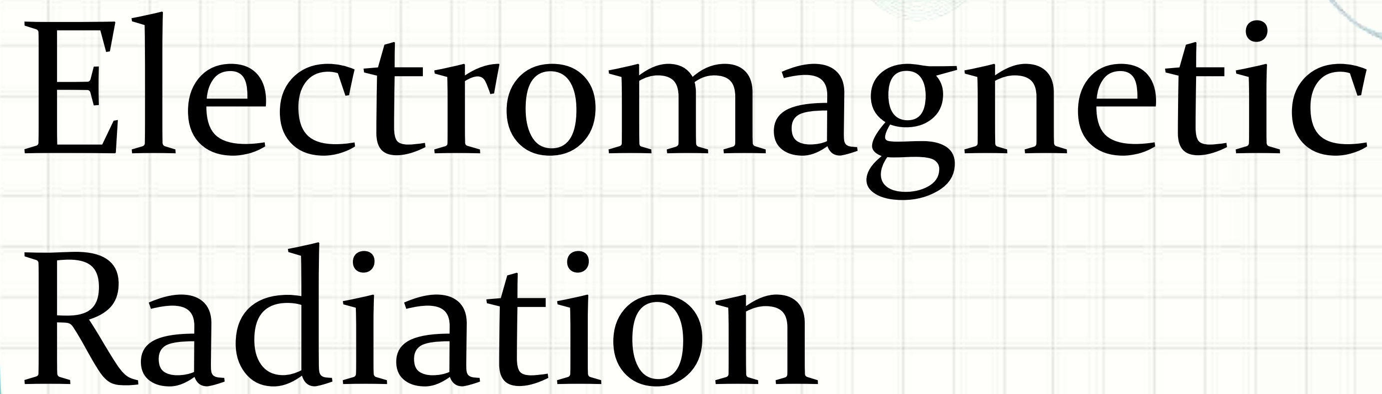
Spectroscopy

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

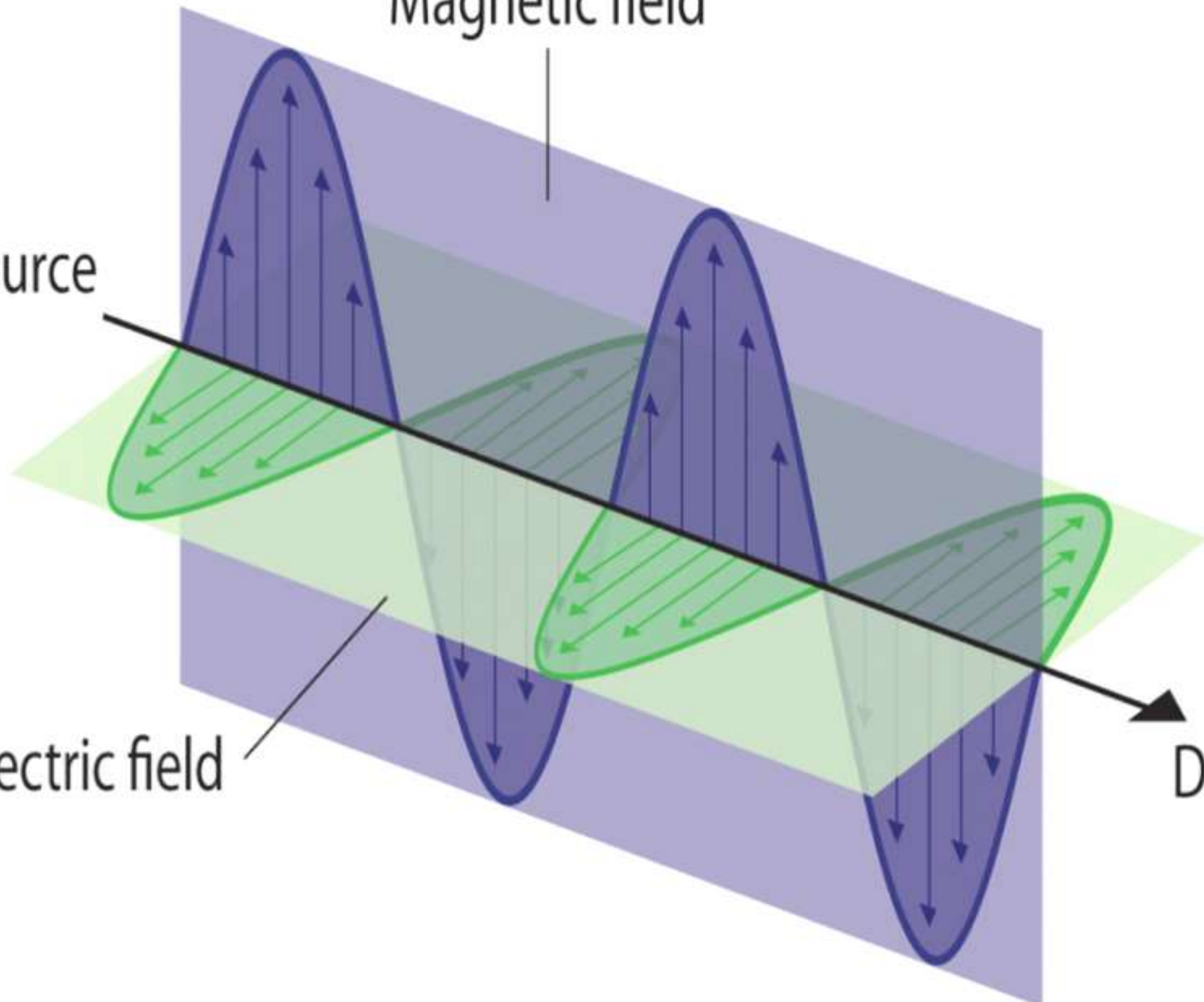
OR

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


Electromagnetic
Radiation

Electromagnetic Radiation

- Electromagnetic radiation consist of discrete packets 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.


Magnetic field
Source
Electric field
Direction

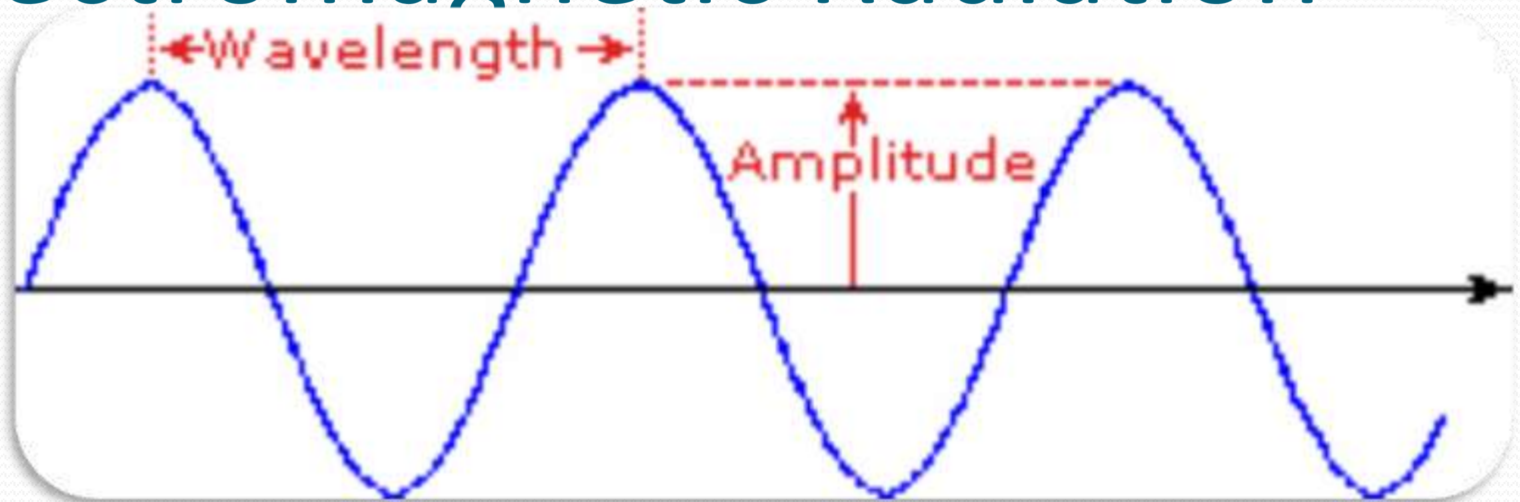
Electromagnetic Radiation

- 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 Hz = 1 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



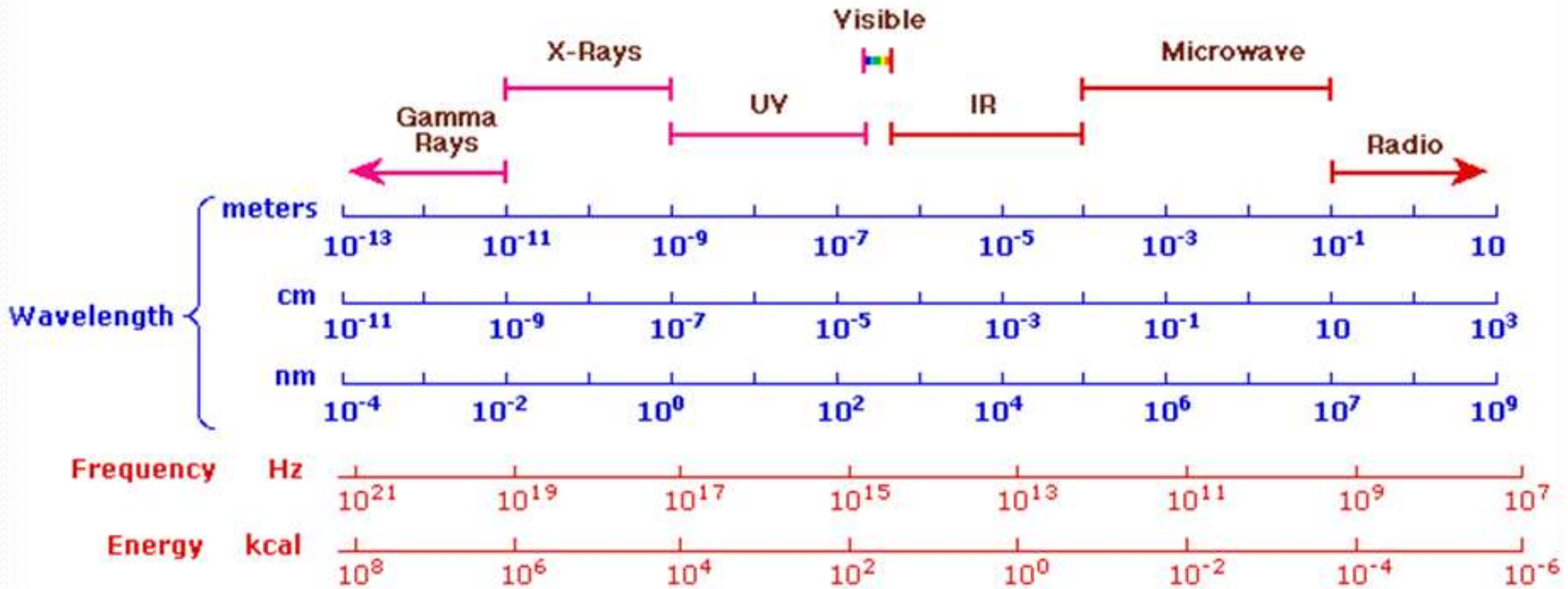
- The relationship between wavelength & frequency can be written as:

$$c = \nu \lambda$$

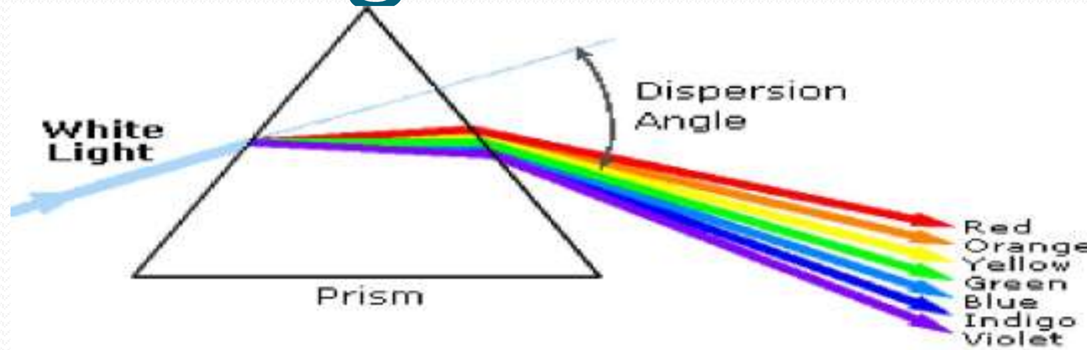
- As photon is subjected to energy, so

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

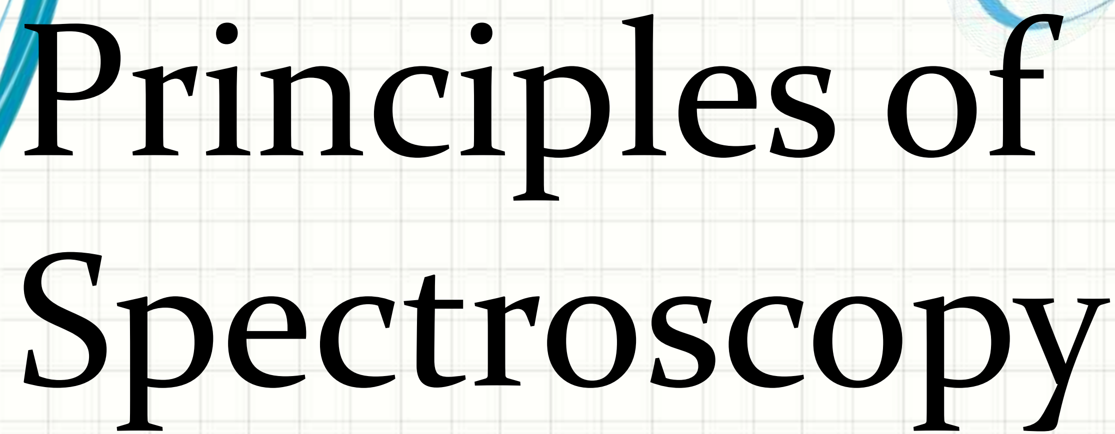
Electromagnetic Radiation



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.

Principles of Spectroscopy

1. Absorption Spectroscopy:

- An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.

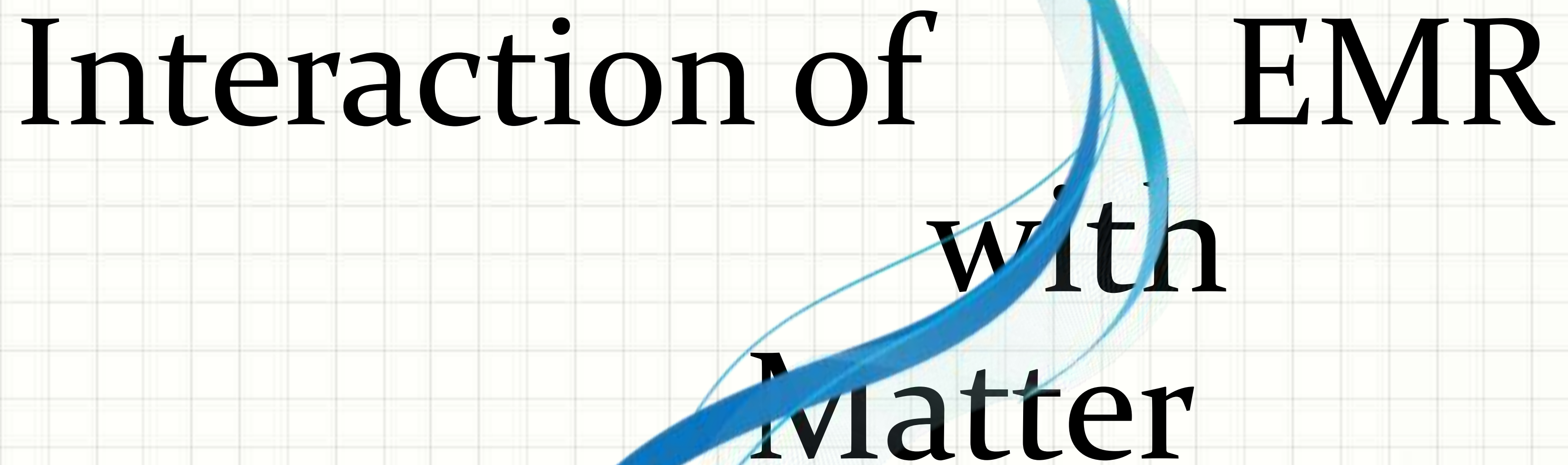
- e.g. UV (185 - 400 nm) / Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 μm)

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

Interaction of EMR with matter

1.Electronic Energy Levels:

- At room temperature the molecules are in the lowest energy levels E_o.

- 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:

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

ΔE = 35 to 71 kcal/mole

Interaction of EMR with matter

2. Vibrational Energy Levels:

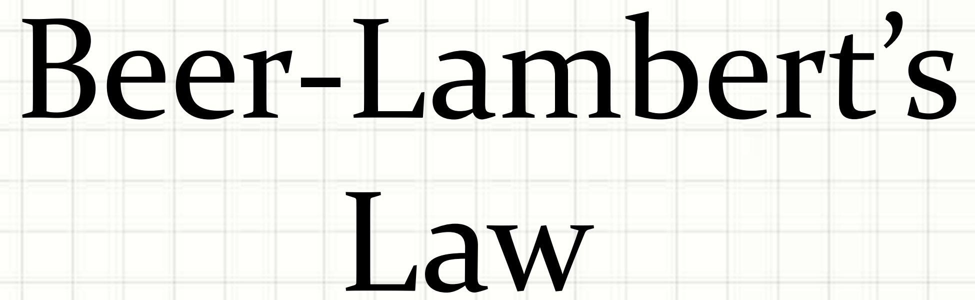
- These are less energy level than electronic energy levels.
- The spacing between energy levels are relatively small i.e. 0.01 to 10 kcal/mole.

e.g. when IR radiation is absorbed, molecules are excited from one vibrational level to another or it vibrates with higher amplitude.

3. Rotational Energy Levels:

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

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


Beer-Lambert's
Law

Beer Lamberts Law:

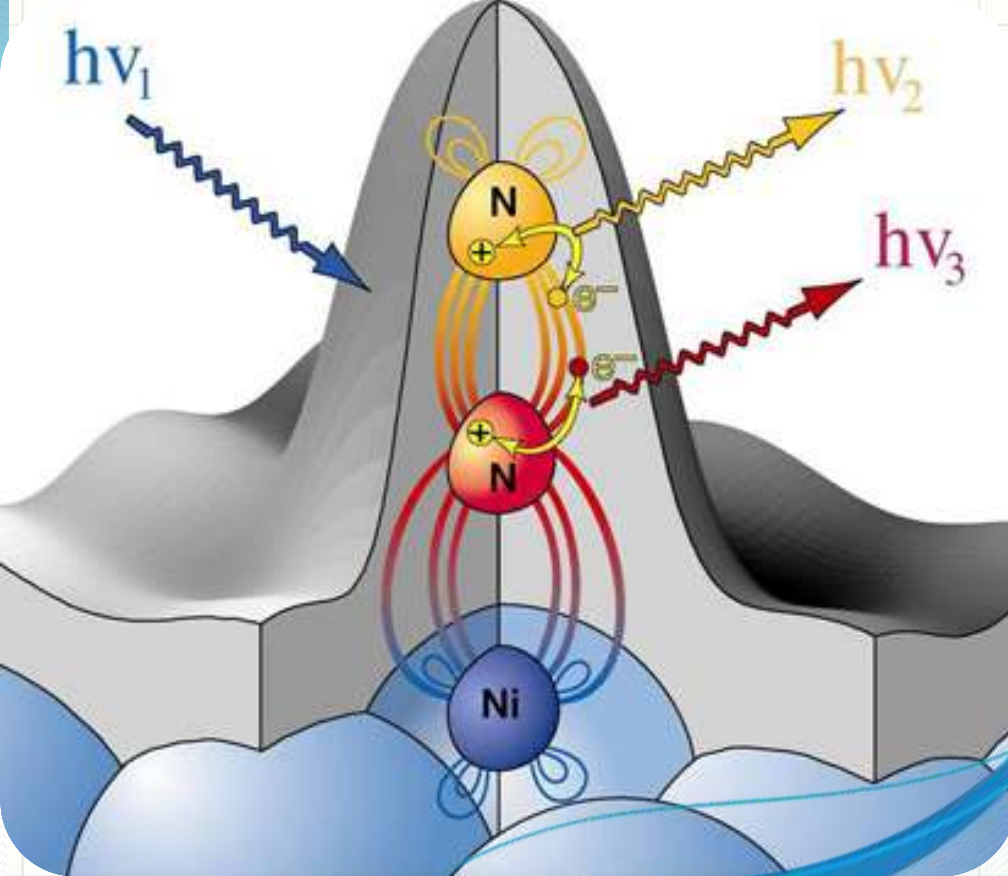
$$A = \varepsilon\, b\, c$$

A=absorbance

ε =molar absorbtivity with units of L /mol.cm

b=path length of the sample (cuvette)

c =Concentration of the compound in solution, expressed in mol /L



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

1

• σ → σ* 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 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 $\pi \rightarrow \pi^*$ 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 $n \rightarrow \sigma^*$ 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).

4

• n → π* 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

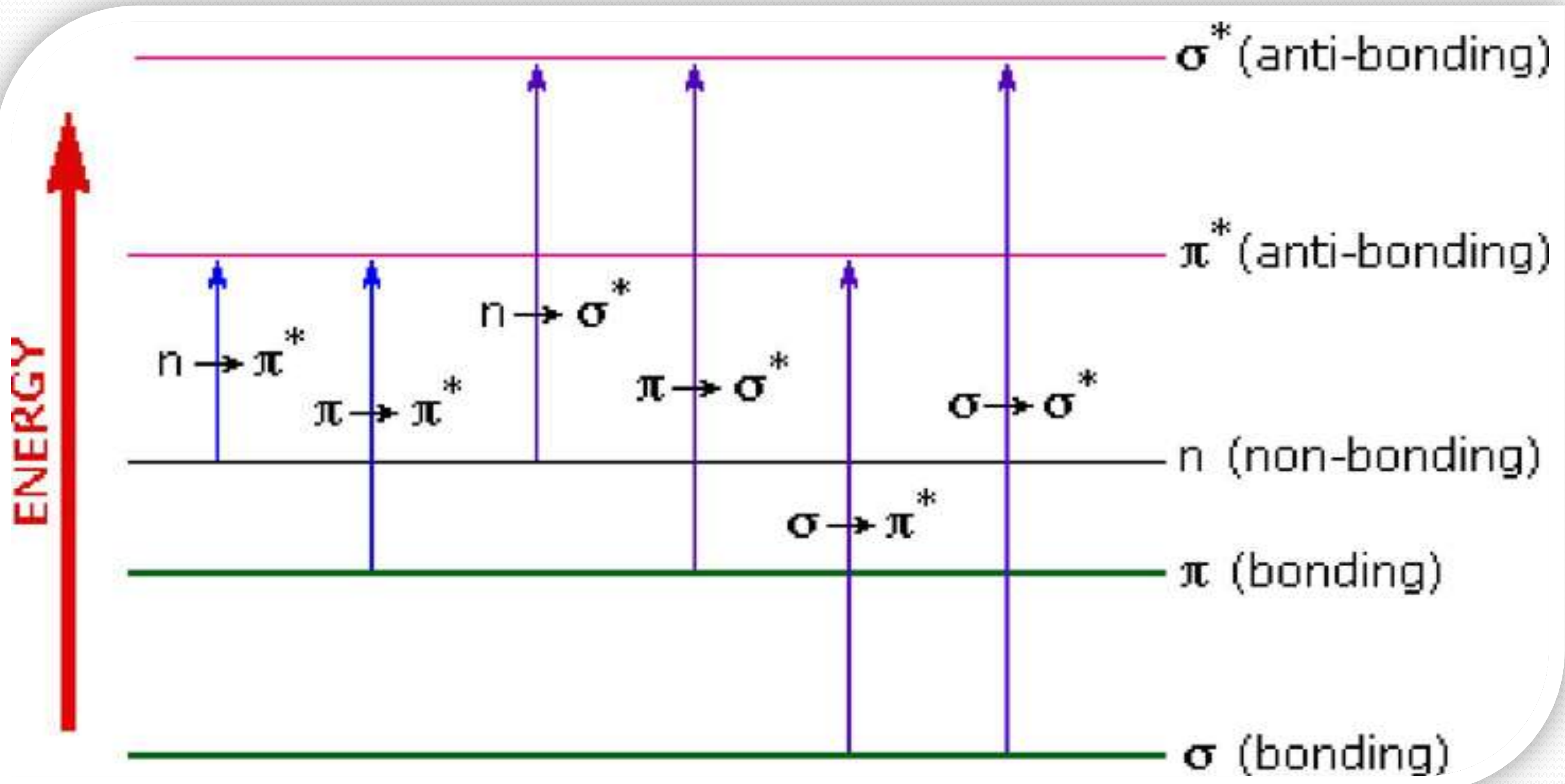
& • $\pi \rightarrow \sigma^*$ transition 6

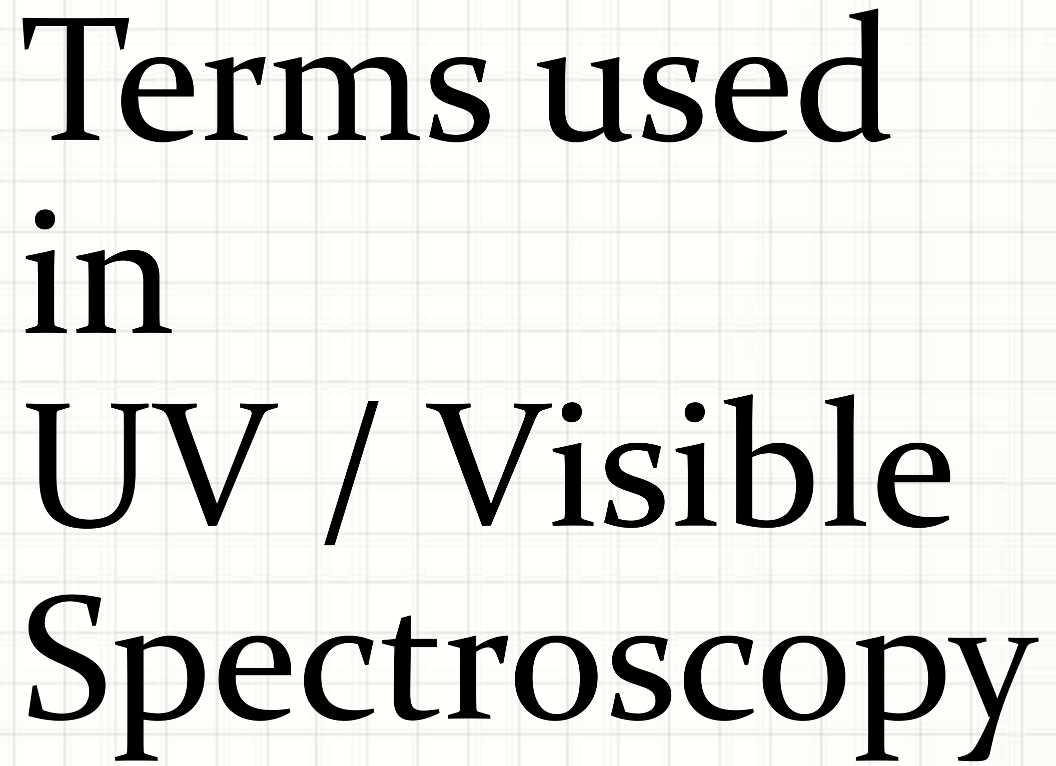
- These electronic transitions are forbidden transitions & are only theoretically possible.

- Thus, $n \rightarrow \pi^*$ & $\pi \rightarrow \pi^*$ 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.

The possible electronic transitions can graphically shown as:




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. NO_2, N=O, C=O, C=N, C≡N, C=C, C=S, etc

Auxochrome

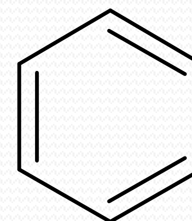
The functional groups attached to a chromophore which modifies the ability of the chromophore to absorb light , altering the wavelength or intensity of absorption.

OR

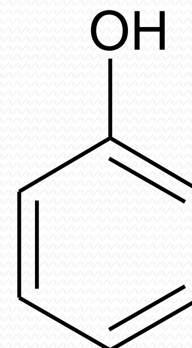
The functional group with non-bonding electrons that does not absorb radiation in near UV region but when attached to a chromophore alters the wavelength & intensity of absorption.

Auxochrome

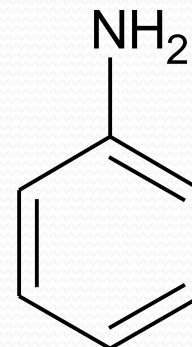
e.g. Benzene λ_{max} = 255 nm



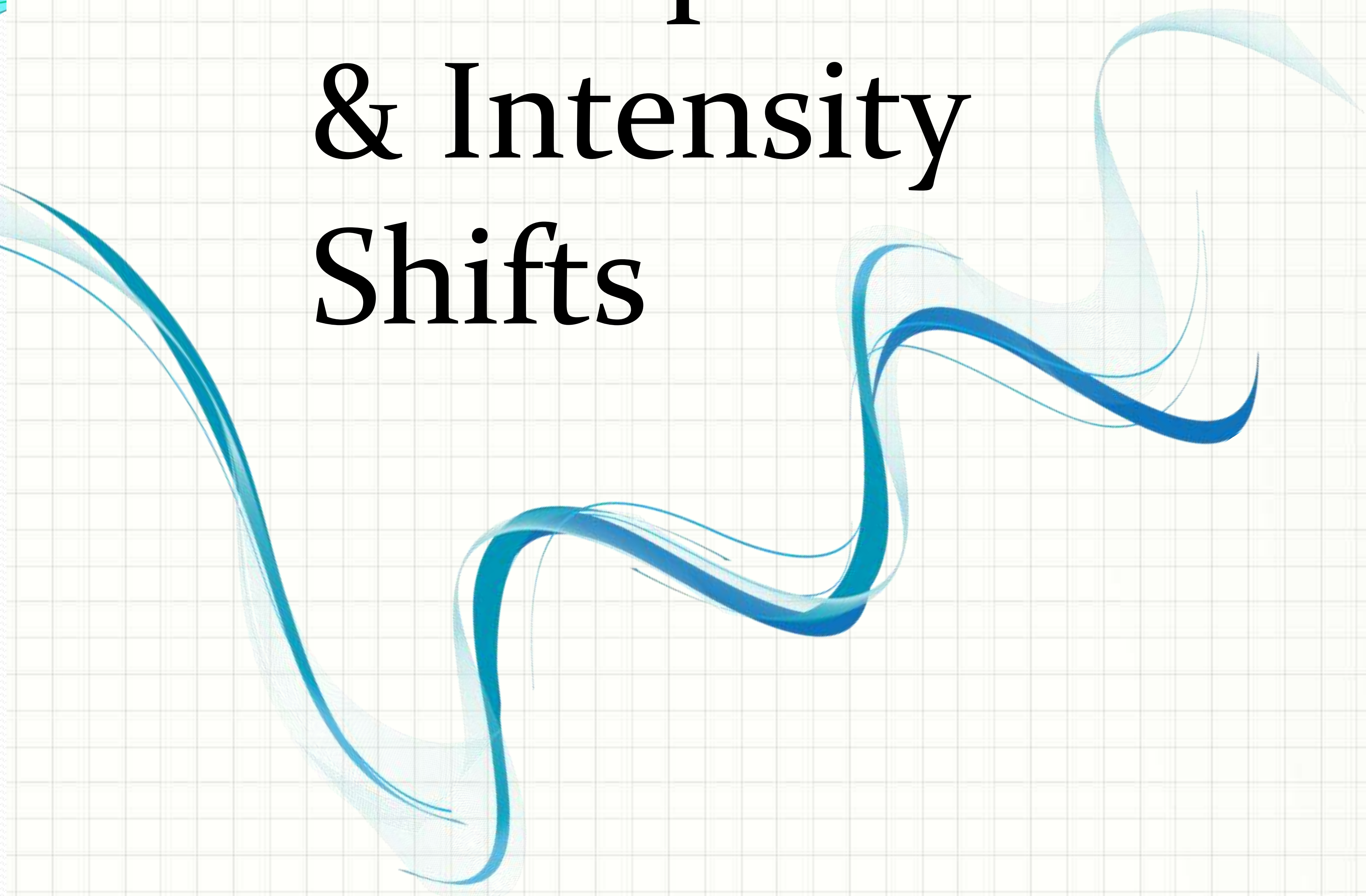
Phenol λ_{max} = 270 nm



Aniline λ_{max} = 280 nm



Absorption & Intensity Shifts



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

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.

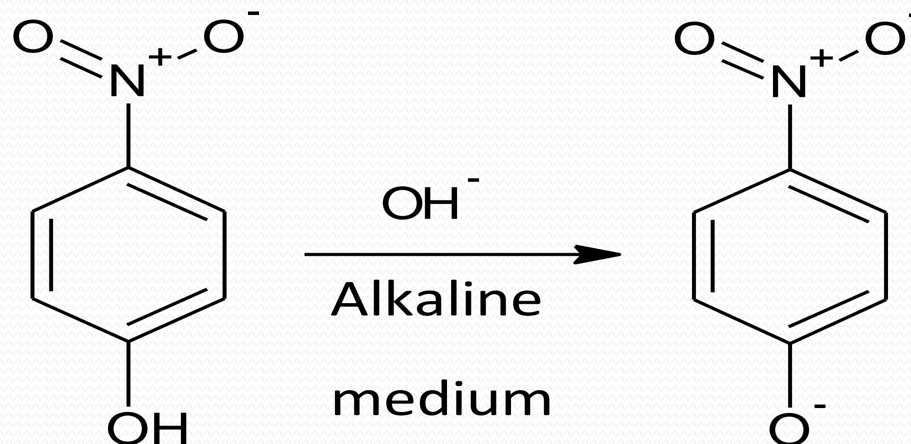
- The effect is due to presence of an auxochrome or by the change of solvent.

- e.g. An auxochrome group like –OH, $-OCH_3$ 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.



p-nitrophenol

λ_{max} = 255 nm λ_{max} = 265 nm

2

• Hypsochromic Shift (Blue Shift)

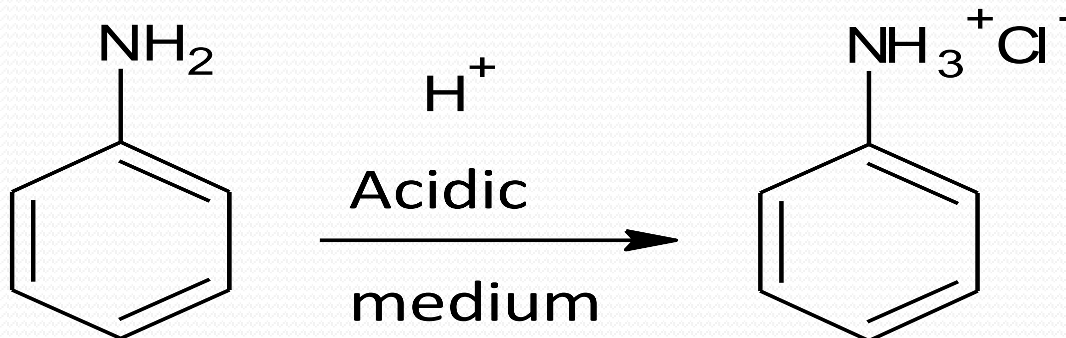
- When absorption maxima (λ_{max}) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.

- 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

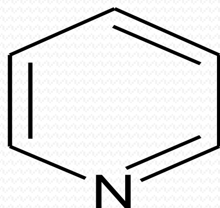
λ_{max} = 280 nm

λ_{max} = 265 nm

3

• Hyperchromic Effect

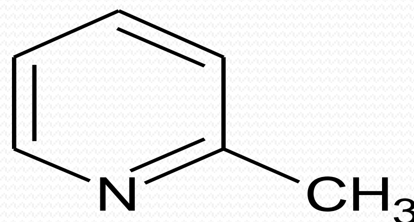
- When absorption intensity (ε) of a compound is increased, it is known as hyperchromic shift.
- If auxochrome introduces to the compound, the intensity of absorption increases.



Pyridine

λ_{max} = 257 nm

ε = 2750



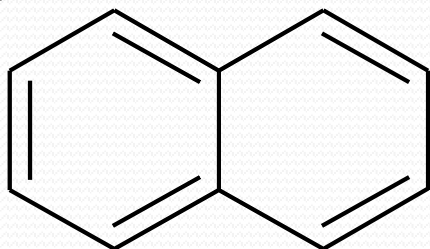
2methylpyridine

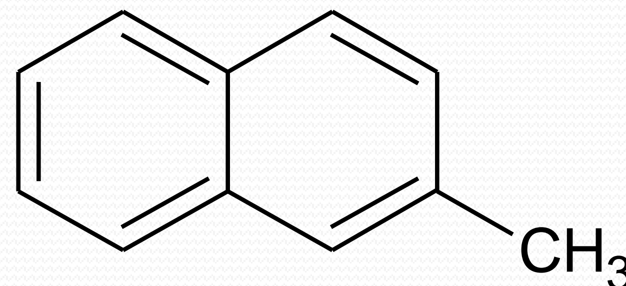
λ_{max} = 260 nm

ε = 3560

4 • Hypochromic Effect

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





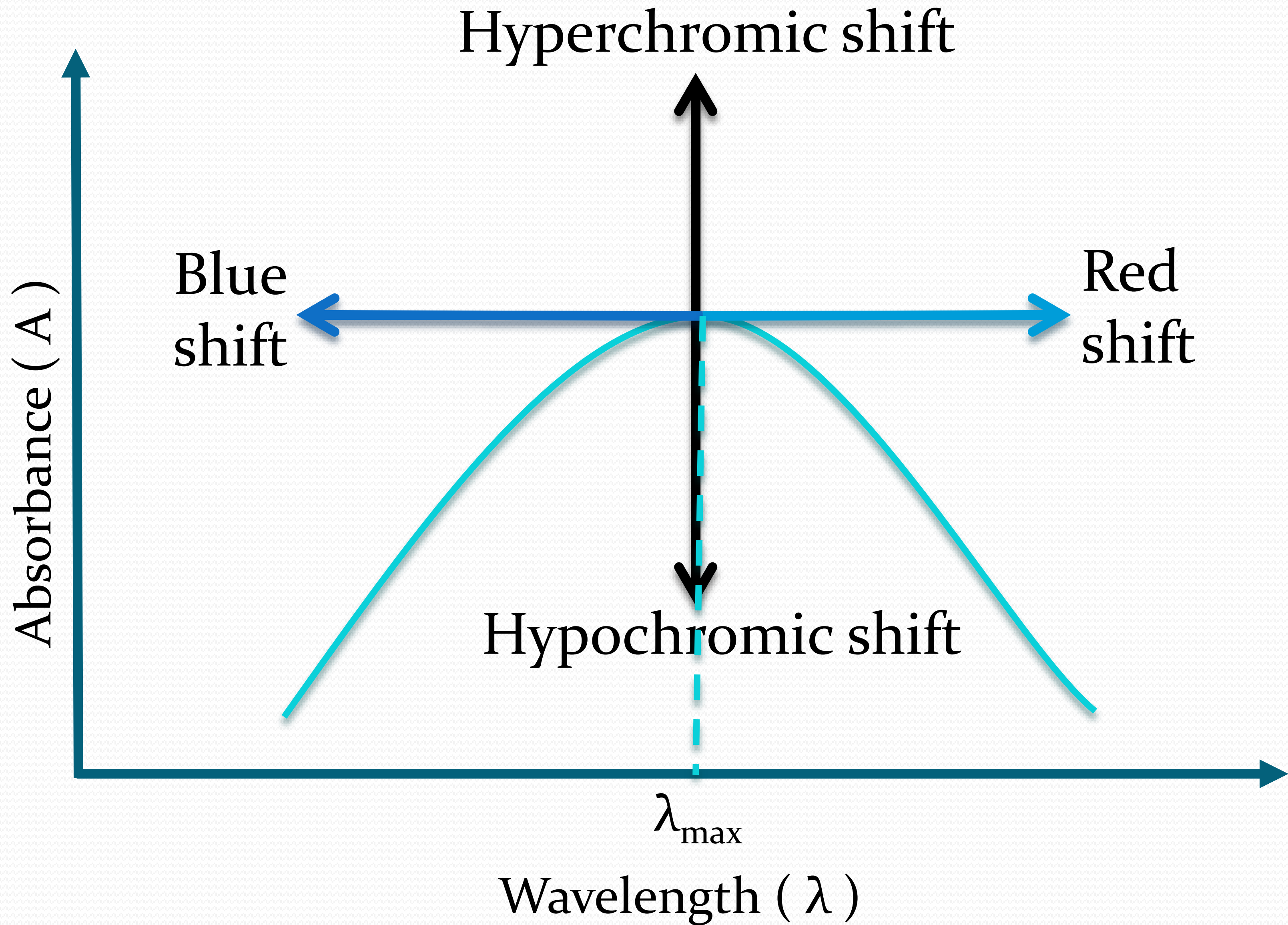
Naphthalene

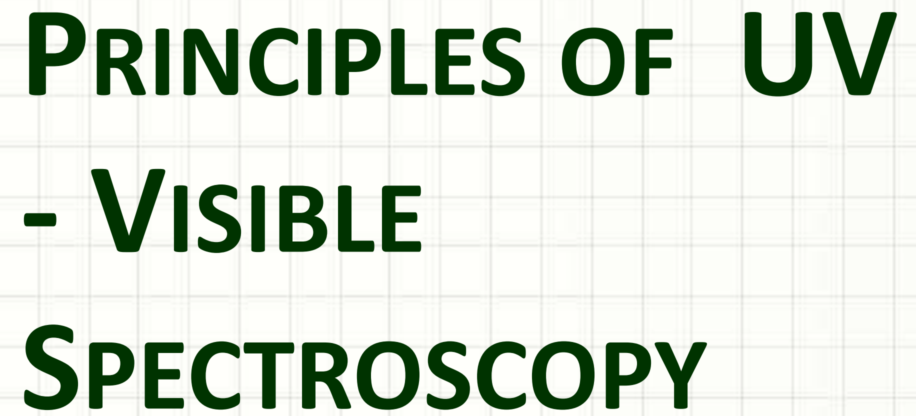
2-methyl naphthalene

$\varepsilon = 19000$

$\varepsilon = 10250$

Shifts and Effects




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

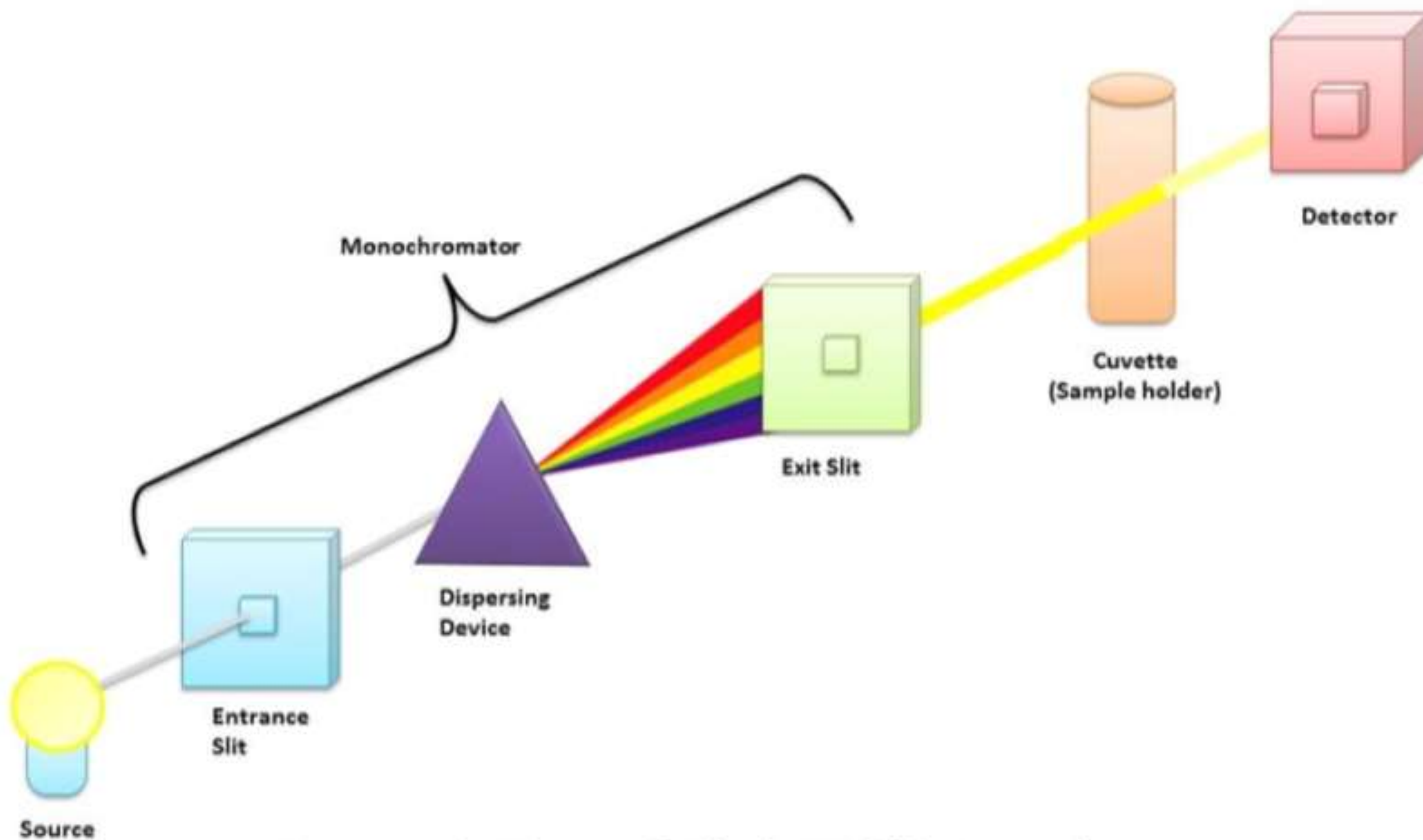
Far UV Region: below 200 nm

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

Instrumentation

Components of UV-Visible spectrophotometer

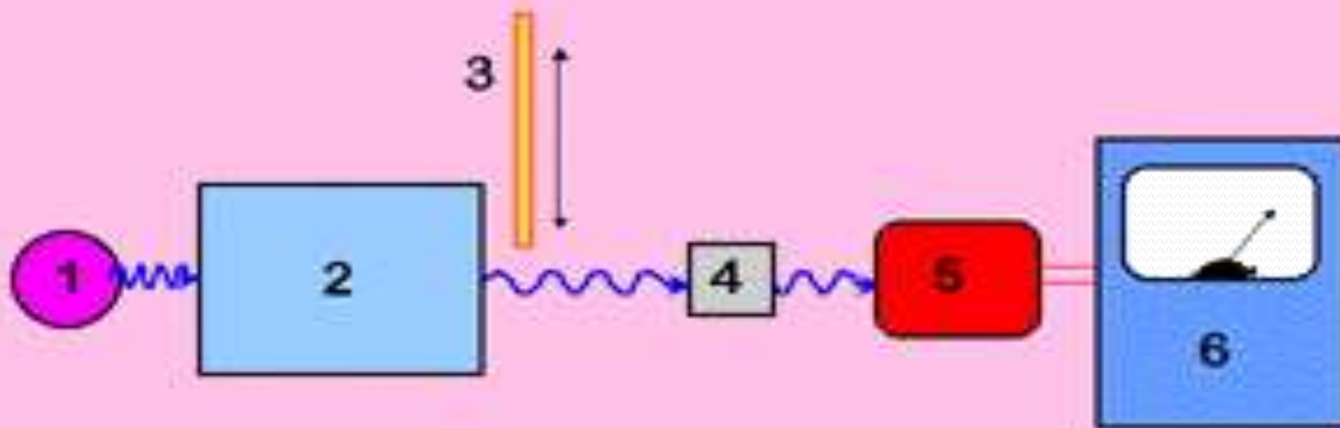
- **Source**
- **Filters & Monochromator**
- **Sample compartment**
- **Detector**
- **Recorder**


Monochromator
Detector
Cuvette
(Sample holder)
Exit Slit
Dispersing
Device
Entrance
Slit
Source
Representative Diagram of a Simple UV-Visible Spectrophotometer

Five Basic Optical Instrument Components

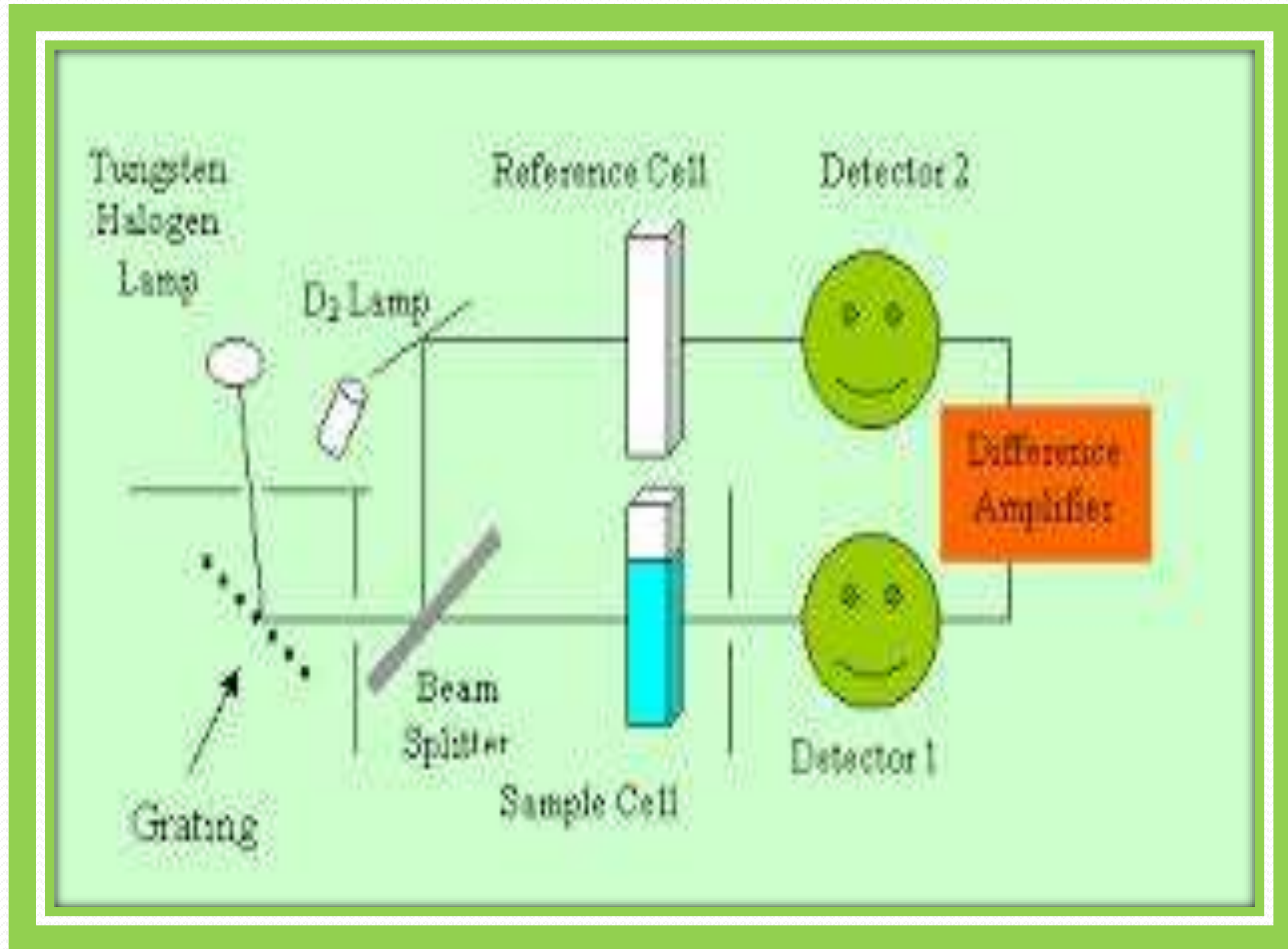
- 1) Source – A stable source of radiant energy at the desired wavelength (or □ range).
- 2) Wavelength Selector – A device that isolates a restricted region of the EM spectrum used for measurement (monochromators, prisms & filters).
- 3) Sample Container – A transparent container used to hold the sample (cells, cuvettes, etc).
- 4) Detector/Photoelectric Transducer – Converts the radiant energy into a useable signal (usually electrical).
- 5) Signal Processor & Readout – Amplifies or attenuates the transduced signal and sends it to a readout device as a meter, digital readout, chart recorder, computer, etc.

Single beam spectrophotometer



1 - light source
2 - wavelength selector
3 - shutter
4 - sample cell
5 - detector
6 - readout

Double Beam Spectrophotometer



LIGHT SOURCES

Various UV radiation sources are as follows

a. Deuterium lamp
b. Hydrogen lamp
c. Tungsten lamp
d. Xenon discharge lamp
e. Mercury arc lamp

Various Visible radiation sources are as follow

a. Tungsten lamp
b. Mercury vapour lamp
c. Carbonone lamp

Wavelength Selectors

- Wavelength selectors output a limited, narrow, continuous group of wavelengths called a *band*.

Two types of wavelength selectors:

A) **Filters**

B) **Monochromators**

A)Filters –

Two types of filters:

a) Interference Filters

b) Absorption Filters

Cont..

B. Monochromators

- Wavelength selector that can continuously scan a broad range of wavelengths.
- Used in most scanning spectrometers including UV, visible, and IR instruments.

PRISM TYPE → **Refractive type**, **Reflective type**

GRATING TYPE → **Diffraction type**, **Transmission Type**

SAMPLE COMPARTMENT

- Spectroscopy requires all materials in the beam path other than the analyte should be as transparent to the radiation as possible.
- The geometries of all components in the system should be such as to maximize the signal and minimize the scattered light.
- The material from which a sample cuvette is fabricated controls the optical window that can be used.
- Some typical materials are:
- Optical Glass - 335 - 2500 nm
- Special Optical Glass – 320 - 2500 nm
- Quartz (Infrared) – 220 - 3800 nm
- Quartz (Far-UV) – 170 - 2700 nm

Detectors

- After the light has passed through the sample, we want to be able to detect and measure the resulting light.
- These types of detectors come in the form of transducers that are able to take energy from light and convert it into an electrical signal that can be recorded, and if necessary, amplified.
- **Three common types of detectors are used**
  - ✓ Barrier layer cells
  - ✓ Photo emissive cell detector
  - ✓ Photomultiplier

SUMMARY

- Types of source, sample holder and detector for various EM region

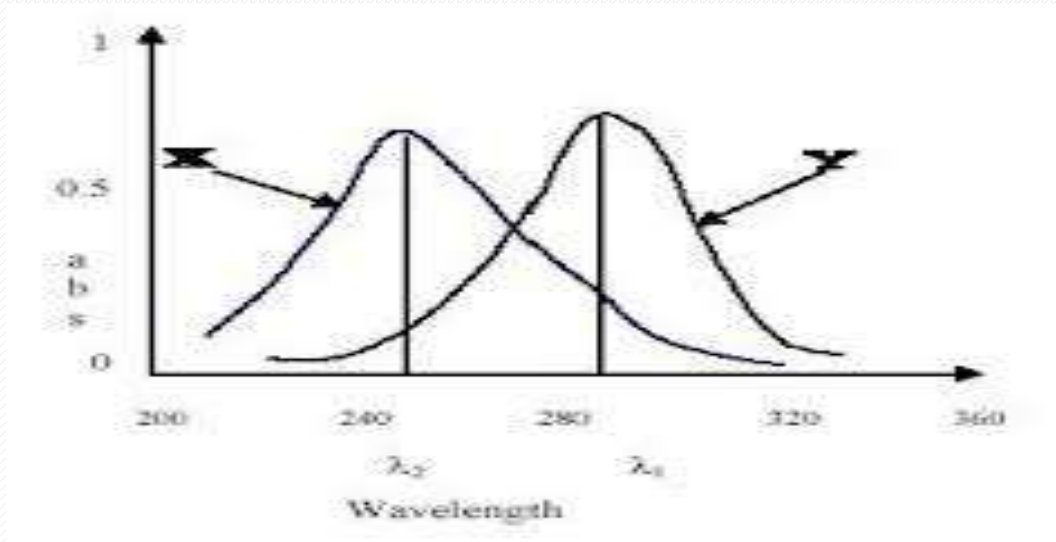
REGION	SOURCE	SAMPLE HOLDER	DETECTOR
Ultraviolet	Deuterium lamp	Quartz/Fused silica	Phototube, PM tube, diode array
Visible	Tungsten lamp	Glass/Quartz	Phototube, PM tube, diode array

DIFFERENT UV-VISIBLE SPECTROPHOTOMETRIC METHODS FOR MULTICOMPONENT ANALYSIS

- (a) Simultaneous equation method
- (b) Absorbance ratio method
- (c) Geometric correction method
- (d) Orthogonal polynomial method
- (e) Derivative spectrophotometry
- (f)Difference spectrophotometry

(a) Simultaneous equation method:

- If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ-max of the other (λ_1 and λ_2), it may be possible to determine both the drugs by the simultaneous equations method.



The information required is

- The absorptivities of X at λ_1 and λ_2, aX_1 and aX_2.
- The absorptivities of Y at λ_1 and λ_2, aY_1 and aY_2.
- The absorbances of the diluted sample at λ_1 and λ_2, A_1 and A_2.

Let, C_x and C_y be the concentration of X and Y respectively in the sample.

- The absorbance of the mixture is the sum of the individual absorbances of X and Y

At λ_1 $A_1 = aX_1^* C_x + aY_1^* C_y$ ....(1)

At λ_2 $A_2 = aX_2^* C_x + aY_2^* C_y$ ....(2)

Multiply the equation (1) with aX_2 and (2) with aX_1

$A_1 aX_2 = aX_1 C_x aX_2 + aY_1 C_y aX_2$ ....(3)

$A_2 aX_1 = aX_2 C_x aX_1 + aY_2 C_y aX_1$ ....(4)

$A_1 aX_2 - A_2 aX_1 = aY_1 C_y aX_2 - aY_2 C_y aX_1$

$A_1 aX_2 - A_2 aX_1 = C_y (aY_1 aX_2 - aY_2 aX_1)$

$C_y = (A_1 aX_2 - A_2 aX_1) / (aY_1 aX_2 - aY_2 aX_1)$ ....(5)

Same way we can derive

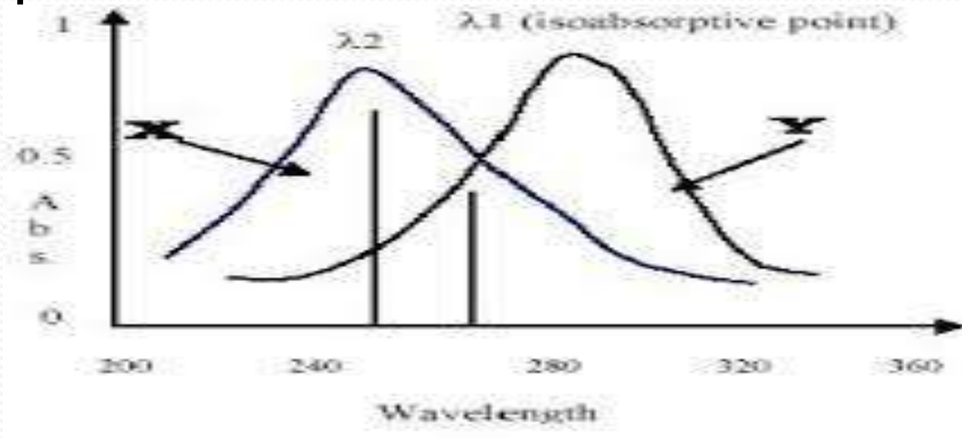
$C_x = (A_2 aY_1 – A_1 aY_2) / (aY_1 aX_2 - aY_2 aX_1)$............ (6)

These equations are known as simultaneous equations and by solving these simultaneous equations we can determine the concentration of X and Y in the sample.

(b) Absorbance ratio method:

The absorbance ratio method is a modification of the simultaneous equations procedure.

In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the λ-max of one of the components (λ2) and other being a wavelength of equal absorptivity of two components (λ1), i.e. an iso-absorptive point.



- At λ_1 $A_1 = aX_1^* C_x + aY_1^* C_y$ .... (1)
- At λ_2 $A_2 = aX_2^* C_x + aY_2^* C_y$................(2)

Now divide (2) with (1)

$$A_2/A_1 = (aX_2^* C_x + aY_2^* C_y)/(aX_1^* C_x + aY_1^* C_y)$$

- Divide each term with $(C_x + C_y)$

$$A_2/A_1 = (aX_2^* C_x + aY_2^* C_y) / (C_x + C_y) / (aX_1^* C_x + aY_1^* C_y) / (C_x + C_y)$$

Put $F_x = C_x / (C_x + C_y)$ and $F_y = C_y / (C_x + C_y)$

$$A_2/A_1 = [aX_2 F_x + aY_2 F_y] / [aX_1 F_x + aY_1 F_y]$$

Where F_x is the fraction of X and F_y is the fraction of Y i.e. $F_y = 1-F_x$

Therefore,

$$A_2/A_1 = [aX_2 F_x + aY_2 (1-F_x)] / [aX_1 F_x + aY_1(1-F_x)]$$

$$= [aX_2 F_x + aY_2 - aY_2F_x] / [aX_1 F_x + aY_1 - aY_1F_x]$$

At iso-absorptive point

$aX_1 = aY_1$ and $C_x = C_y$

There fore $A_2/A_1 = [aX_2 F_x + aY_2 – aY_2 F_x] / aX_1$

$= (aX_2 F_x/ aX_1) + (aY_2/ aX_1) –(aY_2 F_x/ aX_1)$

Let $Q_x = aX_2/aX_1$, $Q_y = aY_2/aY_1$ and absorption ratio $Q_m = A_2/A_1$

$Q_m = F_x Q_x + Q_y - F_x Q_y$

$= F_x (Q_x-Q_y) + Q_y$

$F_x = (Q_m – Q_y) / (Q_x – Q_y)$ ....(3)

From the equations (1) $A_1 = aX_1 (C_x + C_y)$

there fore $C_x + C_y = A_1 / aX_1$

There fore $C_x = (A_1/aX_1) – C_y$ ....(4)

From the equation (3)

$C_x / (C_x + C_y) = (Q_m – Q_y) / (Q_x – Q_y)$

There fore $C_x / (A_1 / aX_1) = (Q_m – Q_y) / (Q_x – Q_y)$

There fore $C_x = [(Q_m – Q_y) / (Q_x – Q_y)] X (A_1 / aX_1)$ ....(5)

(c) Geometric correction method:

- A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin.
- The simplest of this procedure is the three point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

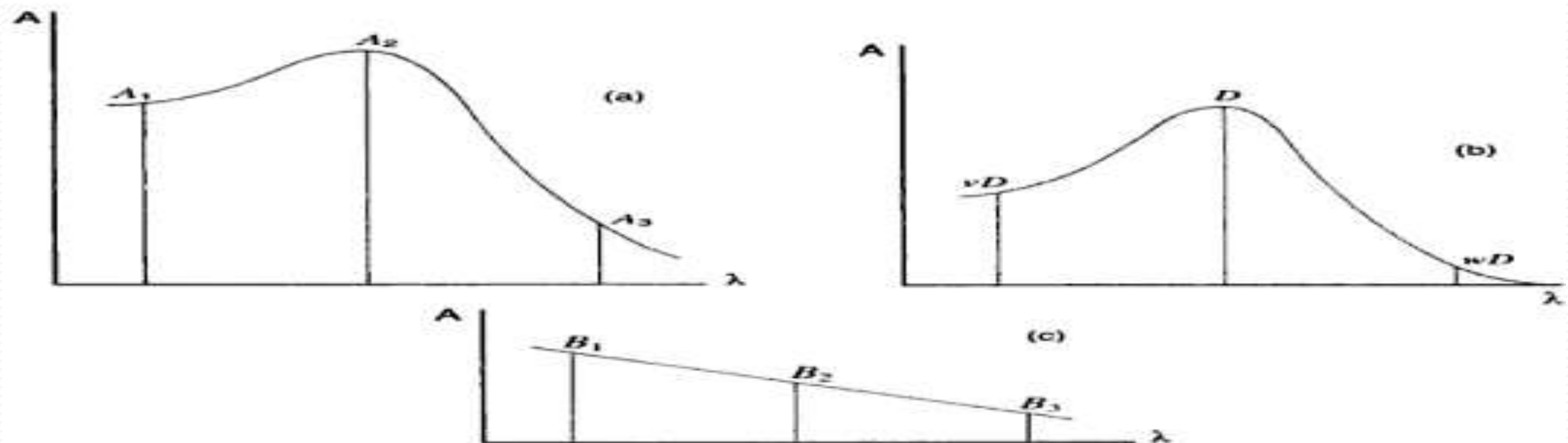


Fig. 7.5(a) The absorption spectrum of a solution of a drug in the presence of linear irrelevant absorption. (b) The individual spectrum of the drug. (c) The individual spectrum of the linear irrelevant absorption

If the wavelengths λ_1, λ_2 and λ_3 are selected to that the background absorbances B_1 , B_2 and B_3 are linear, then the corrected absorbance D of the drug may be calculated from the three absorbances A_1 , A_2 and A_3 of the sample solution at λ_1, λ_2 and λ_3 respectively as follows,

Let v D and w D be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively, i.e. v and w are the absorbance ratios vD/D and wD/D respectively.

$$B_1 = A_1 - vD,\ B_2 = A_2 - D \text{ and } B_3 = A_3 - wD$$

Let y and z be the wavelengths intervals ($\lambda_2 - \lambda_1$) and ($\lambda_3 - \lambda_2$) respectively

$$D = y(A_2 - A_3) + z(A_2 - A_1) / y(1-w) + z(1-v)$$

- This is a general equation which may be applied in any situation where A_1, A_2 and A_3 of the sample, the wavelength intervals y and z and the absorbance ratio v and w are known.

(d) Orthogonal polynomial method:

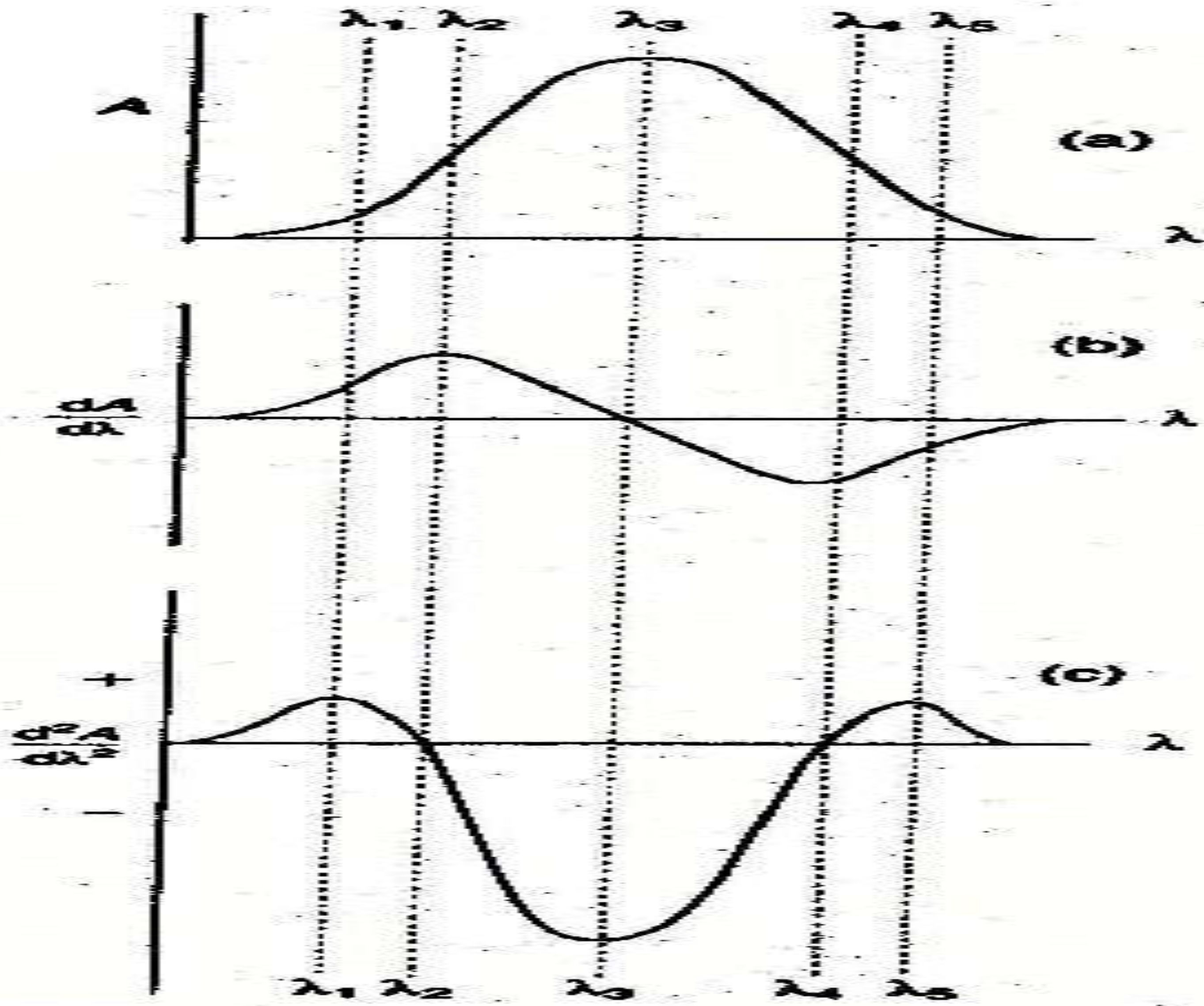
The technique of orthogonal polynomials is another mathematical correction procedure, which involves more complex calculations than the three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows

$$A(\lambda) = p\,P(\lambda) + p_1 P_1(\lambda) + p_2 P_2(\lambda) \ldots\ldots p_n P_n(\lambda)$$

- Where A denotes the absorbance at wavelength λ belonging to a set of n+1 equally spaced wavelengths at which the orthogonal polynomials, $P(\lambda)$, $P_1(\lambda)$, $P_2(\lambda)$ ….. $P_n(\lambda)$ are each defined.

(e)Derivative Spectroscopy:

- For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy.
- Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D o spectrum.



λ1 λ2 λ3 λ4 λ5
A
(a)
λ
(b)
dA/dλ
λ
(c)
+
d²A/dλ²
λ
—
λ1 λ2 λ3 λ4 λ5

- The first derivative D 1 spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ vs. λ. . The maximum positive and maximum negative slope respectively in the D spectrum correspond with a maximum and a minimum respectively in the D 1 spectrum. The λmax in D spectrum is a wavelength of zero slope and gives $dA/d\lambda = 0$ in the D 1 spectrum.

- The second derivative D 2 spectrum is a plot of the curvature of the D spectrum against wavelength or a plot of $d^2A/d\lambda^2$ vs. λ. The maximum negative curvature in the D spectrum gives a minimum in the D 2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima called satellite bands in the D 2 spectrum. The wavelength of maximum slope and zero curvature in the D spectrum correspond with cross-over points in the D 2 spectrum.

(f)Difference Spectroscopy:

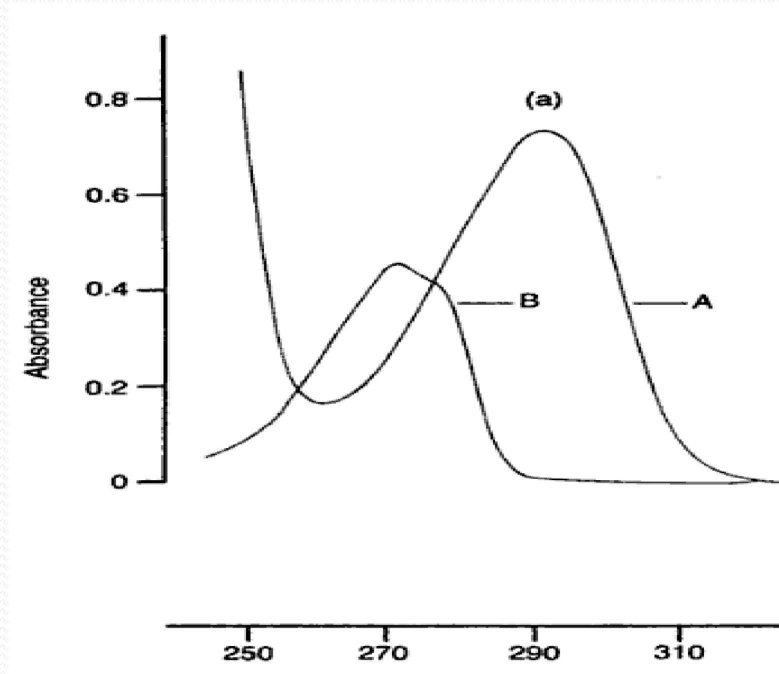
- Difference spectroscopy provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture.

  The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance (Δ A) between two equimolar solutions of the analyte in different forms which exhibit different spectral characteristics.
- The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that:

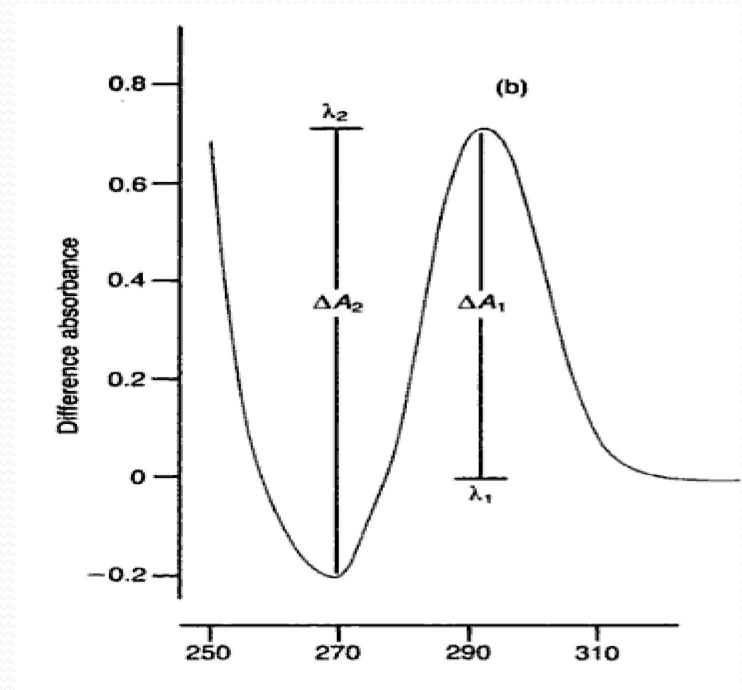
A) Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.

B) The absorbance of the interfering substances is not altered by the reagents.

- The simplest and most commonly employed technique for altering the spectral properties of the analyte properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers



A



B

A)The Spectrum of compound in A(acid) and B(Base)

B) The difference spectrum of B relative to A

Conclusion:

- 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.

Reference Books

- Introduction to Spectroscopy
  - Donald A. Pavia
- Elementary Organic Spectroscopy
  - Y. R. Sharma
- Practical Pharmaceutical Chemistry
  - A.H. Beckett, J.B. Stenlake

RESOURCES

- http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/UV-Vis/spectrum.htm
- http://en.wikipedia.org/wiki/Ultraviolet%E2%80%93visible_spectroscopy
- http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/uvvisab1.htm

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