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.

Theory of UV Visible Spectroscopy…

Why we use UV spectroscopy ?

1. Detection of functional groups.
2. Detection of impurities.
3. Qualitative analysis.
4. Quantitative analysis.
5. Single compound without chromophore.
6. Drugs with chromophoric reagent.

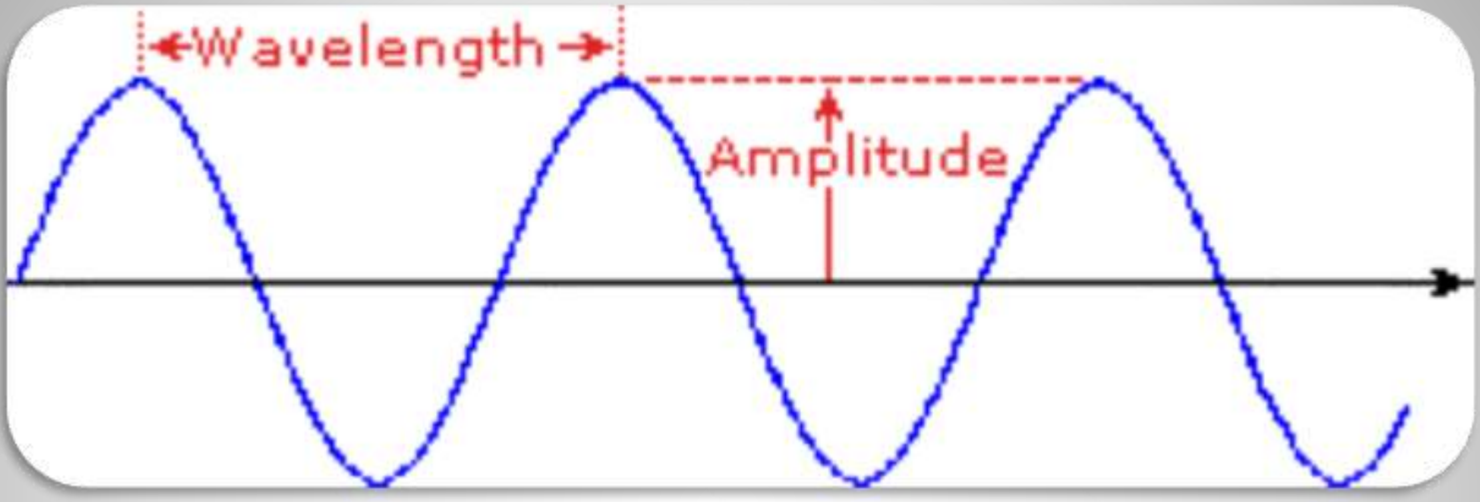
Electromagnetic Radiation

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

- 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.



The relationship between wavelength & frequency can be written as:

$$c = \nu \lambda$$

As photon is subjected to energy, so

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

ABSORBANCE LAWS

BEER'S LAW

" The intensity of a beam of monochromatic light decrease exponentially with the increase in concentration of the absorbing substance" .

Arithmetically;

$$- dI/ dc \; \bar{\alpha} \; I$$

$$I= Io. e^{-kc} \text{ -------- } \quad eq\ (1)$$

LAMBERT'S LAW

"When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light"

mathematically;

$$-dI/\ dt\ \bar{\alpha}\ I$$

$$-In\ .\ I = kt+b \text{ --------------- eq(2)}$$

the combination of eq 1 & 2 we will get

$$A= Kct$$

$$A= \mathcal{E}ct \qquad (K=\mathcal{E})$$

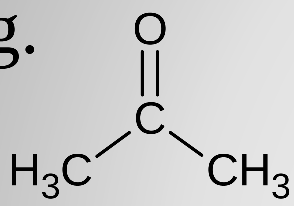
Chromophore…

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

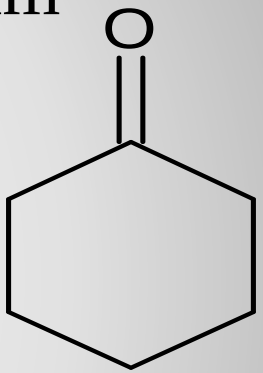
OR

The functional groups containing multiple bonds capable of absorbing radiations above 200 nm due to n → π* & π → π* transitions.

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



Acetone which has λ_{max} = 279 nm



and that cyclohexane has λ_{max} = 291 nm.

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

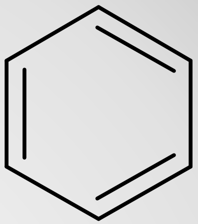
Auxochrome…

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

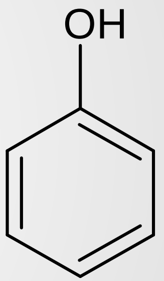
OR

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.

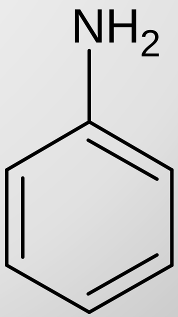
e.g. Benzene λ_{max} = 255 nm



Phenol λ_{max} = 270 nm



Aniline λ_{max} = 280 nm



Principle 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.

- Ultraviolet absorption spectra arise from transition of electron with in a molecule from a lower level to a higher level.

- A molecule absorb ultraviolet radiation of frequency (ϑ), the electron in that molecule undergo transition from lower to higher energy level.

  The energy can be calculated by the equation,

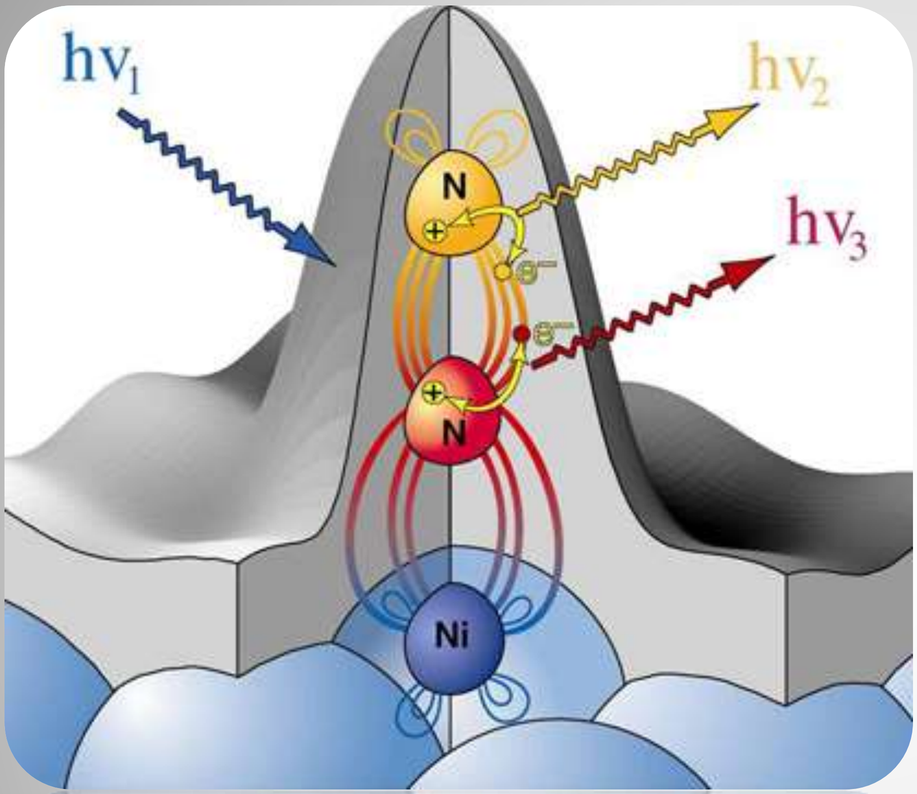
$$E=h\vartheta \text{ erg}$$

$$E_1 - E_o = h\vartheta$$

Etotal = Eelectronic + Evibrational + Erotational

The energies decreases in the following order:

Electronic $\gg$ Vibrational $\gg$ Rotational



Electronic Transitions

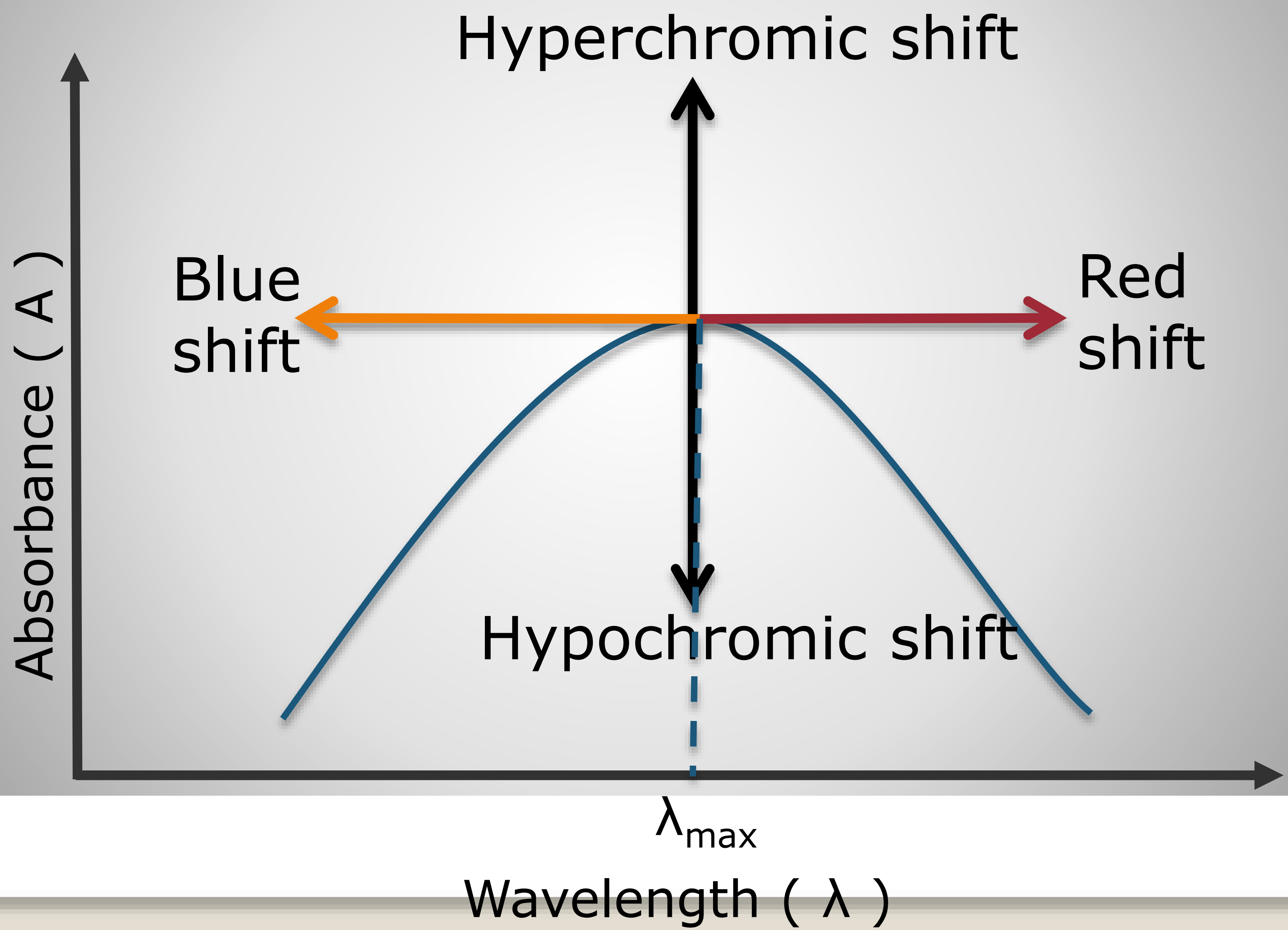
The possible electronic transitions are

- 1- $\sigma \rightarrow \sigma^*$ transition
- 2- $\pi \rightarrow \pi^*$ transition
- 3- $n \rightarrow \sigma^*$ transition
- 4- $n \rightarrow \pi^*$ transition

Absorption & Intensity Shifts

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

Shifts and Effects



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.

Hypsochromic Shift (Blue Shift)

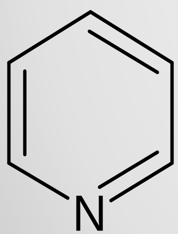
- When absorption maxima (λ_{max}) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.

- The effect is due to presence of an group causes removal of conjugation or by the change of solvent.

Hyperchromic Effect

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

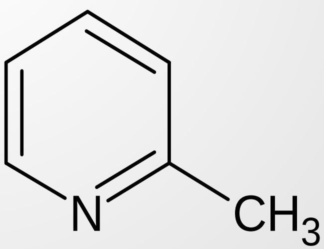
- If auxochrome introduces to the compound, the intensity of absorption increases.



Pyridine

λ_{max} = 257 nm

ε = 2750



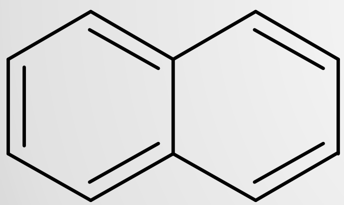
2-methyl pyridine

λ_{max} = 260 nm

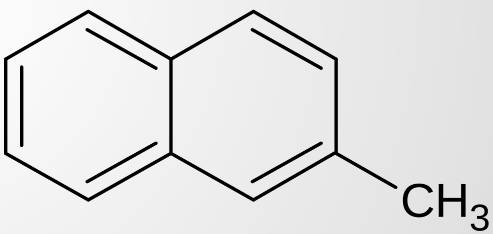
ε = 3560

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

Applications of UV / Visible Spectroscopy

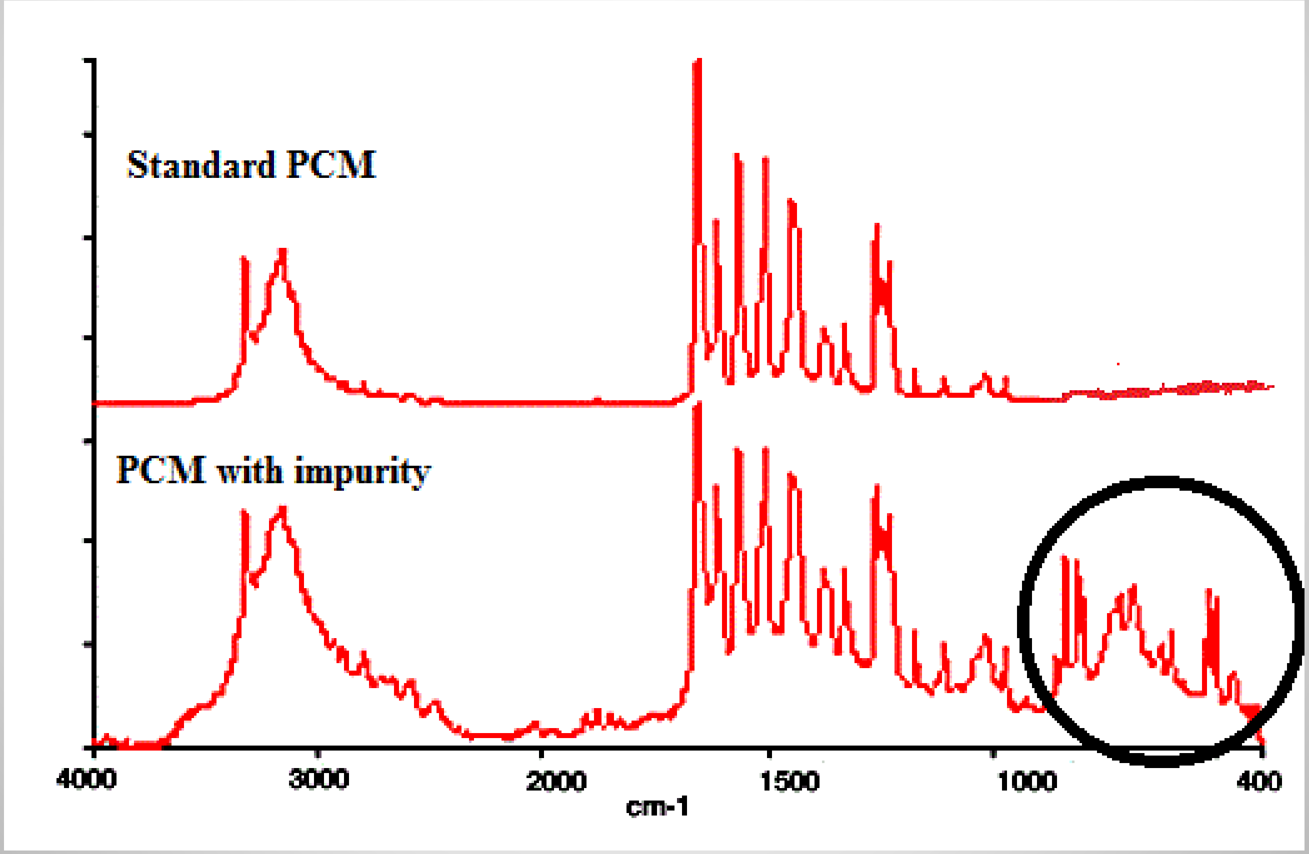
Applications

- 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.

1. Detection of Impurities.

- UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

U.V. Spectra of Paracetamol (PCM)



2. Structure elucidation of organic compounds.

- UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms.
- From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

3. Quantitative analysis

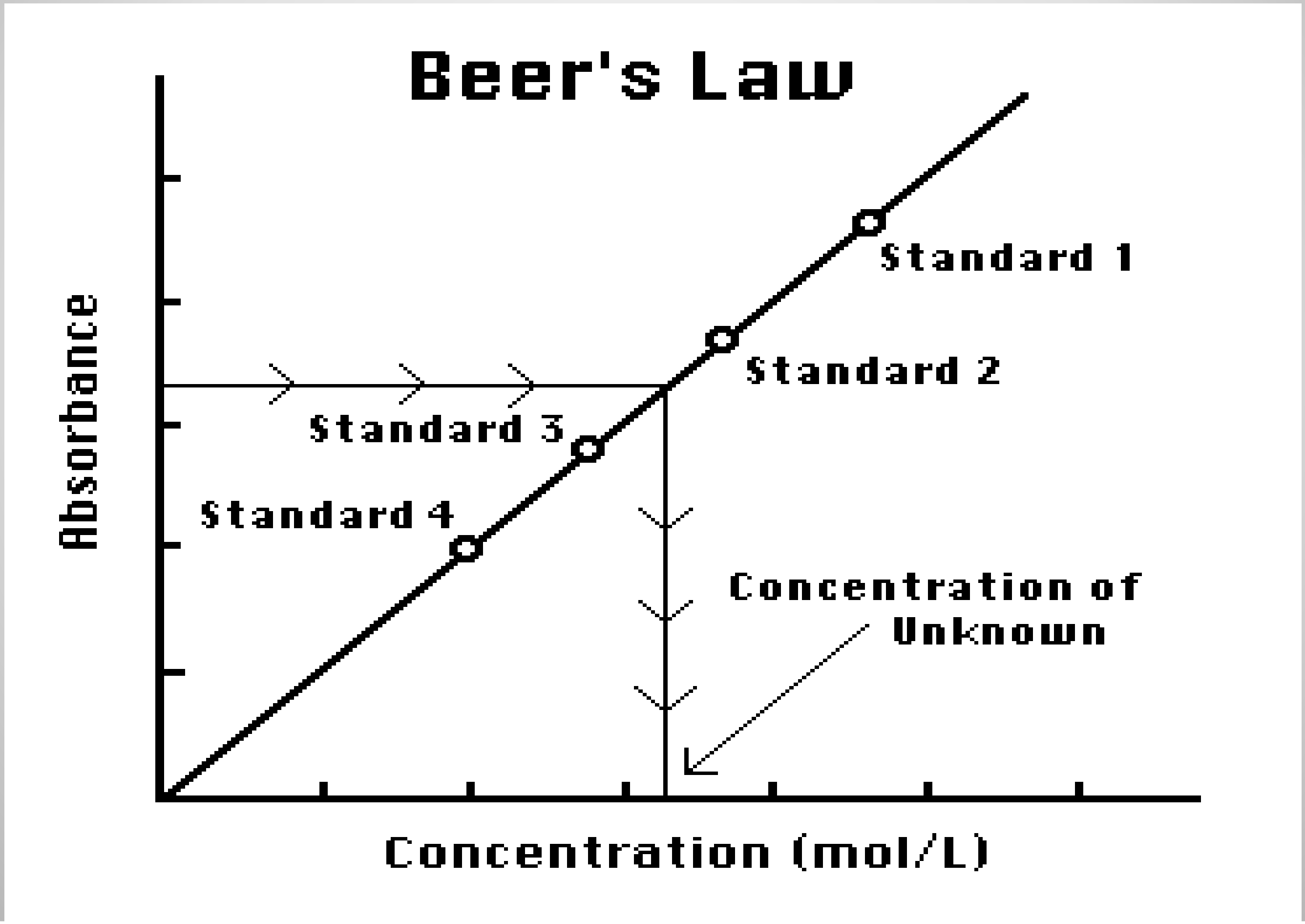
- UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.
- $A = \log I_0 / I_t = \log 1/T = -\log T = abc = \varepsilon bc$

Where : ε -is extinction co-efficient,

c- is concentration,

b- is the length of the cell that is used in UV spectrophotometer.

Beer’s Law…

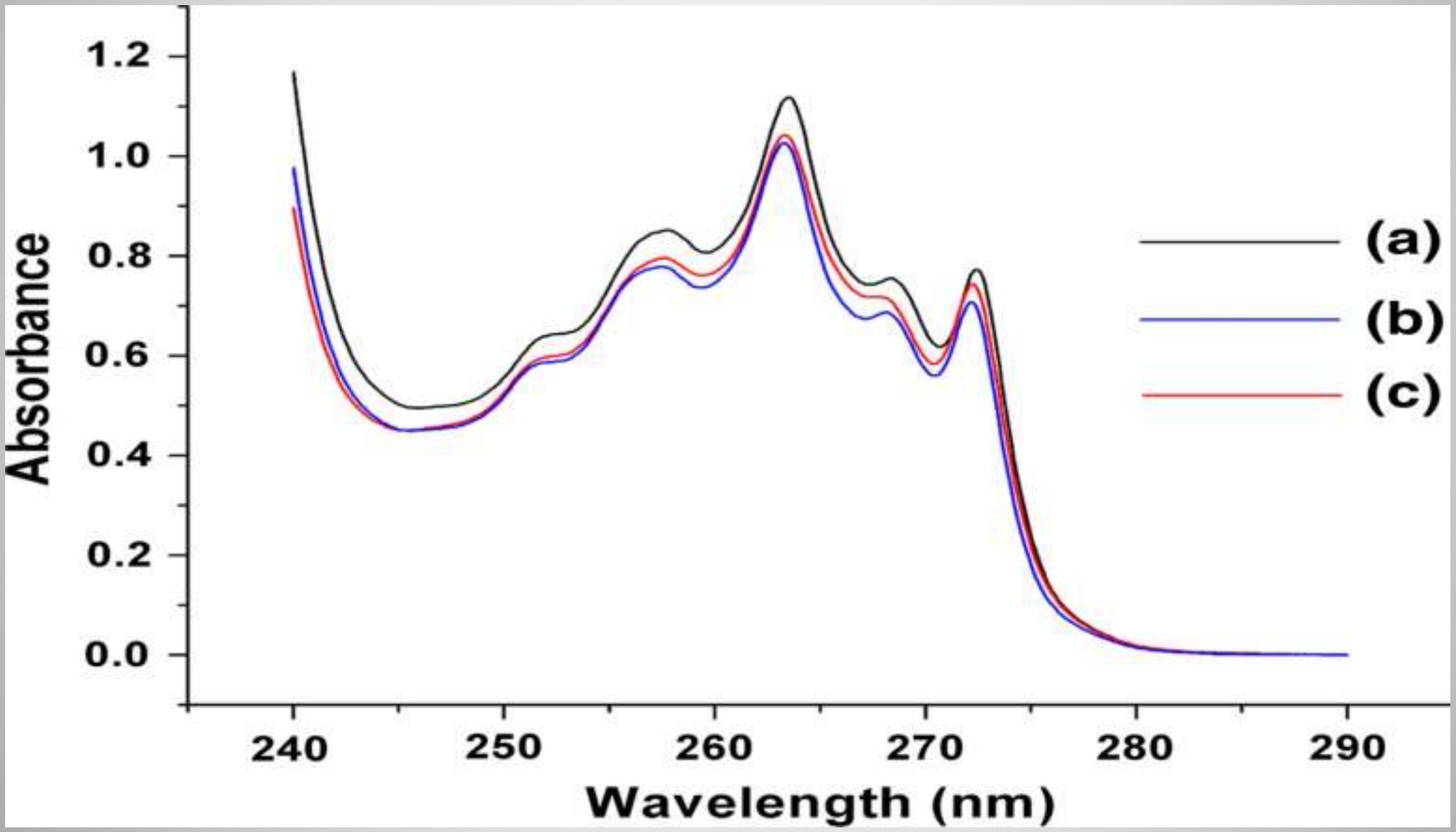


Beer's Law
Absorbance
Standard 1
Standard 2
Standard 3
Standard 4
Concentration of Unknown
Concentration (mol/L)

4. Qualitative analysis

- UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

U.V. Spectra's of Ibuprofen



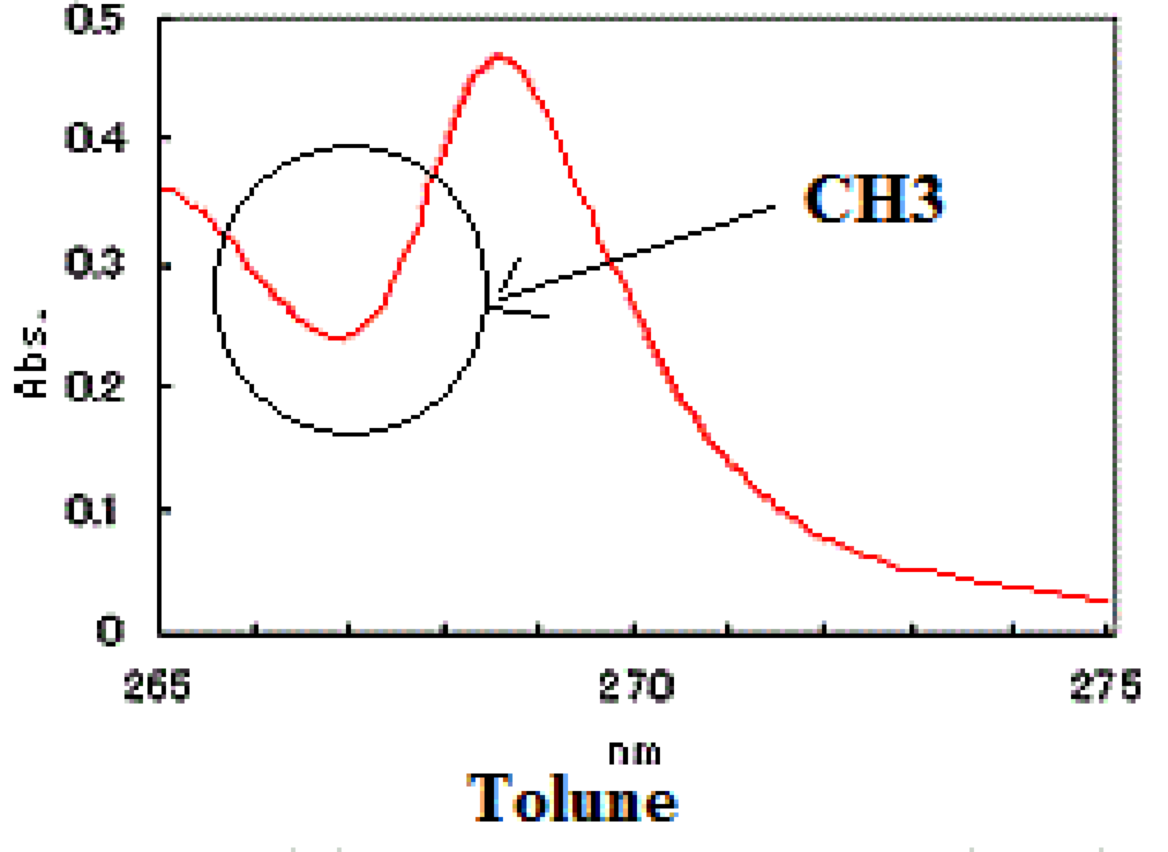
5. Chemical kinetics

- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

6. Detection of Functional Groups

- This technique is used to detect the presence or absence of functional group in the compound
- Absence of a band at particular wavelength regarded as an evidence for absence of particular group

Toluene.



7. Quantitative analysis of pharmaceutical substances

- Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.
- Diazepam tablet can be analyzed by 0.5% H_2SO_4 in methanol at the wavelength 284 nm.

8. Molecular weight determination

- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.

9. As HPLC Detector

- A UV/Vis spectrophotometer may be used as a detector for HPLC.

References

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T H A N K Y O U