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Session name : Introduction, Electronic Excitation, Terms used in U.V. Spectroscopy, Isolated and conjugated dienes

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T.Y. B.Sc. Chemistry

CH-607 Organic Chemistry-II

Ultra Violet (U.V.) and Visible Spectroscopy

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Ultra Violet (U.V.) and Visible Spectroscopy

SESSION AGENDA

- Introduction
- Spectrophotometer
- Electronic transition
- Chromophore, Auxochrome, Bathochromic and Hypsochromic shift
- Isolated and Conjugated Diene

LEARNING OBJECTIVES

Students should be able to understand

- Basic principles of U.V. Spectroscopy
- Electronic excitation (transition)
- Chromophore, Auxochrome, Bathochromic and Hypsochromic shift
- Hyperchromic shift and hypochromic shift
- Isolated and Conjugated Diene

Ultra Violet (U.V.) and Visible Spectroscopy

- Ultra violet spectroscopy provides information about the structure of the molecule regarding the presence of double bonds, triple bonds and conjugated double bonds.
- It can be used to differentiate conjugated and isolated dienes
- To differentiate carbonyl compounds and α - β unsaturated carbonyl compounds
- It also gives idea about stereochemistry of geometrical isomers

Ultra Violet (U.V.) and Visible Spectroscopy

- Both U.V and Visible spectra belong to **electronic excitation** (transition)
- When electron gain energy, they excited from lower energy level (ground state)to higher energy level(excited state). The energy is supplied in the form of light.
- As energy levels of the molecules are quantized, the amount of energy required for promotion of electron from ground state to excited state is fixed.

Electromagnetic Radiations

- Ultra violet region is from 200-400 nm
- Visible region is from 400-800 nm
- Vacuum Ultraviolet

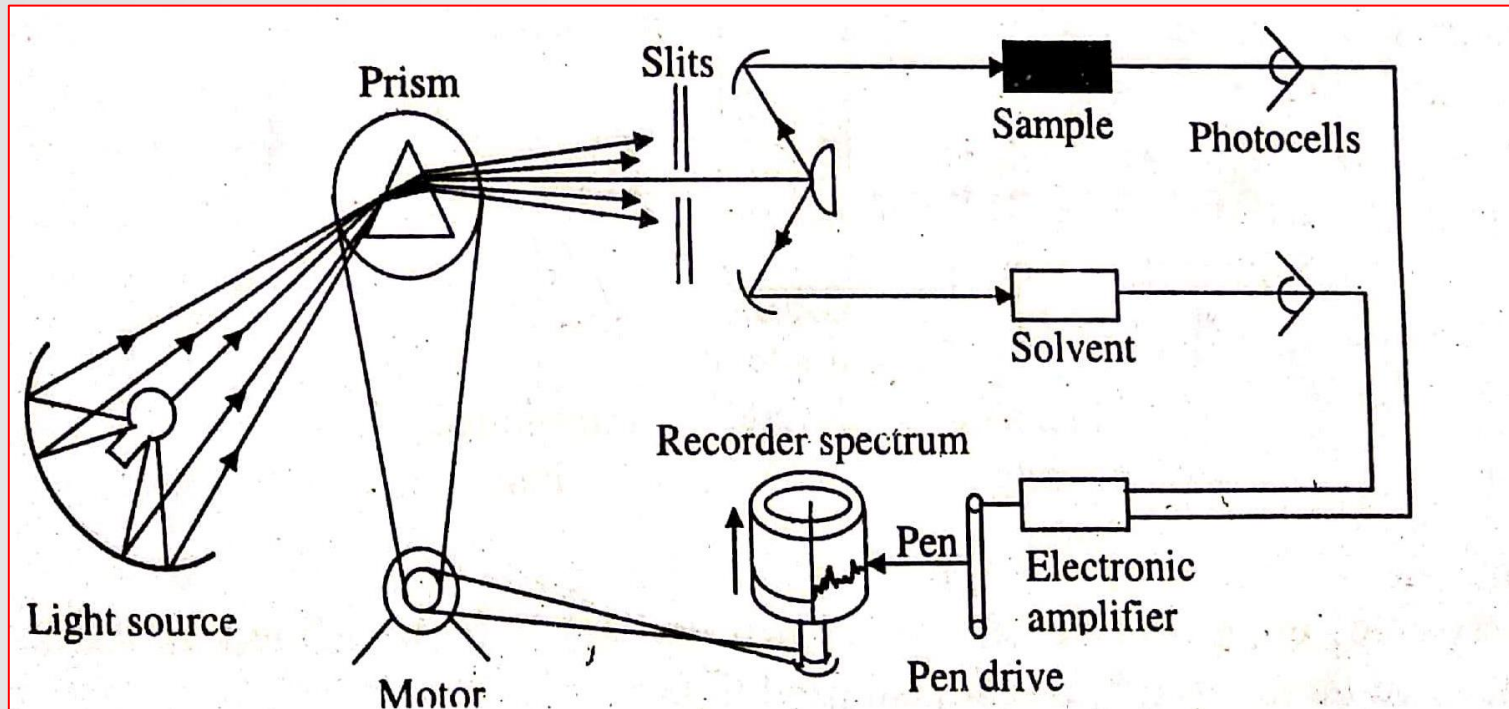
U.V. Spectroscopy can not be used below 200 nm as radiations are absorbed by the air, due to presence of moisture and also due to electronic transition in atmospheric oxygen, nitrogen and carbon dioxide.

In order to study the transition below 200 nm (far U.V. 15-200 nm) one must used vacuum apparatus. This region is also called as vacuum region

Ultra Violet (U.V.) and Visible Spectroscopy

Spectrophotometer

The instrument used to get spectrum of molecule is called spectrophotometer.



Spectrophotometer



Source: -

Deuterium or hydrogen discharge lamp:

It is used for 180 nm to 400 nm. wavelength

Tungsten lamp:

It is used for wavelength greater than 375 nm.

Sample container: -

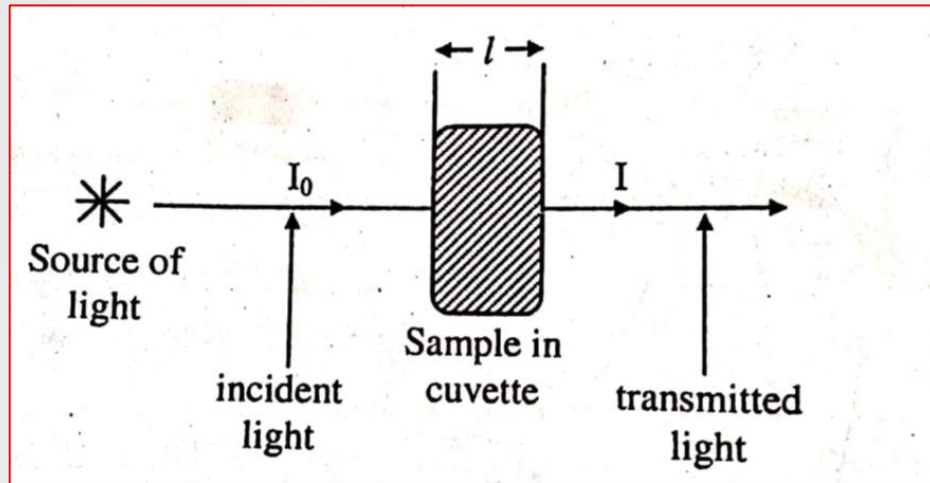
Quartz or fused silica cell with 1 cm path length is always used as container.

Sample and solvent: -

Few mg of pure sample is dissolved in about 100 ml of non-absorbing solvent like cyclohexane, 1,4 dioxane, water or 95% ethyl alcohol.

Beer-Lambert's Law

When a beam of monochromatic light is passed through a solution containing absorbing species, the decrease in intensity of light with path length is proportional to the concentration of the solution and the intensity of incident light.



When incident light strike on sample kept in cuvette, part of it absorbed and remaining part is transmitted.

Beer-Lambert Law

Absorbance is given as,

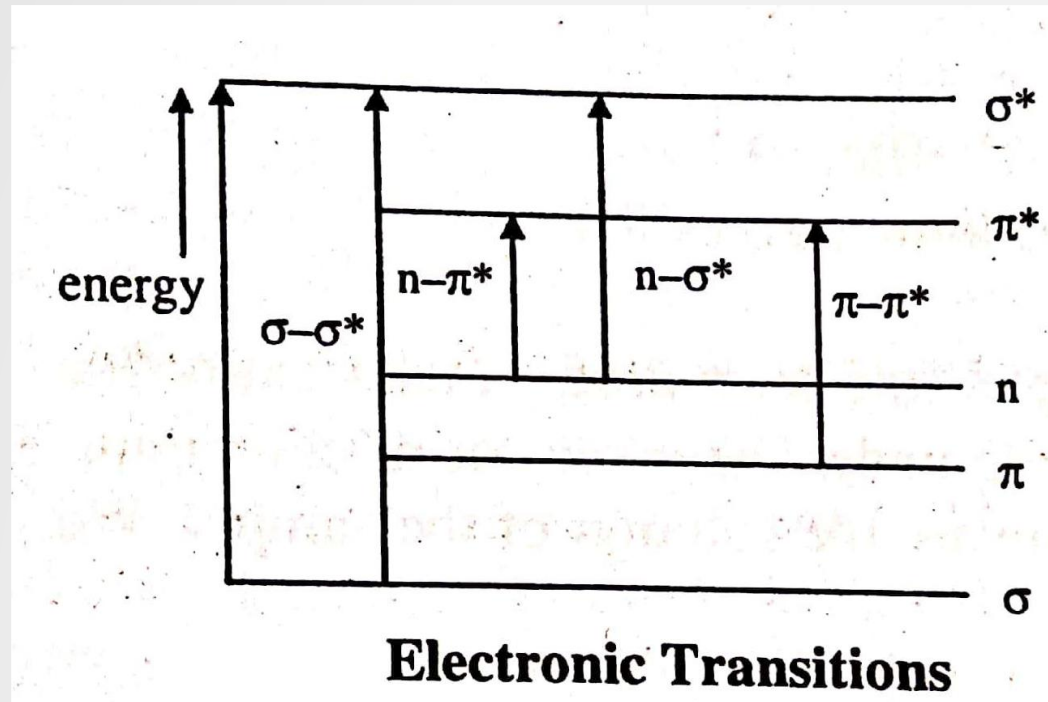
$$A = \log I_0 / I$$

$$A = \epsilon bC$$

- Where,
- I_0 = Intensity of incident light
 - I = Intensity of transmitted light
 - A = Absorbance
 - ϵ = Extinction Coefficient (molar absorptivity)
 - b = Path Length of cell in cm
 - C = Conc. of solution in mole/lit.

Electronic Excitation

Promotion of electrons from ground state molecular orbital (σ , π , n) to higher energy excited state orbitals (σ^* , π^*) is called electronic excitation.

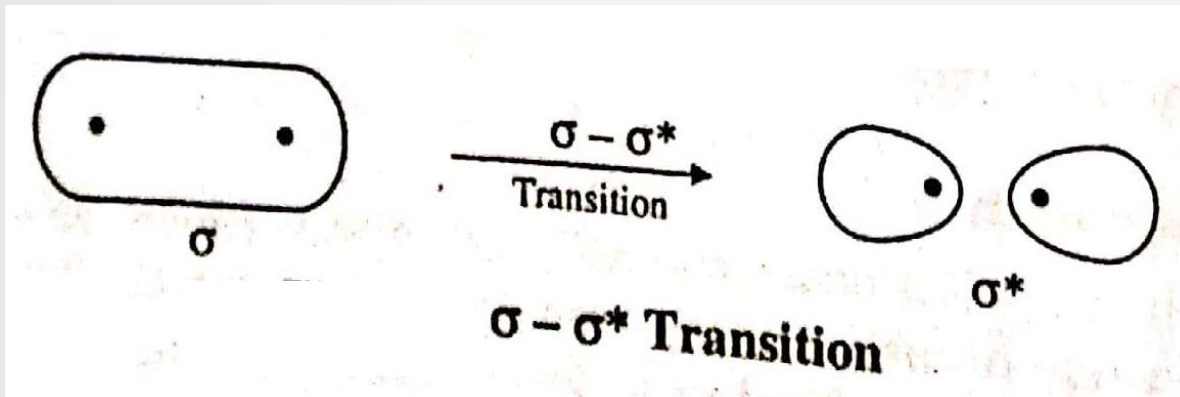


Types of an Electronic Excitation

1) $\sigma - \sigma^*$ transition: -

Promotion of electron from bonding σ to antibonding σ^* orbital. This transition requires high energy.

Example – Methane, ethane etc.

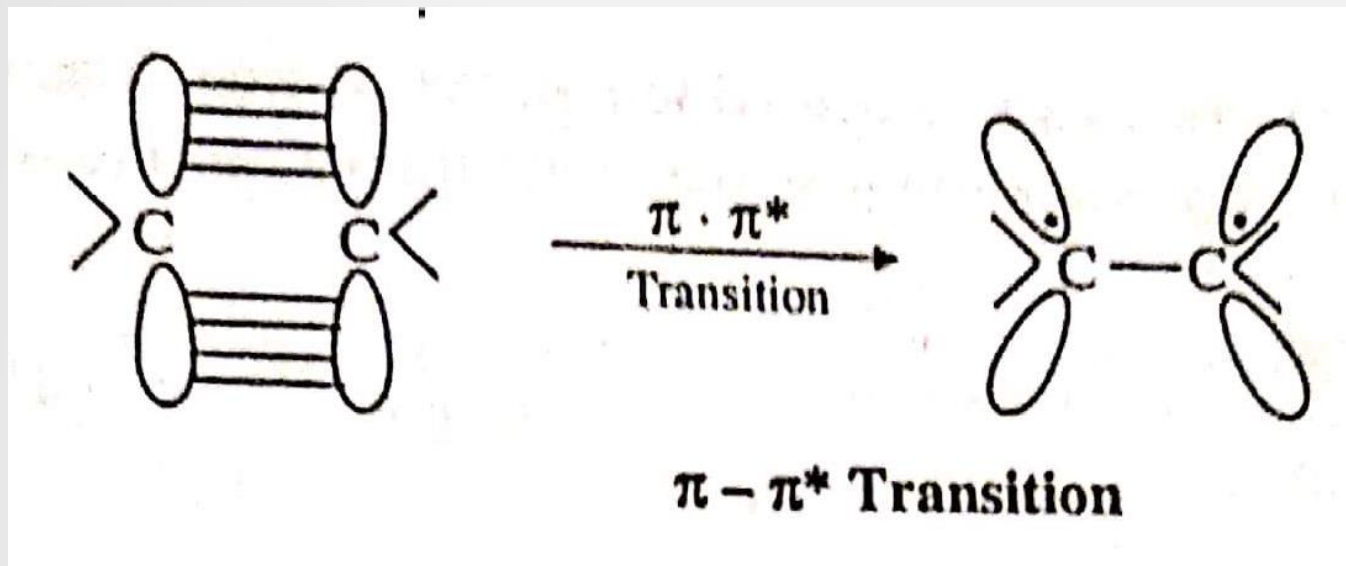


Types of an Electronic Excitation

2) π - π^* transition: -

Promotion of electron from bonding π orbital to antibonding π^* orbital. Molecules containing multiple bonds undergoes this type of transition.

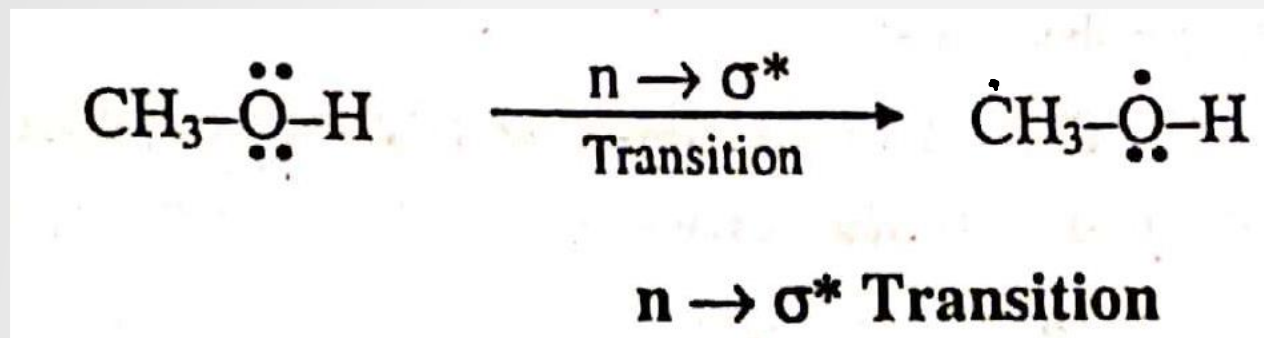
Example – Alkenes, alkynes, carbonyl compounds



Types of an Electronic Excitation

3) $n\text{-}\sigma^*$ transition: -

In $n\text{-}\sigma^*$ transition promotion of electrons from non-bonding molecular orbital (n) to higher energy antibonding orbital (σ^*) take place. Compound containing non-bonding electron pair present on hetero atom (O, N, S) show this transition.



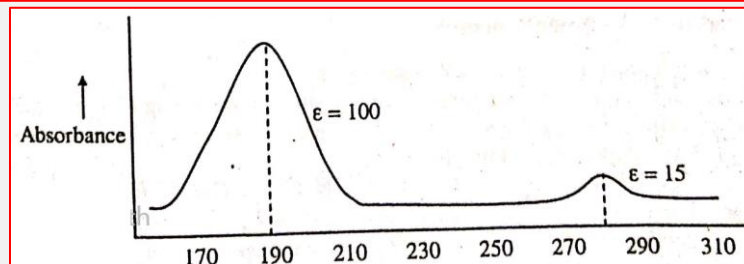
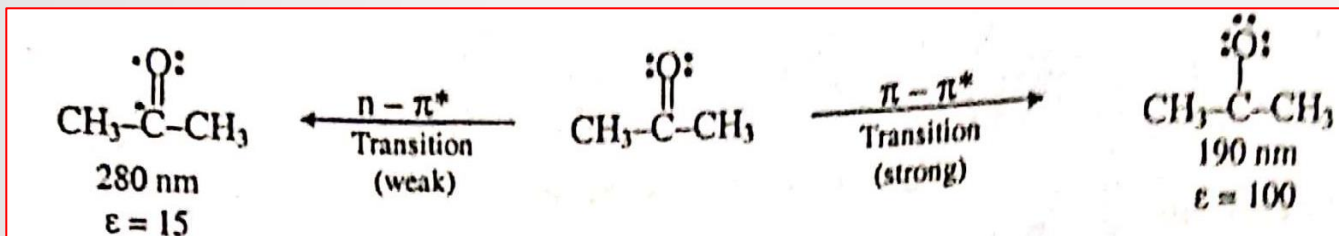
Types of an Electronic Excitation

4) $n - \pi^*$ transition: -

This transition requires minimum energy.

promotion of electrons from non-bonding molecular orbital (n) to higher energy antibonding orbital (π^*) take place.

Example – Acetone shows two absorption peaks one at 280 nm and other at 190 nm as follows.



Terms used in U.V. Spectroscopy

1) Chromophore: -

The part of the molecule responsible for imparting colour is called chromophore.

It is an isolated functional group capable of absorbing U.V. radiation (chroma = colour, phore = bearing)

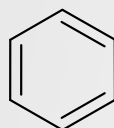
Examples-

$-N=O$, $-NO_2$, $-N=N-$, $-C=O$, $-C=N$, $-C=C-$, $-C=S$ etc

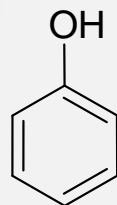
Terms used in U.V. Spectroscopy

2) Auxochrome

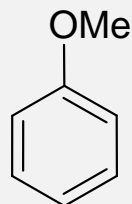
It is the functional group which does not absorb radiation in U.V. region, but when attach to chromophore cause a shift in absorption to longer wavelength. Some common auxochromes are -OH, -OCH₃, -NH₂



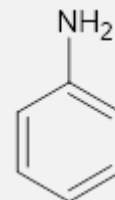
Chromophore
 $\lambda_{\max} = 255 \text{ nm}$



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



Anisole
 $\lambda_{\max} = 269 \text{ nm}$



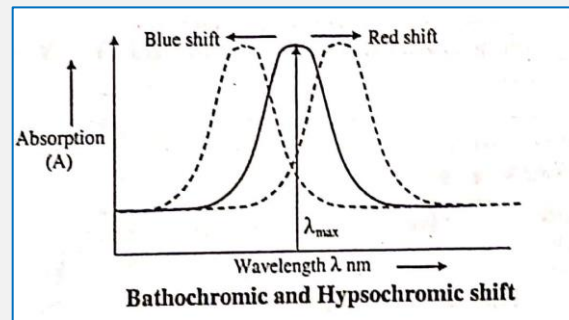
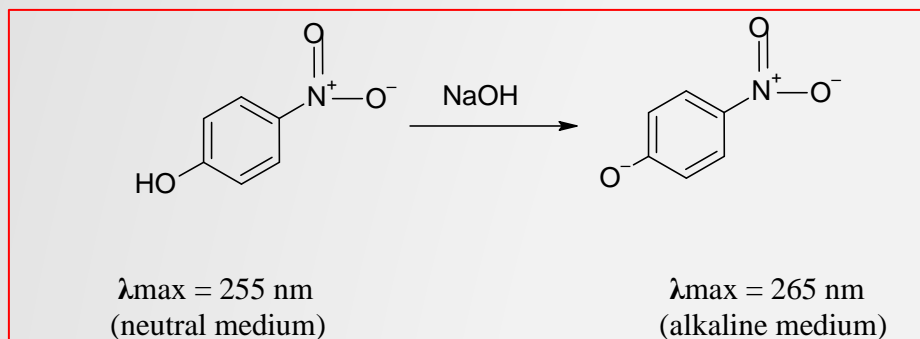
Aniline
 $\lambda_{\max} = 280 \text{ nm}$

Terms used in U.V. Spectroscopy

3) Bathochromic shift (Red Shift): -

An absorption to longer wavelength is known as bathochromic shift or red shift.

Example –p nitro phenol shows red shift in alkaline medium.



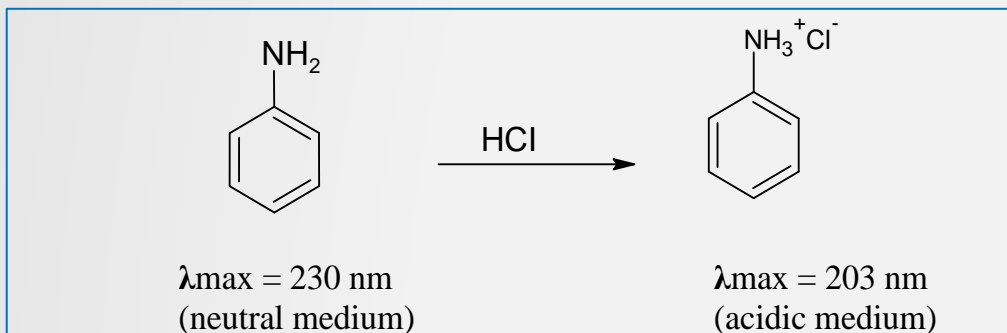
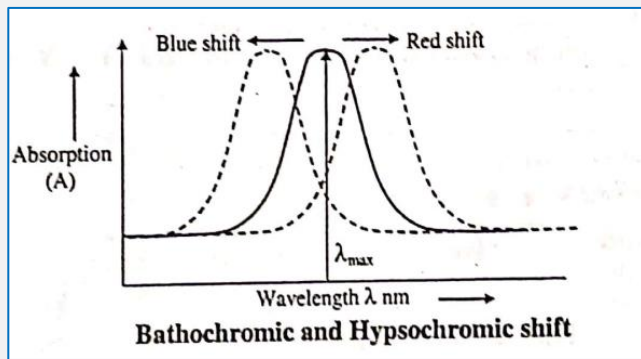
This bathochromic shift arises due to electron donating resonance effect. (negatively charged oxygen have more effect than neutral oxygen)

Terms used in U.V. Spectroscopy

4) Hypsochromic shift: -

An absorption to **shorter wavelength** is known as hypsochromic shift or blue shift.

Example – Aniline shows blue shift in acidic medium. (in acidic medium lone pair of electron present on nitrogen is not available for donation structure II)

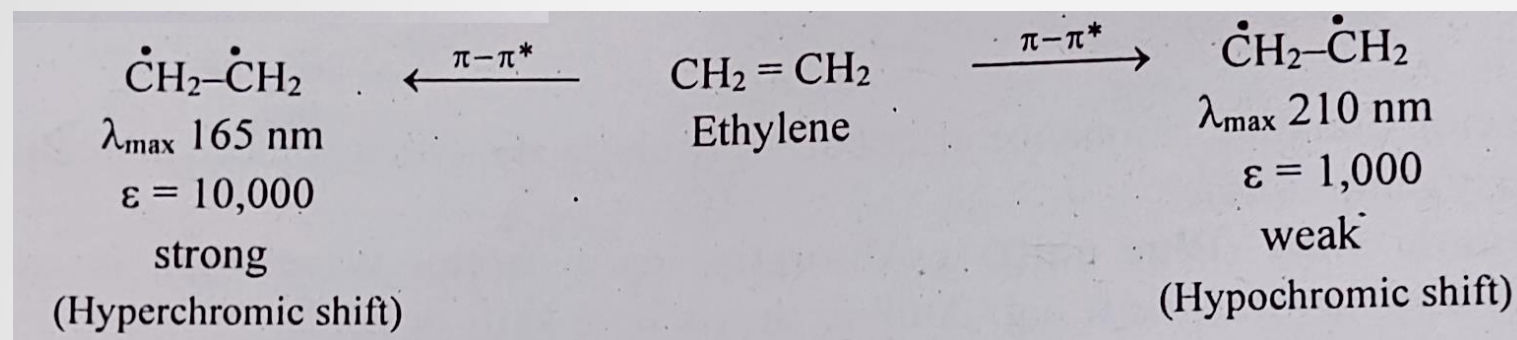


Terms used in U.V. Spectroscopy

Hyperchromic shift and Hypochromic shift

Strong intensity peak is called hyperchromic shift while weak intensity peak is called as hypochromic shift.

Example: -

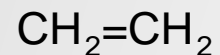


Peak at 165 nm is 10 times more intense than peak at 210 nm.

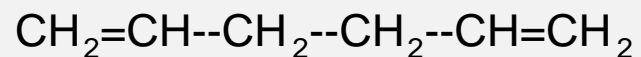
Effect of conjugation on position of U.V. band

A) Similar chromophore (Dienes): -

When two double bonds are separated by at least three single bonds then π - π^* transition take place approximately in the same region (165 nm to 200 nm), such dienes are called as isolated dienes.



Ethylene
 $\lambda_{\text{max}} = 165 \text{ nm}$



1,5 hexadiene
 $\lambda_{\text{max}} = 165 \text{ nm}$

Effect of conjugation on position of U.V. band

When two double bonds are conjugated then absorption take place at longer wavelength.



Ethylene

$\lambda_{\text{max}} = 165 \text{ nm}$



1,3 butadiene

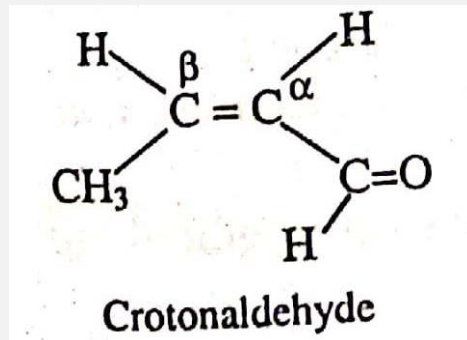
$\lambda_{\text{max}} = 220 \text{ nm}$

In conjugated system there is more stabilization by resonance. Transition of delocalized electron is very much easier and less energetic. Hence absorption occur at longer wavelength.

Effect of conjugation on position of U.V. band

B) Different chromophore: -

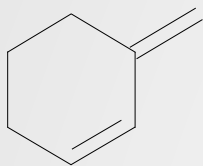
Unsaturated carbonyl compound is called enone. In enone carbon-carbon double bond is in conjugation with carbonyl group.



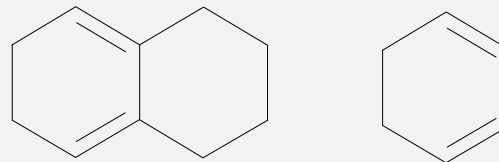
Conjugation decreases the energy for excitation of electron and increases the wavelength of absorption in U.V. region. As number of conjugated double bond increases the energy required for π - π^* transition decreases which increases the λ_{max} value.

Different Diene System

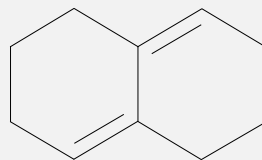
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$
Acyclic conjugated diene



Exocyclic double bond



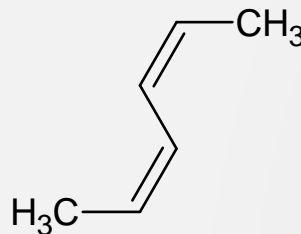
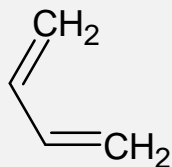
Homoannular diene



Heteroannular diene

Exocyclic double bond: Double bond in which one of the doubly bonded atoms is a part of a ring system.

Cis and trans diene



Student Assignment



1. Explain electronic transition with the help of figure.
2. Explain the terms auxochrome and bathochromic shift..
3. What is isolated and conjugated dienes?
Give examples.

SUMMARY

- Introduction of U.V. Spectroscopy
- Electronic transition
- Chromophore, Auxochrome,
Bathochromic and Hypsochromic shift
- Isolated and Conjugated Diene

PRECAP

- Applications of electronic spectroscopy
- Calculations of λ_{\max} Diene System of by Woodward and Fieser rule



Thank You so Much

U.V. Visible Spectroscopy

Spectrophotometry

Spectrophotometry is the branch of science which deals with the study of **interaction of electromagnetic radiation with matter**.

During this interaction energy is **either absorbed or emitted** by the matter in the form of quanta.

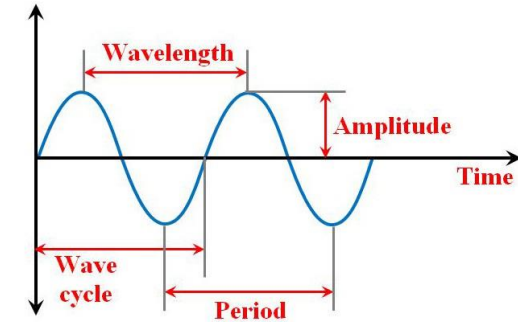
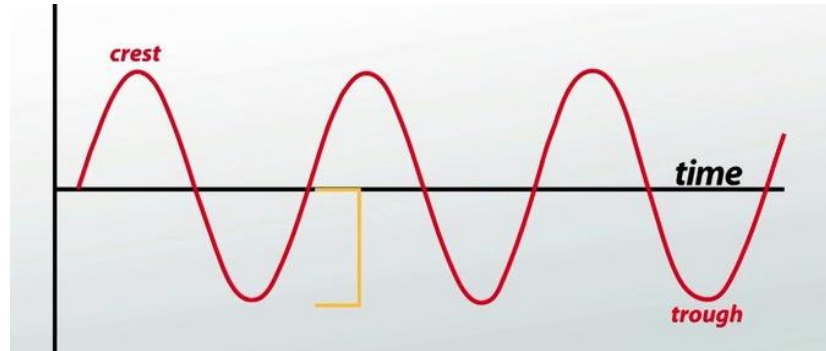
In this technique amount of light absorbed by the solution is measured and concentration of solute present in solution is find out.

This technique was invented by **Arnold J. Beckman** and his colleagues in **1940**.

An instrument used to measures the amount of light absorbed by a sample is called spectrophotometer.

SPECTROPHOTOMETRY

Wave Parameters



1) Wave length (λ) :- The distance between two consecutive crests or troughs is known as wavelength.

Units of measurements:-

Angstrom, Nanometer, Micron are the basic unit of wavelength measurement.

$$1 \text{ \AA} = 10^{-8} \text{ cm} = 10^{-10} \text{ m}$$

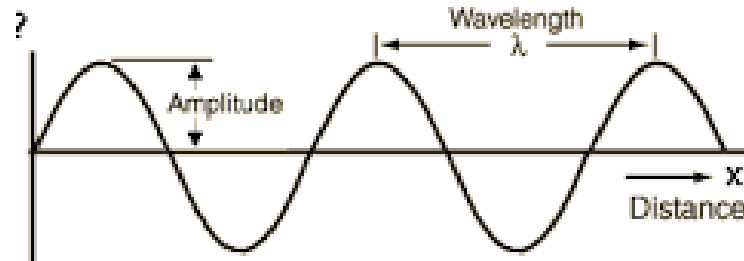
$$1 \text{ nm} = 10^{-7} \text{ cm} = 10^{-9} \text{ m}$$

$$1 \mu = 10^{-4} \text{ cm} = 10^{-6} \text{ m}$$

2) Amplitude (a):- Maximum displacement of the wave from the 'x' axis is called as amplitude of radiation. It is expressed in cm or mm

SPECTROPHOTOMETRY

Wave Parameters



3) Frequency (ν):- Number of wavelength produced (or number of oscillation completed) per unit time is called frequency.

It is measured in Hertz.

1 Hertz = one cycle per second

1 kilohertz = 1000 hertz

1 megahertz = 10^6 hertz

4) Wave number (ν):- No. of waves per centimeter is called wave number.

$$\nu = 1/\lambda \text{ cm}^{-1}$$

Unit: cm^{-1} or Kaisers

Terms used in absorption measurements

1) Energy of radiation /Radiant Energy (E):-

Energy transmitted as electromagnetic radiation is called radiant energy.

$$E = h \nu$$

Where 'h' is plank's constant, 'ν' is frequency

But we know that,

$$c = \nu \lambda$$

$$\nu = c / \lambda$$

Putting value of 'ν' in eq. 1, we get

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

$$E = h \times c \times 1 / \lambda$$

$$E = hc / \lambda \text{ (as h and c are constant)}$$

$$E = 1 / \lambda$$

Thus energy of radiation is inversely proportional to its wavelength and directly proportional to its frequency.

Terms used in absorption measurements

2) Radiant Power (P):-

It is formerly known as intensity.

Radiant power is the rate at which energy is transported in a beam of radiant energy. It can be measured by detector such as photocell or thermocouples.

Radiant power of incident light is given by symbol P_0 and transmitted light is P .

3) Transmittance (T):-

Ratio of radiant power of transmitted light and radiant power of incident light is called as transmittance.

$$\text{Transmittance} = P / P_0 \qquad \% \text{ Transmittance} = P / P_0 \times 100$$

4) Absorbance (A):-

The absorbance is the logarithm to the base 10 of the reciprocal of the transmittance T .

$$A = \log 1/T \qquad A = - \log T$$

$$A = \log P_0 / P$$

Terms used in absorption measurements

5) Absorptivity (a):-

It is also known as the Extinction Coefficient or specific extinction. It is the ratio of absorbance to the product of path length and concentration

$$a = \text{Absorbance} / \text{path length} \times \text{concentration}$$

$$a = A / bC$$

6) Molar absorptivity (€):-

It is also known as the Molar Extinction Coefficient

It is the product of absorptivity and molecular weight of the material.

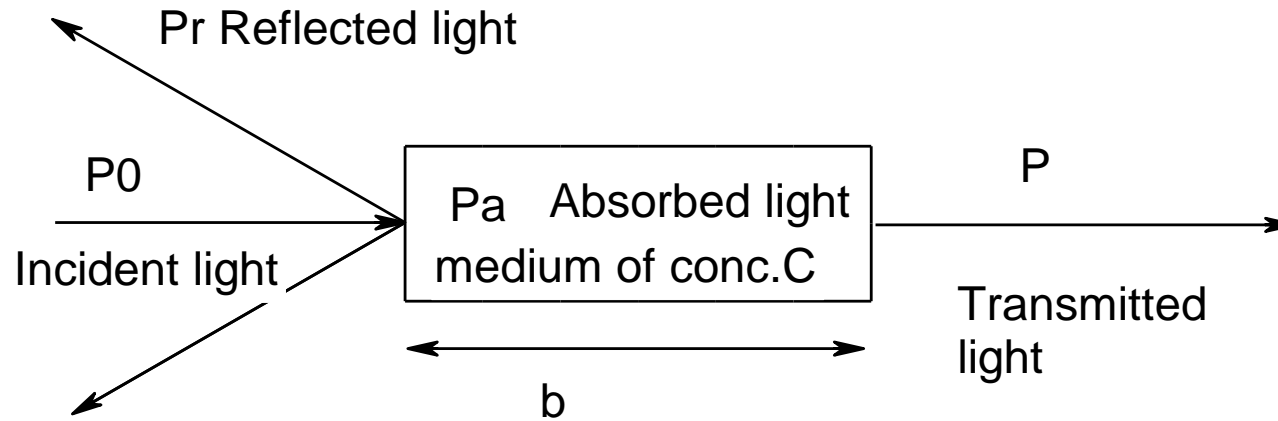
$$\epsilon = a \times M$$

$$\epsilon = A / bC \times M$$

7) Path length (b):-

It is internal diameter of cell in which sample is taken.

Fundamental laws of photometry



$$P_0 = P_r + P_a + P$$

When sample cell is very small then P_r is very small (approx. 4%)

$$\text{Hence, } P_0 = P_a + P$$

Lambert's Law

Lambert Law (1760):-

When a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease in radiant power with the thickness of medium is directly proportional to radiant power of incident light.

$$-\frac{dP}{db} \propto P$$

$$\text{or } -\frac{dP}{db} = k_1 P \quad \text{--- (1)}$$

Rearranging eq (1) we get

$$-\frac{dP}{P} = k_1 \cdot db \quad \text{--- (2)}$$

Integrating eq (2)

$$-\int_{P_0}^P \frac{dP}{P} = k_1 \int_0^b db$$

$$-\ln \frac{P}{P_0} = k_1 b$$

$$= \ln \frac{P_0}{P} = k_1 b$$

$$2.303 \log \frac{P_0}{P} = k_1 b$$

$$\log_{10} \frac{P_0}{P} = \left(\frac{k_1}{2.303}\right) b$$

$$\log_{10} \frac{P_0}{P} = K_1 b$$

$$\log_{10} \frac{P_0}{P} = A$$

$$A = K_1 b$$

Beer's Law

Beer's Law

When a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease of radiant power with the concentration of medium is directly proportional to the radiant power of the incident light.

$$-\frac{dP}{dC} \propto P$$

$$-\frac{dP}{dC} = k_2 P$$

$$-\frac{dP}{P} = k_2 dC$$

$$-\int_{P_0}^P \frac{dP}{P} = k_2 \int_0^C dC$$

$$-\ln \frac{P}{P_0} = k_2 \cdot C$$

$$\ln \frac{P_0}{P} = k_2 \cdot C$$

$$2.303 \log \frac{P_0}{P} = k_2 \cdot C$$

$$\log \frac{P_0}{P} = \left(\frac{k_2}{2.303} \right) C$$

$$\boxed{A = K_2 C} \quad \left(K_2 = \frac{k_2}{2.303} \right)$$

Lambert-Beer's Law

Lambert-Beer's law

For a given system and thickness of the medium, the absorption of medium is directly proportional to the concentration of absorbing species.

It is the combination of Lambert's and Beer's law

Absorbance = constant \times [thickness of the medium] \times [concentration of medium]

When concentration is expressed in gram per liter and thickness of medium in cm then constant is called as **absorptivity**

$$A = \text{Constant} \times b \times C$$

$$A = abC$$

When concentration is expressed in mole per liter and thickness of medium in cm then constant is called as **molar absorptivity (ϵ)**

$$A = \epsilon bC$$

When path length of cell is constant then,

$$A \propto C$$

Quantitative analysis

Determination of unknown concentration can be done by using any one of following method

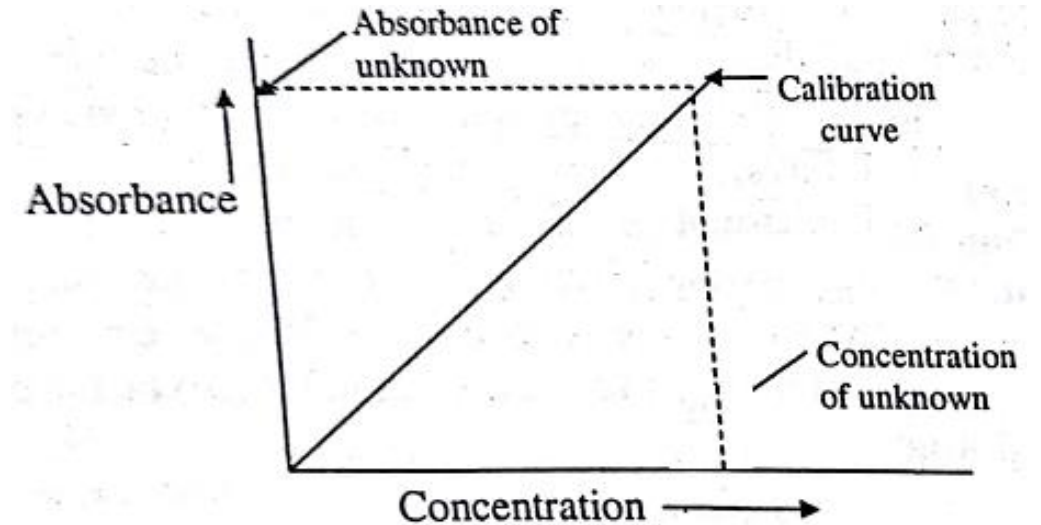
Method I

Series of standard solutions is prepared by using suitable solvent.

100% transmittance or zero absorbance is adjusted by using solvent.

Absorbance of all standard solutions are measured and plot of absorbance verses concentration is plotted to obtain calibration curve.

Absorbance of unknown solution is measured and its concentration is find out as shown in figure.



Quantitative analysis

Method II

Unknown concentration can be find out without constructing calibration curve.

Absorbance of standard solution and unknown solution are measured by using same cuvette.

Thus

$$A_1 = a_1 b_1 C_1 \text{ and } A_2 = a_2 b_2 C_2$$

As absorbing species in both solutions are identical and same cuvette is used for analysis, Hence $a_1 = a_2$ and $b_1 = b_2$

$$\text{As } A_1 = C_1 \text{ and } A_2 = C_2$$

Therefore

$$C_2 = \frac{A_2 \times C_1}{A_1}$$

Quantitative analysis

Method III

Absorbance is given as

$$A = \log P_0 / P$$

$$A = \log 1/T$$

$$A = abC$$

For a given instrument and solution P_0 , a and b remain constant

Therefore

$$C = \frac{\log P_0/P}{ab}$$

Numerical problem

Numerical problem 1

Calculate the frequency and wave number associated with radiation of wavelength 250 nm.

Given: $\lambda = 250 \text{ nm}$ ($250 \times 10^{-7} \text{ cm}$)

Wave number $\nu^- = \frac{1}{\lambda}$

$$\nu^- = \frac{1}{250 \times 10^{-7}}$$

$$\nu^- = 0.004 \times 10^7$$

$$\nu^- = 4 \times 10^4 \text{ cm}^{-1}$$

$$\text{Frequency}(\nu) = \frac{c}{\lambda}$$

$$\text{Frequency}(\nu) = \frac{3 \times 10^{10}}{250 \times 10^{-7}}$$

$$\text{Frequency}(\nu) = 0.012 \times 10^{17}$$

$$\text{Frequency}(\nu) = 1.2 \times 10^{15} \text{ sec}^{-1}$$

Numerical problem

Numerical problem 2

0.01M solution shows 15% transmittance. If path length is 1.5 cm Calculate molar absorptivity.

Percent transmittance of solution is 15%

$$\%T = T \times 100$$

$$15 = T \times 100$$

$$15/100 = T$$

$$T = 0.15$$

$$\text{Now } A = -\log T$$

$$A = -\log 0.15$$

$$\text{Absorbance} = 0.8239$$

Now from Beer's law

$$A = \epsilon \times b \times C$$

$$0.8239 = \epsilon \times 1.5 \times 0.01$$

$$\epsilon = \frac{0.8239}{1.5 \times 0.01}$$

$$\epsilon = 54.92 \text{ L mole}^{-1}\text{cm}^{-1}$$

Numerical problem

Numerical problem 3

0.03M solution shows 12% transmittance. If path length is 1 cm Calculate absorbance and molar absorptivity.

Numerical problem

Numerical problem 3

0.03M solution shows 12% transmittance. If path length is 1 cm Calculate absorbance and molar absorptivity.

Percent transmittance of solution is 12%

$$\%T = T \times 100$$

$$12 = T \times 100$$

$$12/100 = T$$

$$T = 0.12$$

$$\text{Now } A = -\log T$$

$$A = -\log 0.12$$

$$\text{Absorbance} = 0.9208$$

Now from Beer's law

$$A = \epsilon \times b \times C$$

$$0.9208 = \epsilon \times 1 \times 0.03$$

$$\epsilon = \frac{0.9208}{1 \times 0.03}$$

$$\epsilon = 30.69 \text{ L mole}^{-1}\text{cm}^{-1}$$

Numerical problem

Numerical problem

1.5×10^{-4} M Copper sulphate solution shows absorbance of 0.83 at selected wavelength. Calculate concentration of unknown solution of copper sulphate having absorbance 0.23

**Given $A_1 = 0.83$, $C_1 = 1.5 \times 10^{-4}$ M
 $A_2 = 0.23$, $C_2 = ??$**

$$A_1 = abC_1$$

$A_2 = abC_2$ as a and b are constant

$$A_1 = C_1$$

$$A_2 = C_2$$

$$C_2 = \frac{A_2 \times C_1}{A_1}$$

$$C_2 = \frac{0.23 \times 1.5 \times 10^{-4}}{0.83}$$

$$C_2 = 0.4156 \times 10^{-4}$$

$$C_2 = 4.15 \times 10^{-5} \text{ M}$$

Numerical problem

Numerical problem

When 2.0×10^{-4} M solution placed in 4 5 cm length cell show absorbance of 0.35. what will be the absorbance of solution if it is placed in 1 cm path length cell.

Given $A_1 = 0.35$, $b_1 = 5$

$A_2 = ??$, $b_2 = 1$

$$A_1 = ab_1C$$

$A_2 = ab_2C$ as a and C are constant

$$A_1 = b_1$$

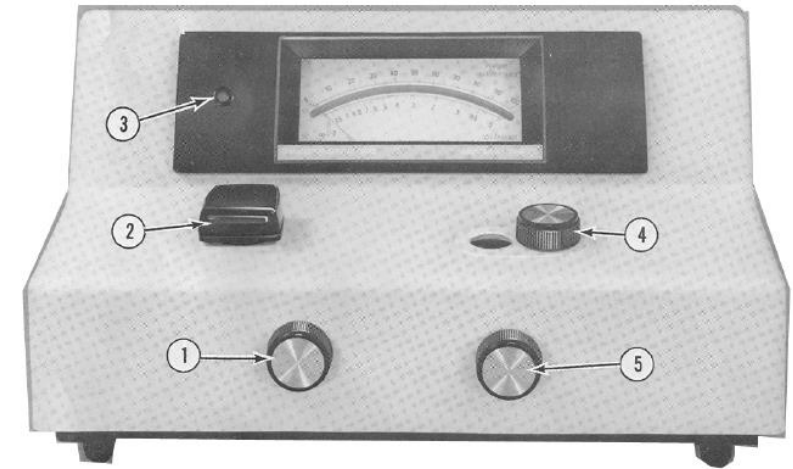
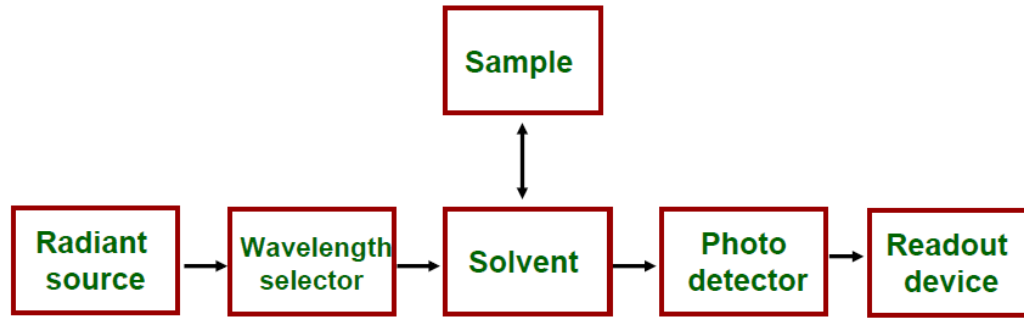
$$A_2 = b_2$$

$$A_2 = \frac{A_1 \times b_2}{b_1}$$

$$A_2 = \frac{0.35 \times 1}{5}$$

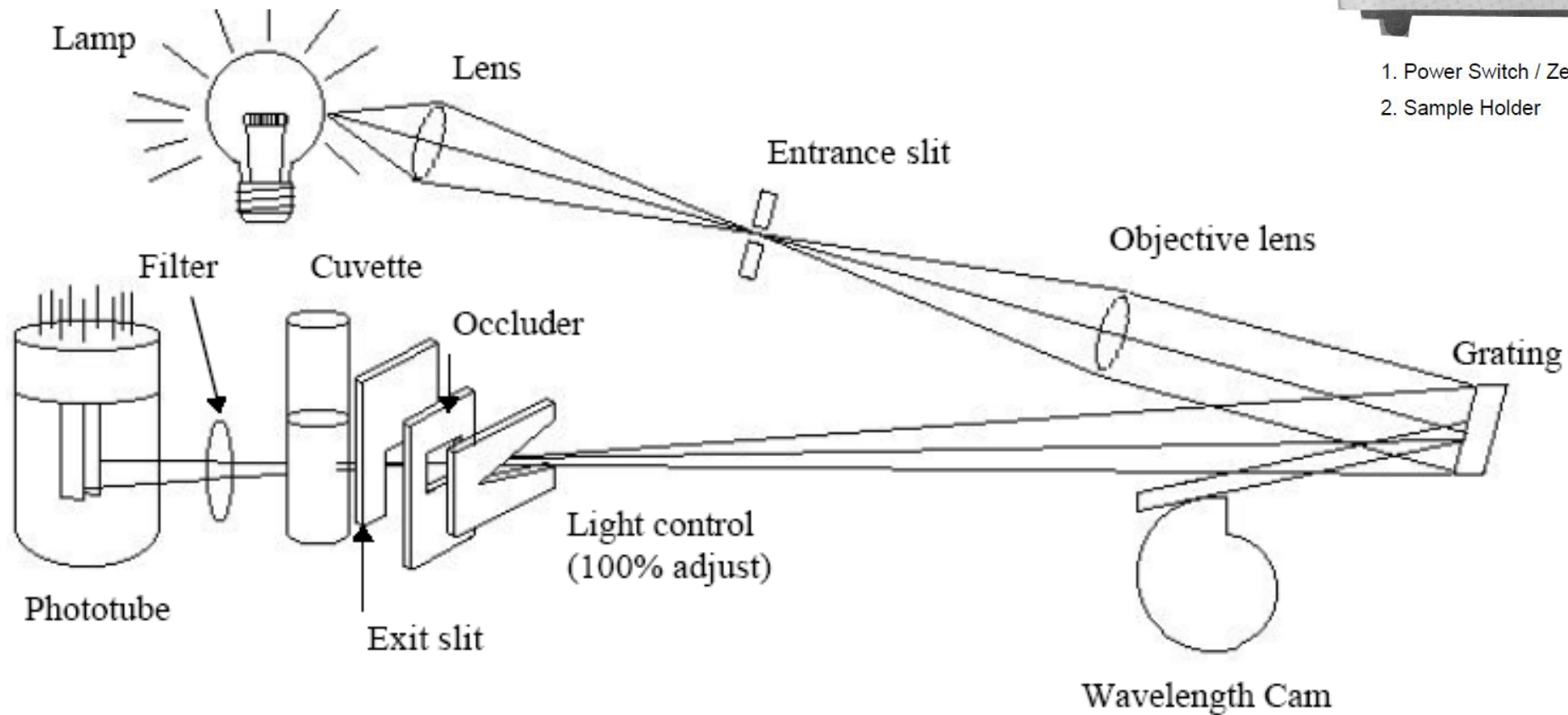
$$A_2 = 0.07$$

Instrumentation



- 1. Power Switch / Zero Control
- 2. Sample Holder

- 3. Pilot Lamp
- 4. Wavelength



Instrumentation

1) Radiation Source:

a) Tungsten lamp

In visible region (400-800 nm) tungsten filament lamp is used as radiation source. Constant power supply is used to avoid fluctuation.

It is most common light source used in spectrophotometer.

Average life time is 1200 hours

Limitations of Tungsten lamp:

At higher temperature it gives maximum portion of radiant energy in near I.R. region.

Example:- at 1725 °C it gives 1% I.R. radiation, while at 2725 °C it gives 15% I.R. radiation.

b) Carbon arc lamp: This lamp is used when more intense source is required.

Instrumentation

1) Radiation Source:

c) Hydrogen lamp: It is generally used in U.V. region.

d) Deuterium lamp: It gives 3 to 5 times more intense light than hydrogen lamp. This lamp is used when high intensity is required.

e) Xenon discharge lamp and Mercury vapor lamp are the other radiation source.

(Can be used in U,V and visible region)

Requirement of good source

It must be stable

It must produce spectrum of light covering entire wavelength

It must produce sufficient intensity

Instrumentation

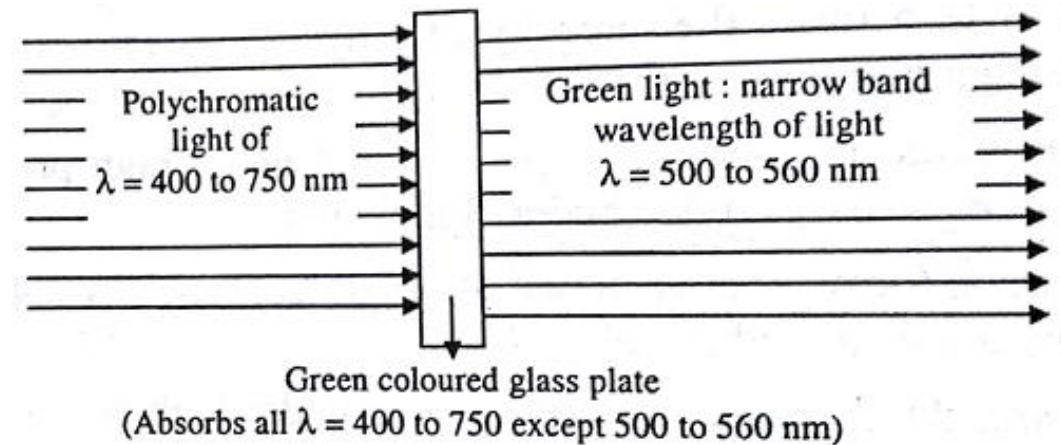
2) Filters and monochromators:

a) **Filter** : Filter permit certain bands of wavelength (bandwidth of ~ 50 nm) to pass through, but absorbs the light of other wavelength.

Types of Filter:

i) Absorption Filter:

This filter absorb unwanted radiation. It consist of solid glass plate having coloured pigments. Sometimes coloured solution of inorganic salt and organic dyes in rectangular glass cell are used as a filter. These filters provide 35 to 50 nm bandwidth.



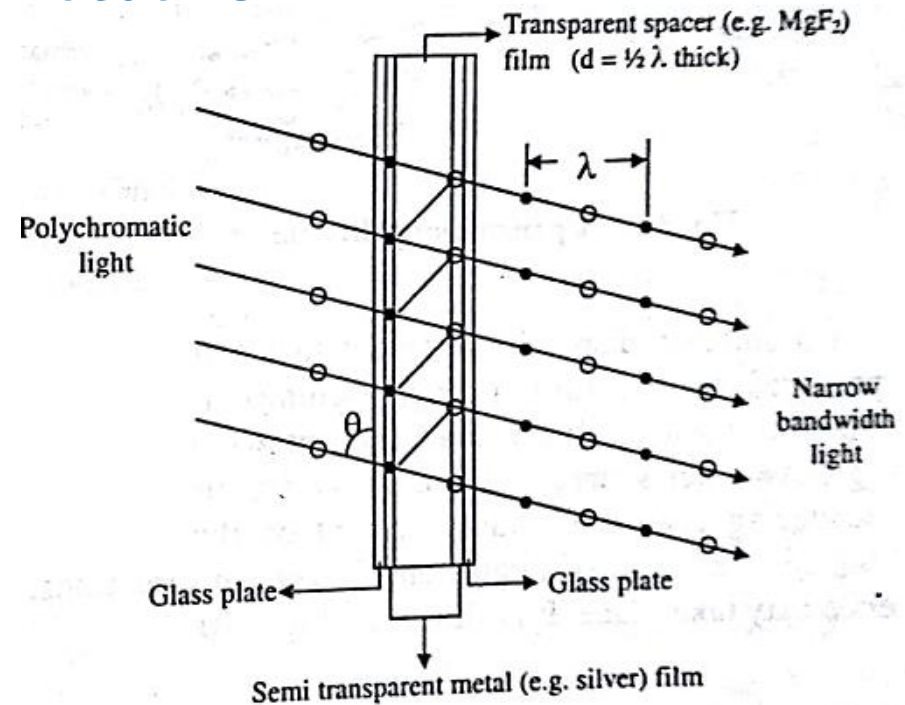
Disadvantage

- 1) They are not very good wavelength selectors and can't be used in instruments utilized in research.
- 2) They allow to pass a broad bandwidth which gives a chance for deviation from Beer's law.

Instrumentation

ii) Interference Filter:

These filter provide approximately 10 nm bandwidth. These are more expensive than absorption filters. It consist of several optical layers deposited on a glass substrate or transparent quartz.



Construction

Semitransparent metal sheet is deposited on glass plate. It is coated with dielectric transparent material like MgF₂. This is followed by another coating of metal and another layer of glass.

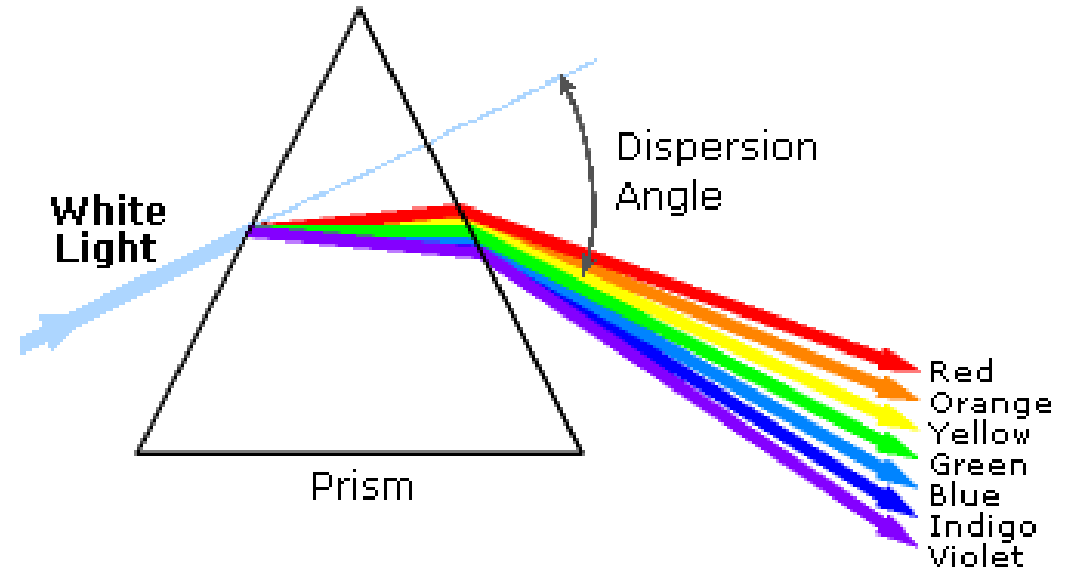
Unwanted radiation is reflected while desire radiation is transmitted through this filter.

Instrumentation

b) Monochromators

They are used for spectral scanning (varying the wavelength of radiation over a considerable range). They can be used for UV/Visible region.

All monochromators consist of slits, mirrors, lenses, gratings or prisms.



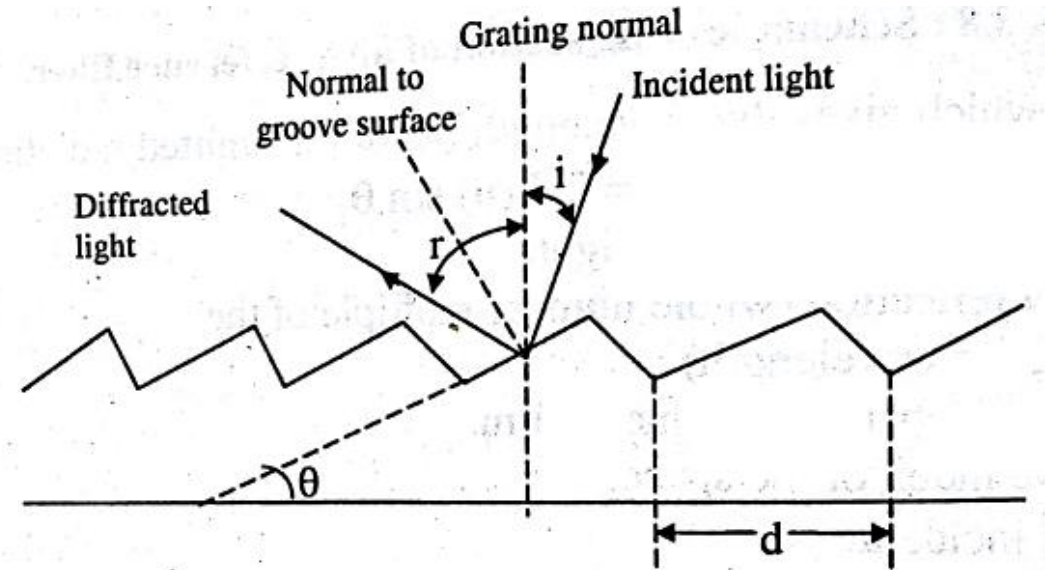
i) Prism:

It is triangular shape piece of glass or quartz. It work on refraction phenomenon. Depending on the wavelength light is refracted at different angle, shorter wavelengths are refracted more than longer wavelength

Instrumentation

ii) Diffraction Grating:

It is dispersing element that can isolate a selected band of wavelength. It is prepared by ruling a large number of parallel equidistance grooves upon highly polished metallic surface. Approximately 15000 to 30000 grooves per square inch are present on diffraction grating, these acts as scattering centers.



Wavelength (λ) is given as

$$n\lambda = d (\sin i + \sin r)$$

Where

d = distance between adjacent grooves

i = angle of incident

r = angle of refraction

n = integer corresponding to the order of radiation that are refracted

Instrumentation

3) Cuvette or sample container

Sample holding cell is called as cuvette. It is made up of glass or quartz. It may be rectangular or cylindrical. Its internal diameter is generally 1 cm.

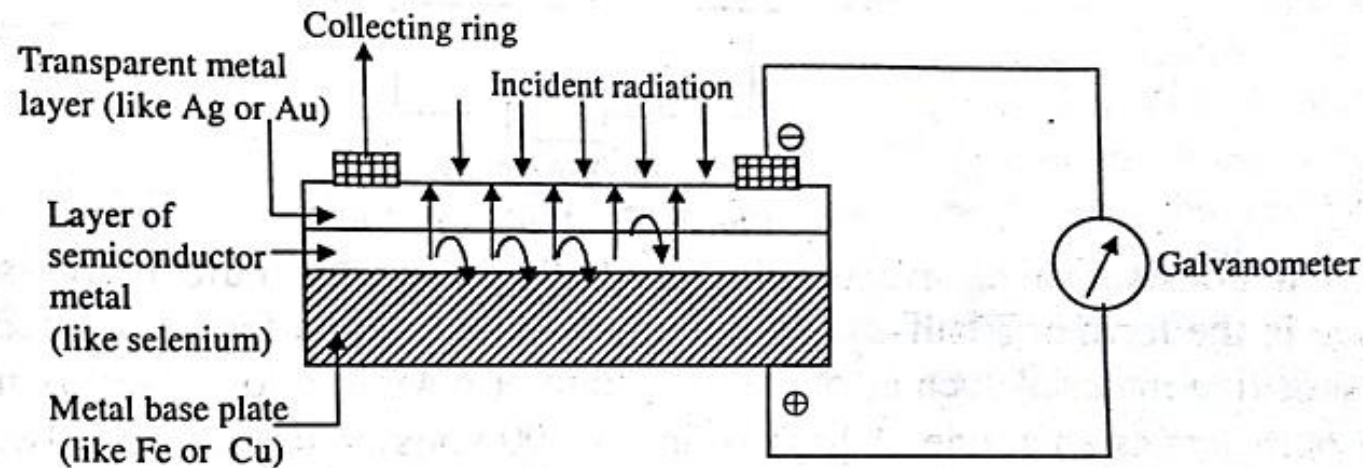
Instrumentation

4) Detectors

a) **Photovoltaic cell:** It is also called as barrier-layer cell or Photonic cell.

Construction:

It consists of metal base plate made up of iron or copper, which act as one electrode. Thin layer of semiconducting material (selenium) is deposited on the surface of the metal plate. This is covered by very thin layer of gold or silver which act as collector electrode.



Instrumentation

Working:

When radiation incident upon the surface of semiconducting material, electrons are ejected at Se-Ag interface. Due to accumulation of electron on silver surface photo current is generated. Magnitude of photocurrent is directly proportional to the radiation.

Characteristics of photovoltaic cell / Advantages

- i) It work without battery
- ii) It generate its own emf
- iii)It is sensitive over whole visible region.
- iv)Current output depends upon wavelength of incident radiation.

Limitations/Disadvantages

- i) Because of low internal resistance current is difficult to amplify.
- ii) It shows fatigue effect

Instrumentation

b) Phototubes / Photoemissive tubes

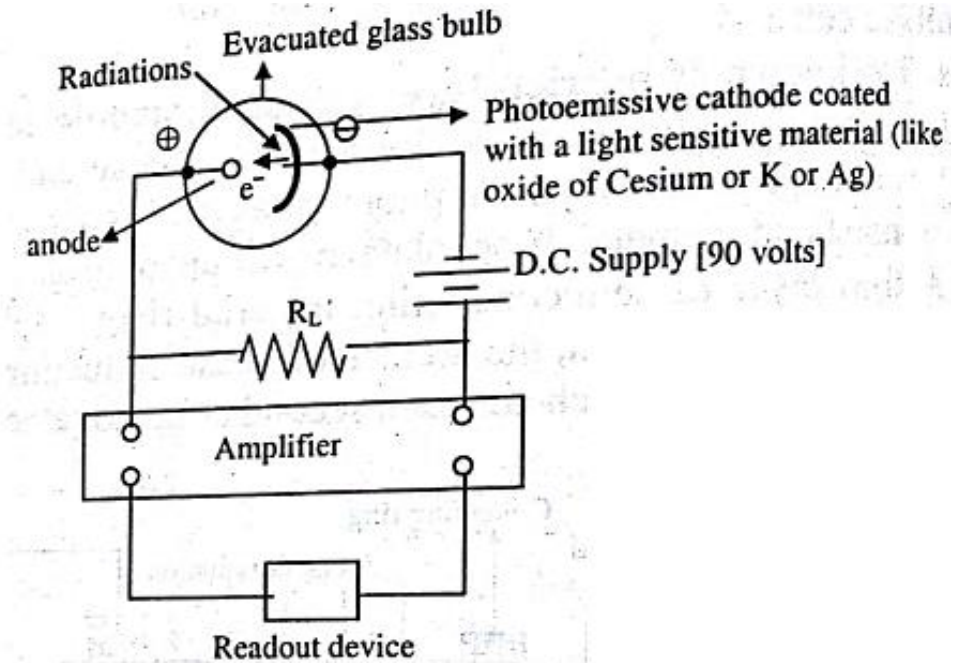
Principle: Emission of electrons from photosensitive solid surface is take place by incident radiation.

Construction:

It consist of glass bulb. Inside this there is photoemissive cathode in the form of half cylinder of metal. Inner surface of cathode is coated with light sensitive material like Cs_2O , Ag_2O and K_2O . Metal ring present in the bulb act as anode.

Working:

When light fall on cathode, electrons are emitted by photoelectric effect. These electrons then flow towards anode in the form of current. This current is proportional to the radiation of light striking on detector.

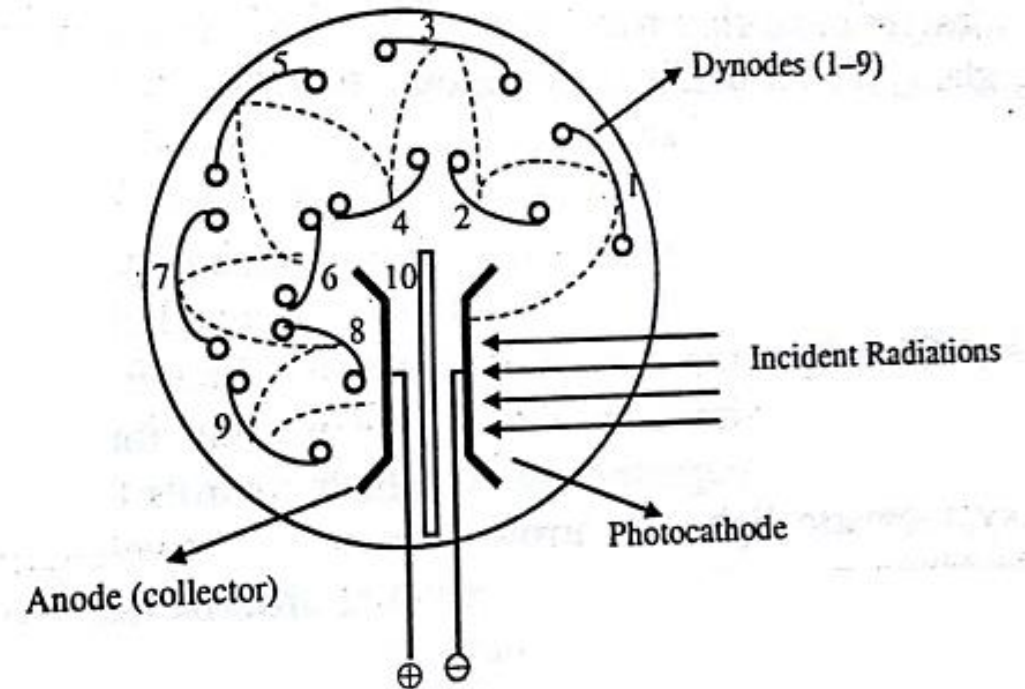


Instrumentation

c) Photomultiplier tubes

Construction:

It contains a photosensitive half cylinder of metal which acts as a cathode. The inner surface of the cathode is coated with a light-sensitive material like Cs_2O , Ag_2O and K_2O . It consists of 9 dynodes which have a coating of cesium metal which emits several electrons (2 to 5). These electrons are collected by the collecting electrode (anode).

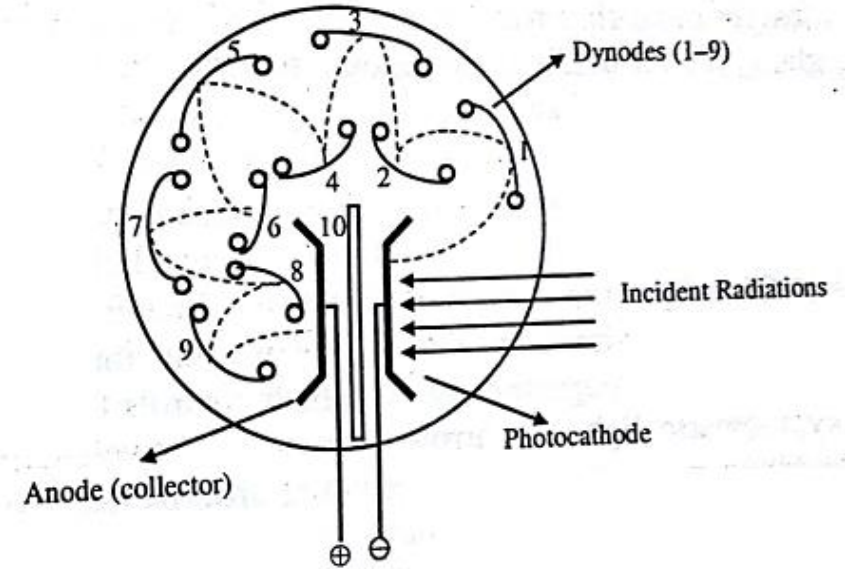


Instrumentation

c) Photomultiplier tubes

Working:

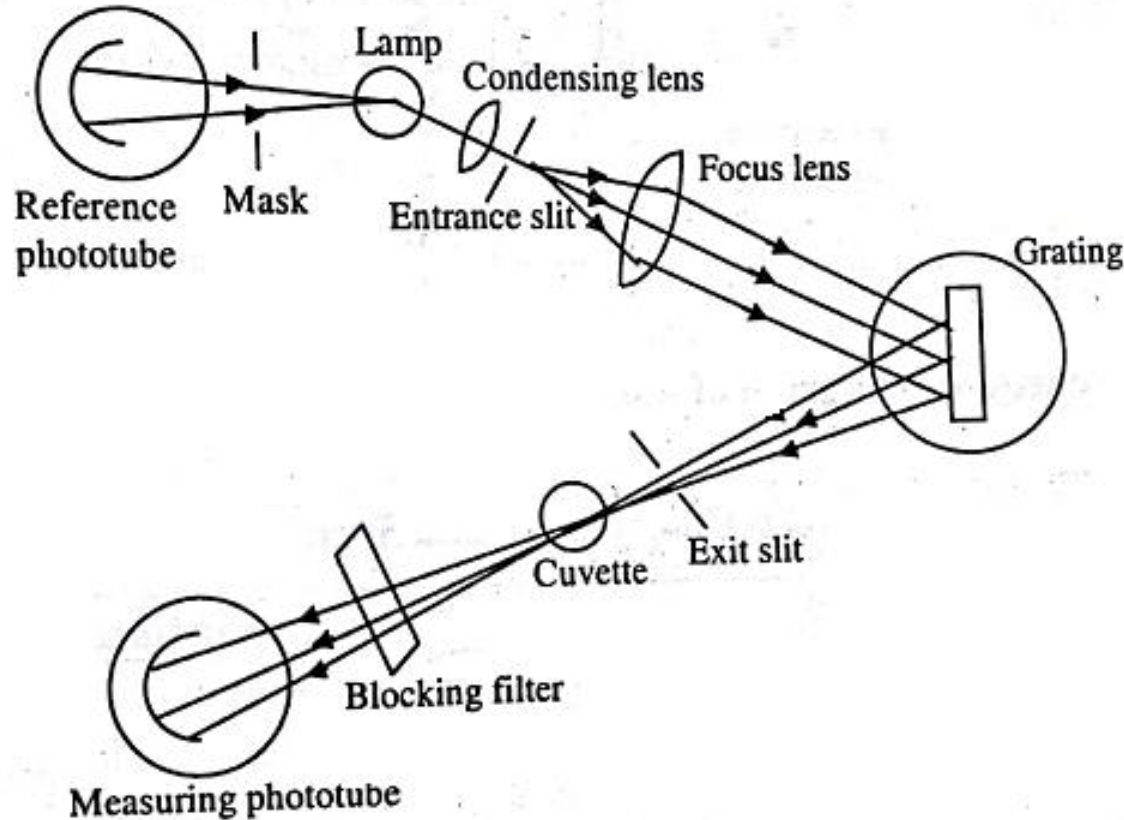
When light strike on cathode surface, it eject electrons due to photoelectric effect. These electrons strike on the surface of first dynode and ejection of 2 to 5 electrons take place. These electrons strike on surface of second dynode and ejection of more electron take place. This process is continued up to 9th dynode. Emitted electrons are collected by collecting electrode and current begin to flow. This current is amplified and measured by read out device.



Advantages:

- 1) It is very fast (response time is 10^{-9} second)
- 2) High sensitivity for U.V. and visible region.
- 3) It can measure the radiant power of 200 times weaker than those measured by photoelectric cell.

Single beam Spectrophotometer



- Spectrophotometer is a combination of spectrometer and photometer.

It is an instrument used to measure the absorbance or transmittance of solution as a function of wavelength.

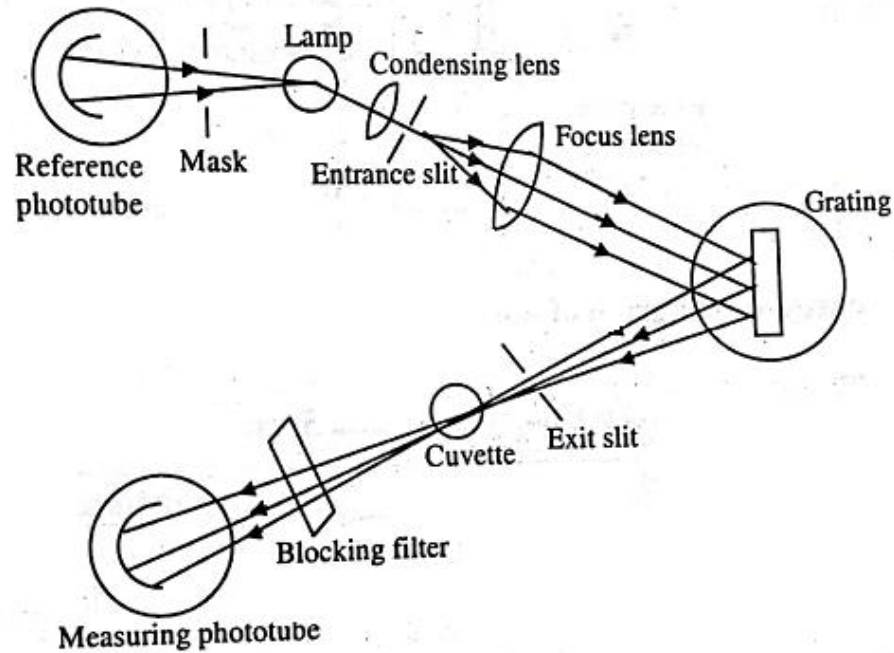
This instrument is generally used in visible region. (340-650 nm)

Diffraction grating is used for converting polychromatic light to monochromatic light having 20 nm bandwidth.

Phototube is used as detector.

Before use zero absorbance or 100% transmittance is adjusted by using solvent in cuvette.

Single beam Spectrophotometer

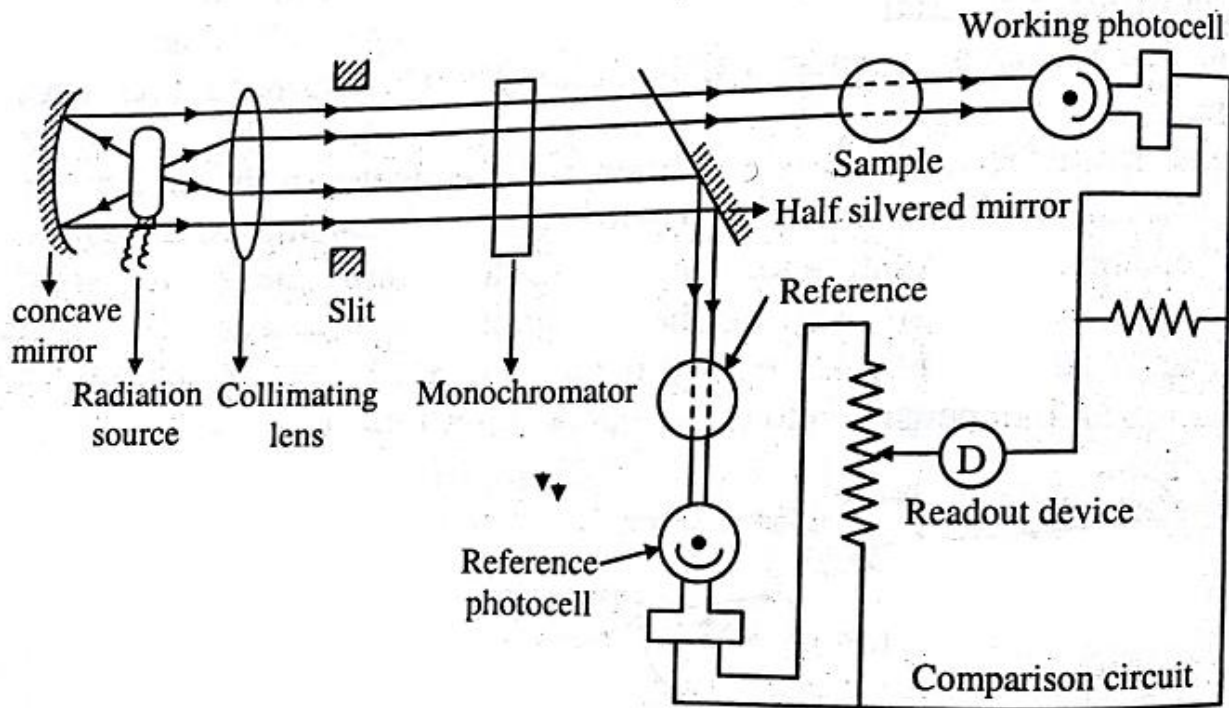


Working:

- 1) λ_{\max} is selected
- 2) Filter is selected in such a way that it should absorb maximum and emit minimum radiation
- 3) Cuvette is filled with solvent and 100% transmittance is adjusted.
- 4) Sample is filled and absorbance is measured

- Source: Tungsten filament lamp
- Collimating lens: It make a beam of light parallel
- Slit: Slit 1 control incoming radiation, while slit 2 control outgoing radiation.
- Filter: Coloured glass is used as a filter which convert polychromatic light in to a desired narrow band of radiation.
- Cuvette: Glass or Quartz tube having 1 cm path length.
- Detector: Photovoltaic cell
- Recorder: Galvanometer is used as read out device

Double beam Spectrophotometer



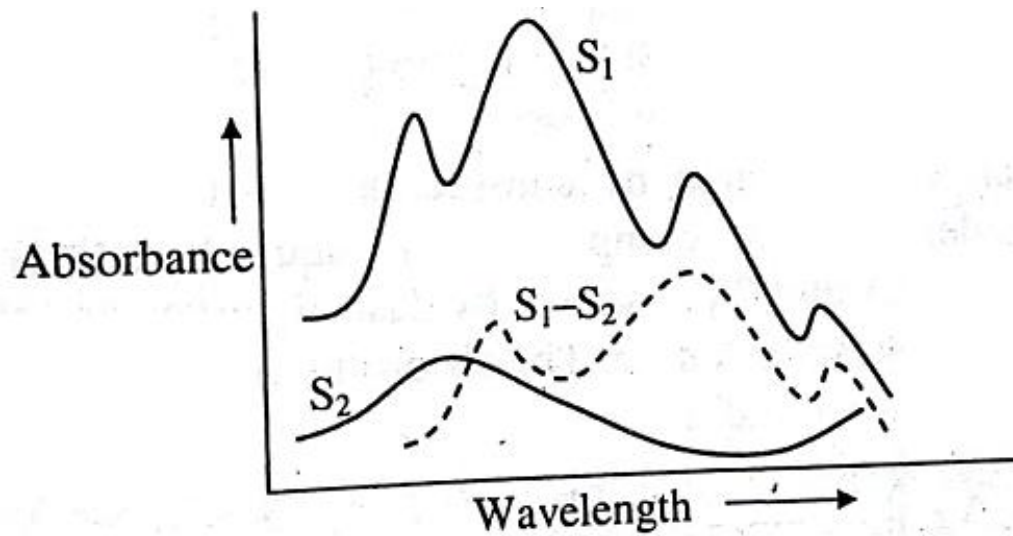
- In double beam spectrophotometer wavelength varies automatically and instrument measure the absorbance as a function of wavelength.
- In this instrument two beam from same source are used. One beam passed through sample solution and strike on working photocell, while other beam passed through blank or reference solution and strike on reference photocell.
- Two detectors are connected by comparison circuit and read out device directly show the absorbance of the sample solution.

Double beam Spectrophotometer

Advantages:

- 1) Automatically compensate the absorbance of blank solution.
- 2) Continuous replacement of blank with sample is not required.
- 3) Reading is independent of fluctuation of electric current.
- 4) As null method is used instrument is not affected by sensitivity of photocell.
- 5) It gives rapid scanning over wide range of wavelength.
- 6) Very good for qualitative analysis when whole spectrum is used.

Additivity of Absorbance



Absorption is an additive property. When solution contain two or more species, then each component absorb independently and total absorbance is given as

$$A = A_1 + A_2 + A_3 + A_4 + \dots + A_n$$

$$A = b (\epsilon_1 C_1 + \epsilon_2 C_2 + \epsilon_3 C_3 + \epsilon_4 C_4 + \dots \epsilon_n C_n)$$

Use of Additivity:

- 1) It permit the subtraction of contribution of absorbance of solvent (blank solution).
- 2) If absorption spectrum of system is known (say S1) and if absorbance of one of two chromophore (say S2) is available then one can draw the spectrum for remaining component (i.e. S1-S2).
- 3) It permit simultaneous determination of two or more species.

Simultaneous spectrophotometric determination

For simultaneous determination

- 1) There should be no chemical interaction between absorbing species.
- 2) Each component must act independently.
- 3) Beer's law should be obeyed by all components at their λ_{max} .
- 4) λ_{max} value of each component must differ widely.

Procedure:

- 1) λ_{max} of each component is measured.
- 2) Solutions of various known concentration are prepared for each component and Beer's law is tested at their λ_{max} .
- 3) Absorbance of solution is measured at chosen λ_{max} .
- 4) Total absorbance value of mixture at each λ_{max} is measured by using cuvette having same path length.
- 5) Observations are inserted in following equation

Simultaneous spectrophotometric determination

5) Observations are inserted in following equation

$$A_{\lambda_1} = (\epsilon_1)_{\lambda_1} C_1 + (\epsilon_2)_{\lambda_1} C_2 + (\epsilon_3)_{\lambda_1} C_3 + \dots + (\epsilon_n)_{\lambda_1} C_n$$

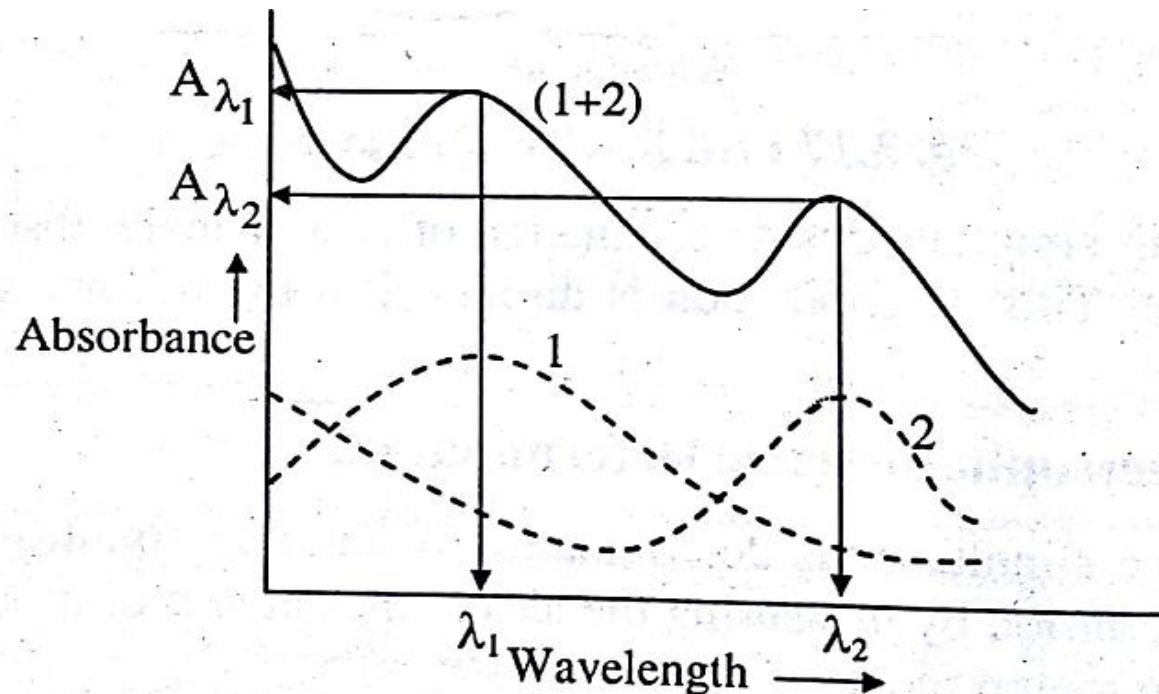
$$A_{\lambda_2} = (\epsilon_1)_{\lambda_2} C_1 + (\epsilon_2)_{\lambda_2} C_2 + (\epsilon_3)_{\lambda_2} C_3 + \dots + (\epsilon_n)_{\lambda_2} C_n$$

$$A_{\lambda_3} = (\epsilon_1)_{\lambda_3} C_1 + (\epsilon_2)_{\lambda_3} C_2 + (\epsilon_3)_{\lambda_3} C_3 + \dots + (\epsilon_n)_{\lambda_3} C_n$$

6) Above equations are solved simultaneously to find out $C_1, C_2, C_3, \dots, C_n$ of component 1, 2, 3, ..., n.

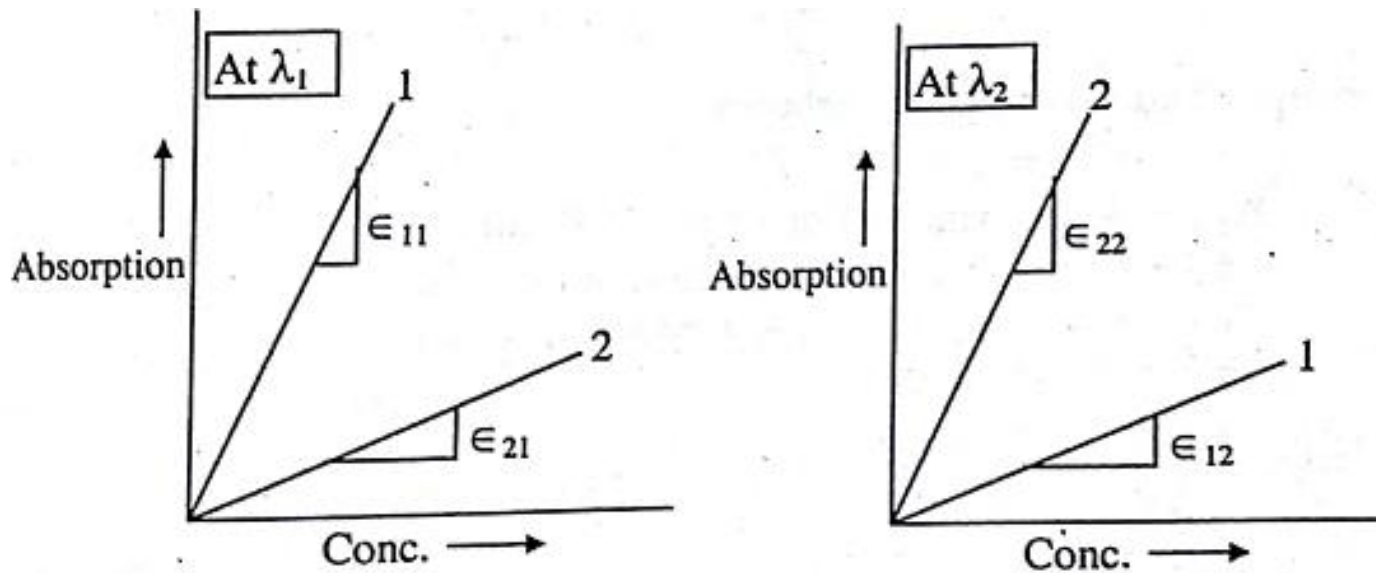
Example: Two component analysis

Absorption spectra of component 1 and 2 are shown by dotted curve while that of mixture was shown by solid curve.



Simultaneous spectrophotometric determination

Calibration curve is plotted at both wavelength λ_1 and λ_2 for compound 1 and 2 and slope is determined.



Calibration curve in two component analysis

$$\begin{aligned} \text{At } \lambda_1: \quad A_{\lambda_1} &= (A_1)_{\lambda_1} + (A_2)_{\lambda_1} \\ &= \epsilon_{11} C_1 + \epsilon_{21} C_2 \\ \text{At } \lambda_2: \quad A_{\lambda_2} &= (A_1)_{\lambda_2} + (A_2)_{\lambda_2} \\ &= \epsilon_{12} C_1 + \epsilon_{22} C_2 \end{aligned}$$

Simultaneously solving above equation, we get,

$$\begin{aligned} C_1 &= \frac{(\epsilon_{22} \times A_{\lambda_1}) - (\epsilon_{21} \times A_{\lambda_2})}{(\epsilon_{11} \times \epsilon_{22}) - (\epsilon_{21} \times \epsilon_{12})} \\ C_2 &= \frac{(\epsilon_{11} \times A_{\lambda_2}) - (\epsilon_{12} \times A_{\lambda_1})}{(\epsilon_{11} \times \epsilon_{22}) - (\epsilon_{21} \times \epsilon_{12})} \end{aligned}$$

Spectrophotometric Titrations

Titration in which absorbance of a solution is used to determine the end point of titration is called **spectrophotometric titration**.

Types:

1) Titration outside the absorptivity cell:

Titration is carried out by usual laboratory method and absorbance after each addition is measured.

2) Titration inside the absorptivity cell:

- a) Cell is modified and capacity of the cell is increased up to 5 to 100 ml.
- b) Optimum wavelength is selected and zero absorbance is adjusted by using solvent.
- c) Solution to be titrated (titrand) is taken in the cell and cell is kept in the instrument.
- d) Known volume of titrant is added to the cell and after every addition absorbance is measured.
- e) Graph of absorbance verses volume of titrant added is plotted to find out the end point of the titration.

Spectrophotometric Titrations

Dilution correction:

According to Beer's law $A = \epsilon b C$, thus absorbance depends on concentration of solution. When titration is carried out there is change in concentration of test solution. Hence correction is required which is called as dilution correction.

Selection of Wavelength:

λ max. is selected in such a way that other absorbing substances present in the solution should not absorb at that wavelength.

Titration curve:

Plot of absorbance versus ml of titrant added is called titration curve. It consists of two lines with different slopes.

Spectrophotometric Titrations

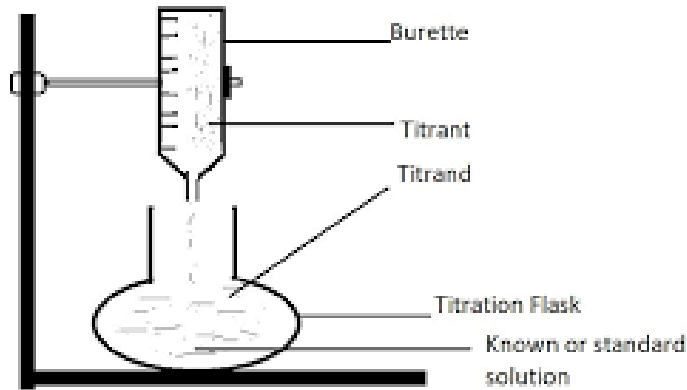
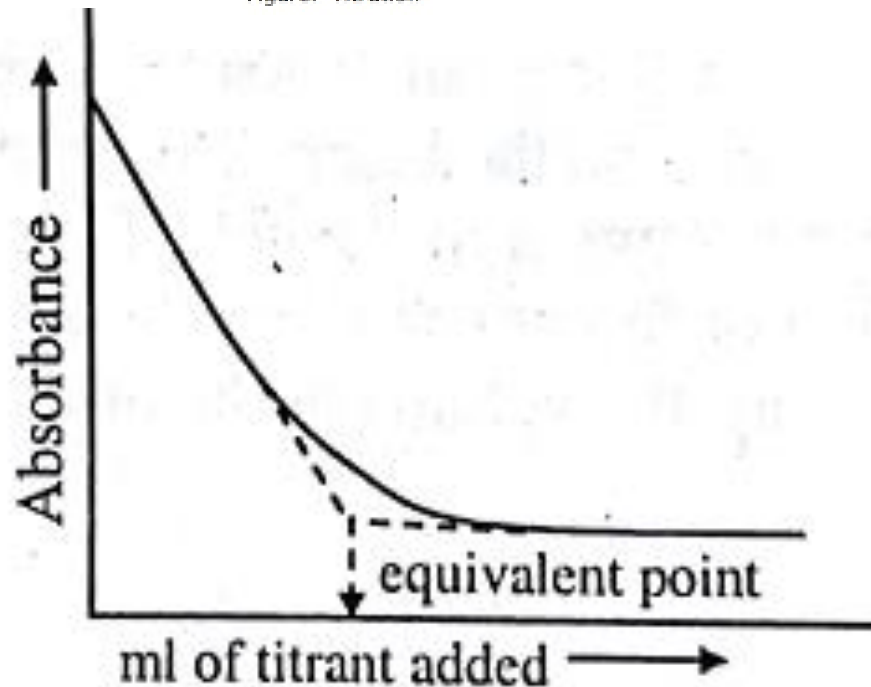


Figure:- Titration



Titration curve:

Plot of absorbance versus ml of titrant added is called titration curve. It consists of two lines with different slopes.

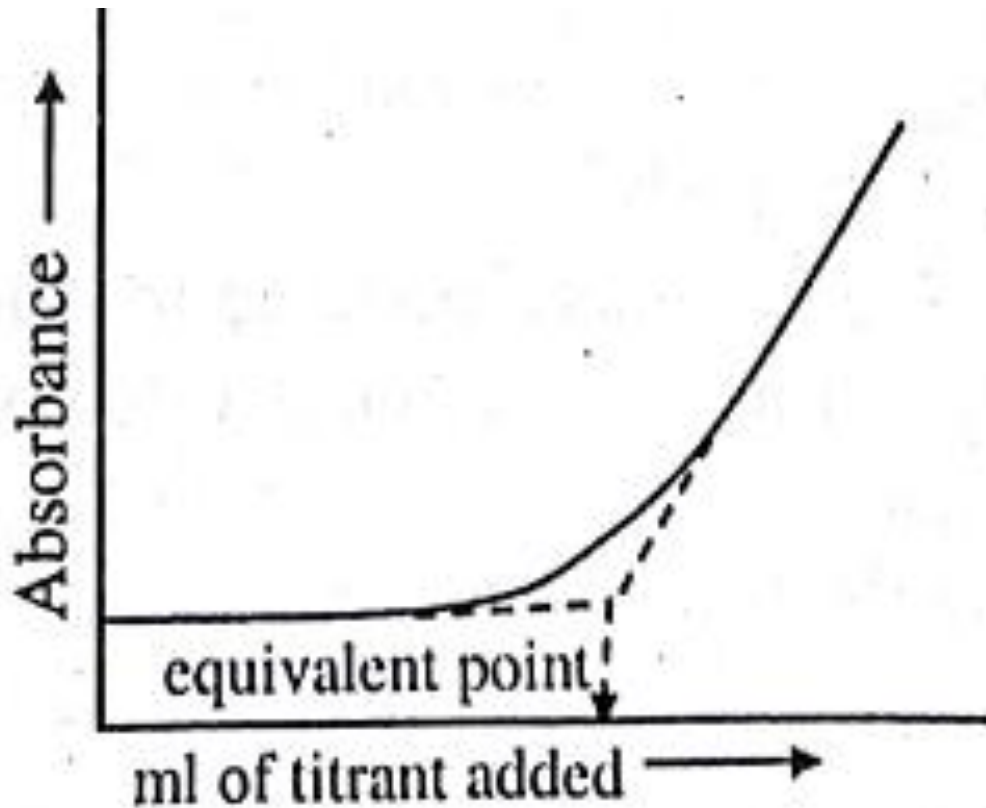
a) Only the titrand absorbs

In this titration, the titrant and the product do not absorb at the selected wavelength.

In this titration, the absorbance does not change after the equivalence point.

Example: Titration of p-Toluidine in butanol with perchloric acid at 290 nm.

Spectrophotometric Titrations



b) Only the titrant absorbs

- In this titration titrand and product does not absorb at selected wavelength.
- In this titration there is no change in absorbance up to the equivalence point. After equivalence point absorbance starts increasing due to accumulation of titrant in the test solution.
- Example: Titration of Arsenic (III) compound with bromide or bromate.

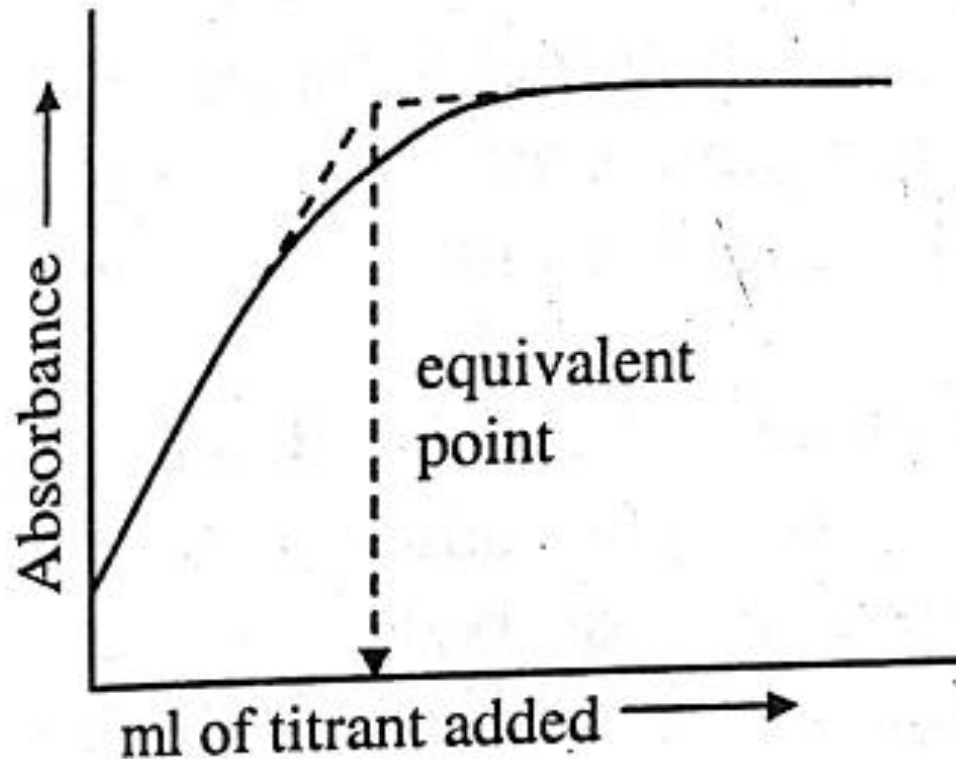
Spectrophotometric Titrations

c) Only the reaction product absorbs

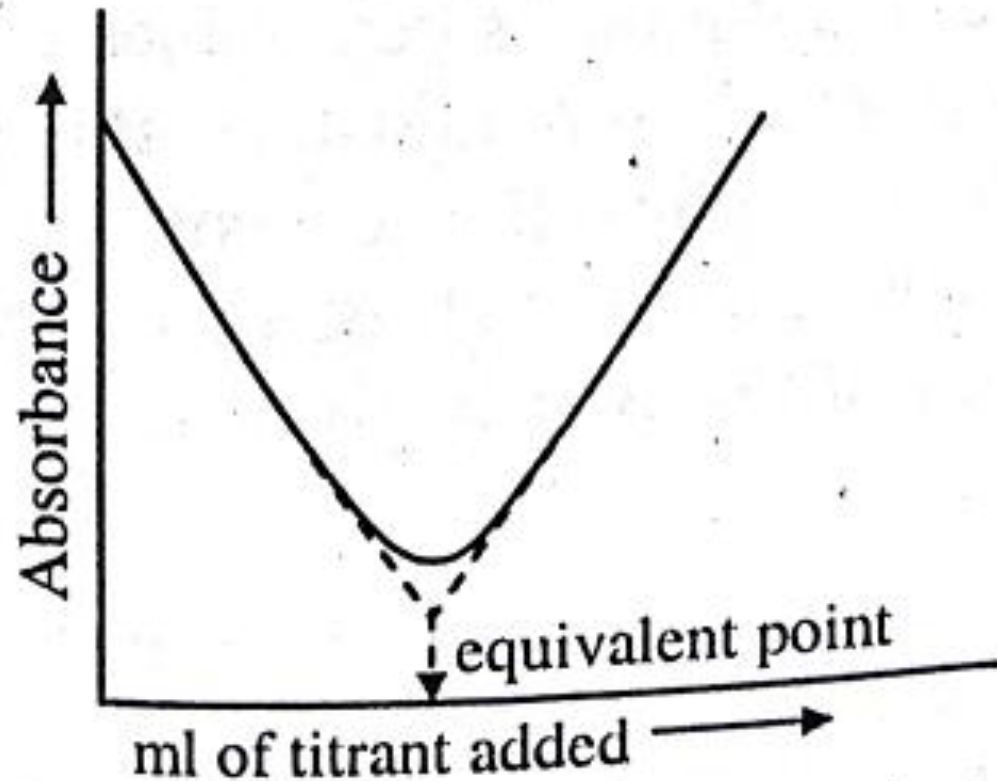
In this titration both titrant and titrand does not absorb at selected wavelength.

In this titration there is gradual increase in absorbance up to the equivalence point. After equivalence point absorbance remain constant.

Example: Titration of cupric salt with EDTA forming Cu-EDTA complex.



Spectrophotometric Titrations



d) Both titrant and titrand absorbs

In this titration both titrant and titrand absorbs but the product does not absorption at selected wavelength.

In this titration there is gradual decrease in absorbance up to the equivalence point. After equivalence point again gradual increase in absorbance is observed.

Example: Titration of coloured titrant producing colourless product.

Spectrophotometric Titrations

Advantages of spectrophotometric titration:

- 1) Method is useful for highly coloured solutions, which cannot be determined by the usual visual indicators.
- 2) End point determination is sharp and accurate.
- 3) Useful for solution with lower and higher ionic strength.
- 4) Other absorbing species do not interfere during actual titration.
- 5) Provide more accurate result than routine analysis.

Applications of Spectrophotometry

1) Quantitative analysis:

Determination of unknown concentration can be done by using any one of following method

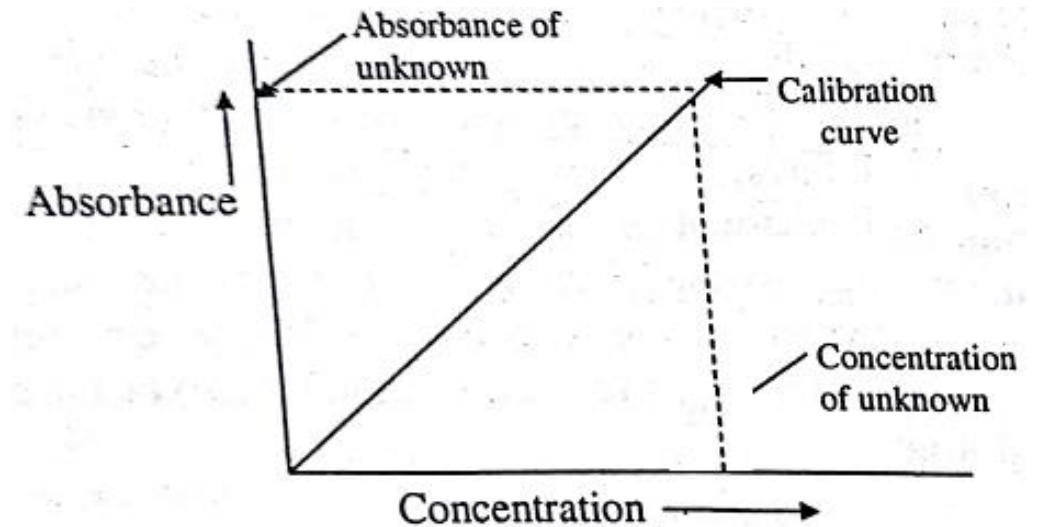
Method I

Series of standard solutions is prepared by using suitable solvent.

100% transmittance or zero absorbance is adjusted by using solvent.

Absorbance of all standard solutions are measured and plot of absorbance verses concentration is plotted to obtain calibration curve.

Absorbance of unknown solution is measured and its concentration is find out as shown in figure.



Applications of Spectrophotometry

1) Quantitative analysis:

Method II

Unknown concentration can be found out without constructing calibration curve.

Absorbance of standard solution and unknown solution are measured by using same cuvette.

Thus

$$A_1 = a_1 b_1 C_1 \text{ and } A_2 = a_2 b_2 C_2$$

As absorbing species in both solutions are identical and same cuvette is used for analysis, Hence $a_1 = a_2$ and $b_1 = b_2$

$$\text{As } A_1 = C_1 \text{ and } A_2 = C_2$$

Therefore

$$C_2 = \frac{A_2 \times C_1}{A_1}$$

Applications of Spectrophotometry

1) Quantitative analysis:

Method III

Absorbance is given as

$$A = \log P_0 / P$$

$$A = \log 1/T$$

$$A = abC$$

For a given instrument and solution P_0 , a and b remain constant

Therefore

$$C = \frac{\log P_0/P}{ab}$$

Applications of Spectrophotometry

1) Quantitative analysis:

Method II

Unknown concentration can be found out without constructing calibration curve.

Absorbance of standard solution and unknown solution are measured by using same cuvette.

Thus

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$$\text{As } A_1 = C_1 \text{ and } A_2 = C_2$$

Therefore

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Applications of Spectrophotometry

1) Quantitative analysis:

Method III

Absorbance is given as

$$A = \log P_0 / P$$

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Applications of Spectrophotometry

2) Spectrophotometric studies of coordinate compound

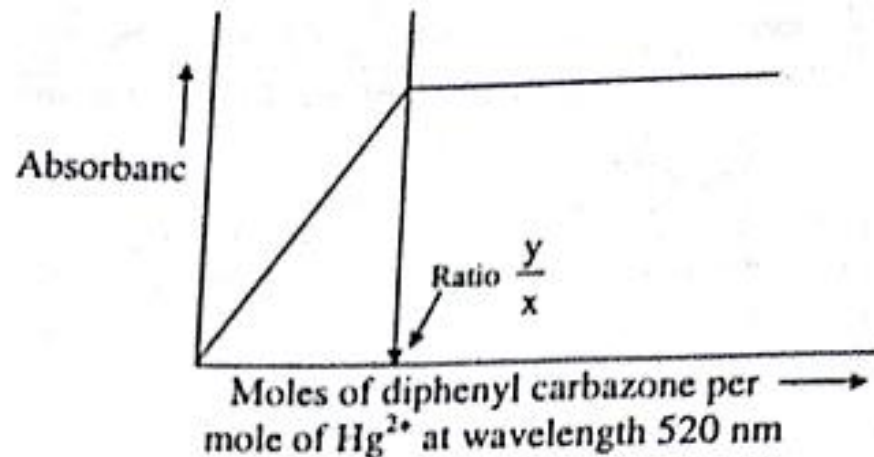
a) Mole ratio method by Ye and Jones

A series of solution is prepared in which the analytical concentration of one reactant is kept constant while that of other is varied.

Absorbance of each solution is measured as usual. A plot of absorbance versus mole ratio of the reactants is plotted.

Straight line from origin is obtained, which becomes parallel to x axis when equivalent amount of constituents are present.

Example: Mercury diphenyl carbazone complex (M_3L_2 Complex)



Moles of M	Moles of L
1	1
2	1
3	1
4	1

Applications of Spectrophotometry

2) Spectrophotometric studies of coordinate compound

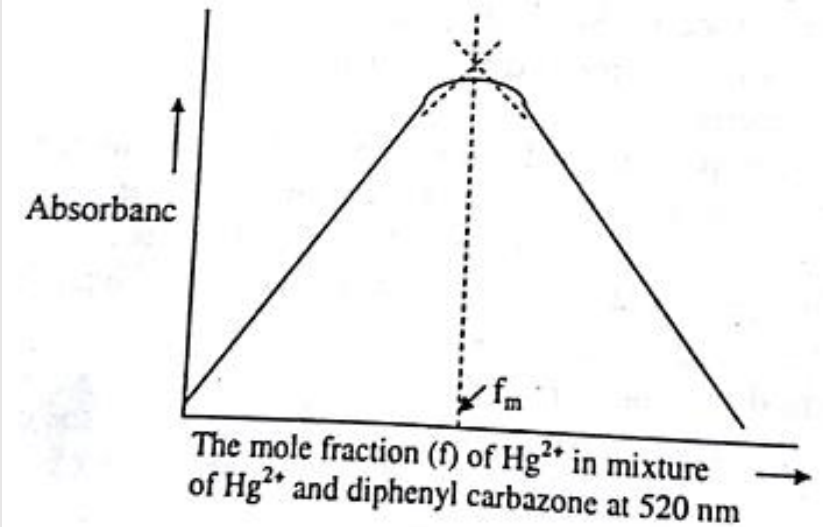
b) Job's continuous variation method:

This method is based on the measurement of a series of solutions in which molar concentrations of two reactants vary but their sum remains constant.

The absorbance of each solution is measured at a suitable wavelength and plotted versus the mole fraction of one reactant.

Curve start from zero absorbance when only one component is present.

Absorbance gradually increases with addition of second component. Maximum absorbance value is obtain when component one is totally reacted, after that absorbance start decreasing as there is no component 1 in solution



When, $f_m = 0.5$

it is 1:1 complex i.e. ML,

$f_m = 0.33$

it is 1:2 complex i.e. ML₂

$f_m = 0.67$

it is 2:1 complex i.e. M₂L

Applications of Spectrophotometry

CM	CL	Total	Mole fraction
0	10	10	0
1	9	10	0.1
2	8	10	0.2
3	7	10	0.3
4	6	10	0.4
5	5	10	0.5
6	4	10	0.6
7	3	10	0.7

Applications of Spectrophotometry

2) Spectrophotometric studies of coordinate compound

c) Slope-ratio method

This method is used for the study weak complexes.

These complexes are form with a large excess of either metal or ligand.

Two sets of solutions are prepared

The first set contains various amounts of metal ion each with the same large excess of ligand.

Second set consists of various amounts of ligand each with the same large excess of metal.

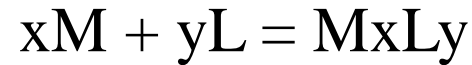
Moles of M	Moles of L
1	large excess (same quantity)
2	
3	
4	

Moles of M	Moles of L
large excess (same quantity)	1
	2
	3
	4

Applications of Spectrophotometry

c) Slope-ratio method

For the reaction,



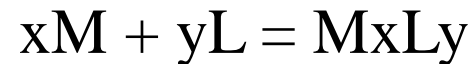
In presence of large excess of ligand, the concentration of product formed is limited by the concentration of the metal,

$$[M_xL_y] \propto [M]$$

$$A = \epsilon b [M] / x$$

Second set consists of various amounts of ligand each with the same large excess of metal.

For the reaction,



In presence of large excess metal, the concentration of product formed is limited by the concentration of the ligand,

$$[M_xL_y] \propto [L]$$

$$A = \epsilon b [L] / y$$

Applications of Spectrophotometry

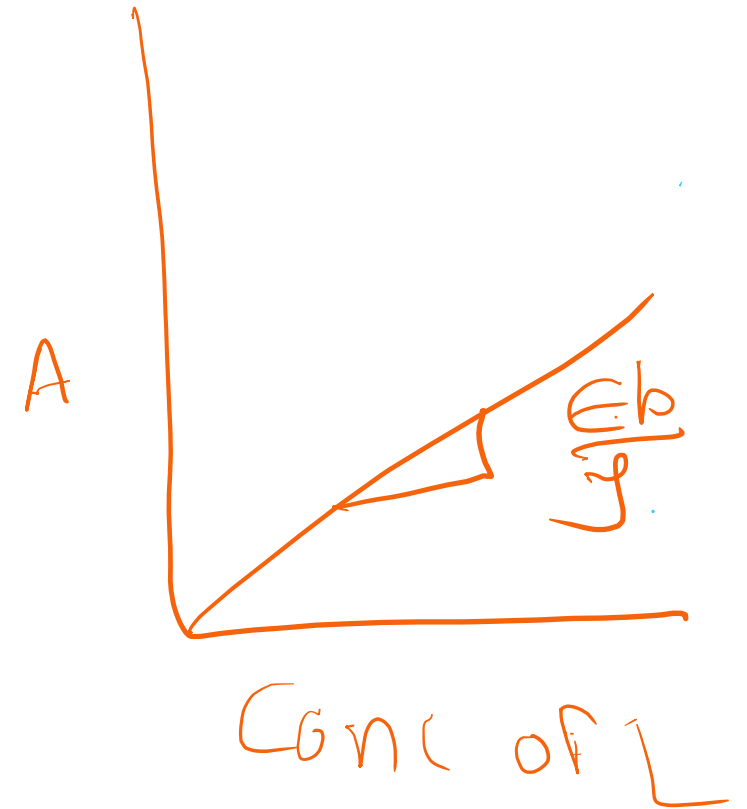
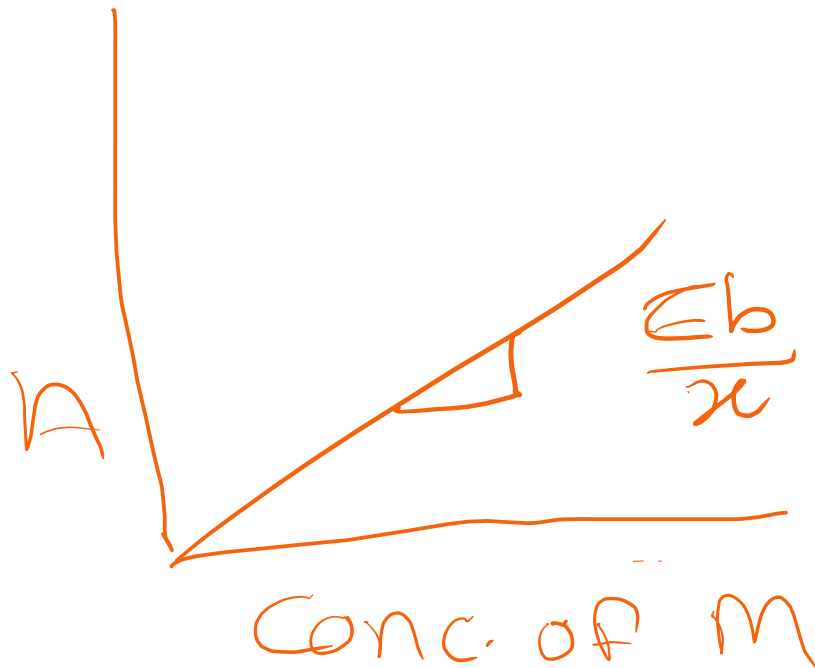
c) Slope-ratio method

Plot of A verses [M] give straight line with slope $\epsilon b / x$

Similarly

Plot of A verses [L] give straight line with slope $\epsilon b / y$

From graph we can find out x and y



Applications of Spectrophotometry

3) Determination of instability constant



If α is the degree of dissociation and C the initial concentration of the complex then at equilibrium,

$$[M] = C\alpha$$

$$[R] = nC\alpha$$

$$\text{and } [MR_n] = C(1 - \alpha).$$

The instability constant of the complex is given by,

$$K_{\text{inst.}} = \frac{(C\alpha)(nC\alpha)^n}{C(1 - \alpha)}$$

The value of α can be evaluated from the following relationship

$$\alpha = \frac{A_m - A_s}{A_m}$$

A_m is the maximum absorbance in presence of large excess of reagent and A_s is the absorbance when metal and reagent present in stoichiometric amount (i.e. 1: n).

Applications of Spectrophotometry

4) Determination of pK value of indicator:

pK value of indicator can be find out by using spectrophotometry.

Consider HMR as an indicator

It's dissociation take place as



According to law of mass action

$$K = \frac{[\text{H}^+][\text{MR}^-]}{[\text{HMR}]}$$

$$\therefore \log K = \log [\text{H}^+] + \log \frac{[\text{MR}^-]}{[\text{HMR}]}$$

$$\therefore -\log K = -\log [\text{H}^+] - \log \frac{[\text{MR}^-]}{[\text{HMR}]}$$

$$\therefore \text{pK} = \text{pH} - \log \frac{[\text{MR}^-]}{[\text{HMR}]}$$

as $\text{pK} = -\log K$ and $\text{pH} = -\log [\text{H}^+]$

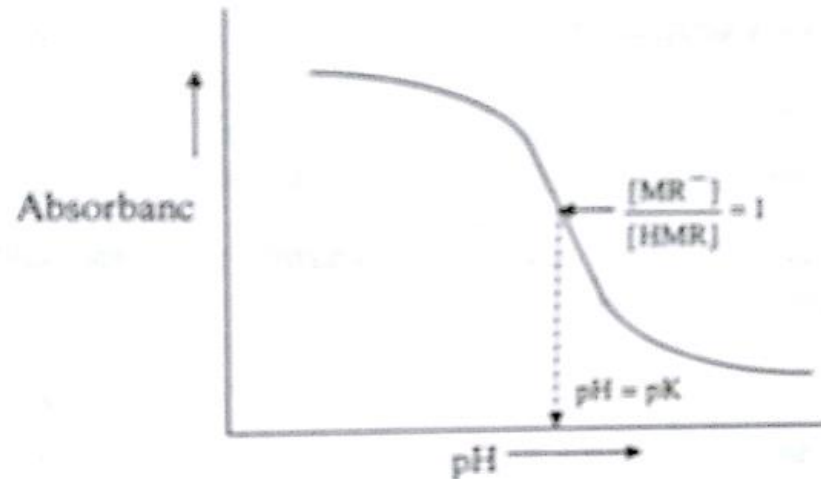
if $[\text{MR}^-] = [\text{HMR}]$ then equation reduces to

$$\text{pK} = \text{pH}$$

Applications of Spectrophotometry

Procedure

Series of solution of known pH is prepared. Equal amount of methyl red indicator is added to these solutions and absorbance is measured. Plot of absorbance versus pH is plotted.



$$\frac{[\text{MR}^-]}{[\text{HMR}]} = 1 \quad \therefore \quad \text{pK} = \text{pH}$$

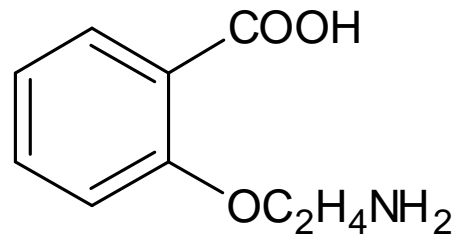
Applications of Spectrophotometry

5) Structure of organic compound

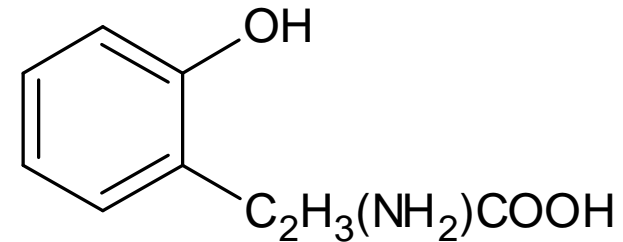
Spectrophotometry is used to find out the correct structure of organic compound.

Example Structure of Tyrosine ($C_9H_{11}O_3N$)

Absorption spectrum of tyrosine is similar to phenol, hence structure II is correct structure.



I



II

6) Structure of Inorganic complex

It is used to distinguish Cis and Trans isomers. (due to different colour)

It also help in study of tetrahedral, octahedral Squair planar structure.

Applications of Spectrophotometry

1) Qualitative Analysis:

i) Position and intensity of absorption band and chromophore

Position of absorption band and their intensity in observed spectrum help us to identify various chromophores like -N=N-, -C=C-, -C=O, -COOH, NO₂ etc.

ii) Shift in position and change in intensity of absorption band and auxochrome.

Auxochromes like -OH, -NH₂, -Or, -NHR increases the λ max value of compound.

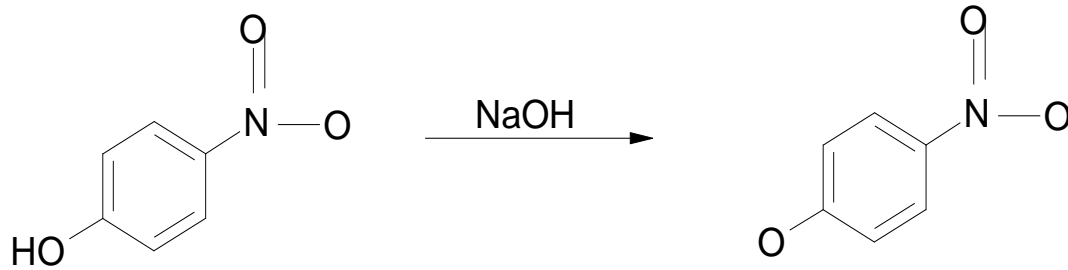
iii) Modification of absorption band due to change in structure or solvent

Applications of Spectrophotometry

1) Bathochromic shift

An absorption to longer wavelength is known as bathochromic shift or red shift.

Example –p nitro phenol shows red shift in alkaline medium.



$\lambda_{\text{max}} = 255 \text{ nm}$

(Neutral medium)

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

(Alkaline medium)

This bathochromic shift arise due to electron donating resonance effect.
(Negatively charged oxygen have more effect than neutral oxygen)

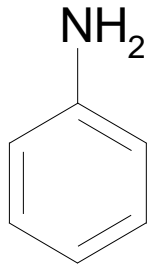
Applications of Spectrophotometry

2) Hypsochromic shift:-

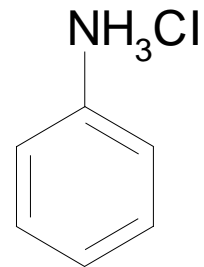
An absorption to shorter wavelength is known as hypsochromic shift or blue shift.

Example:

Aniline shows blue shift in acidic medium.(in acidic medium lone pair of electron present on nitrogen is not available for donation structure II)



$\lambda_{\text{max}} = 230 \text{ nm}$
(Neutral medium)



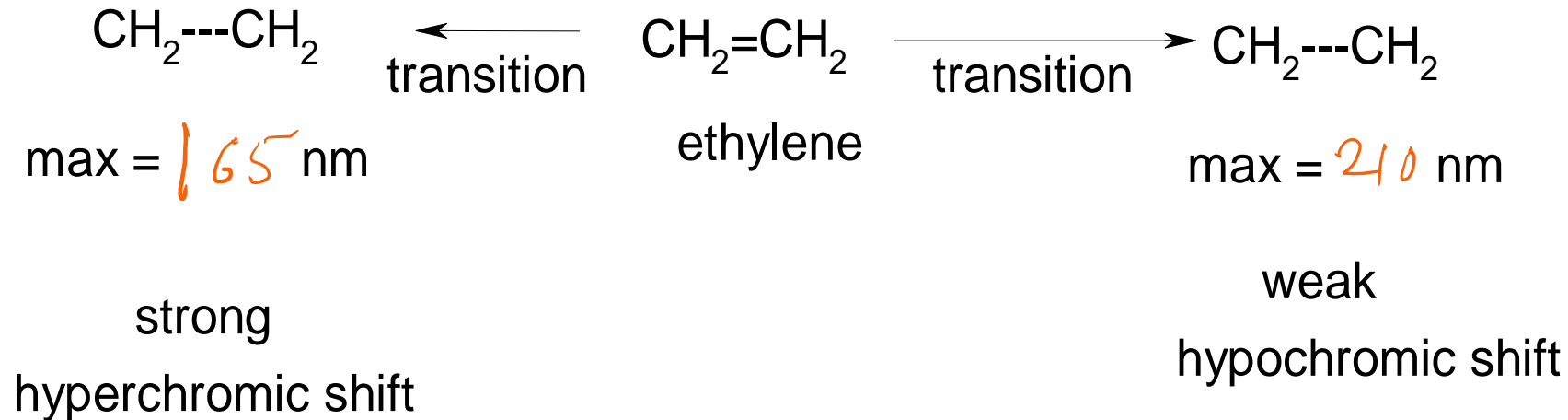
$\lambda_{\text{max}} = 203 \text{ nm}$
(Acidic medium)

Applications of Spectrophotometry

Hyperchromic shift and Hypochromic shift

Strong intensity peak is called hyperchromic shift while weak intensity peak is called as hypochromic shift.

Example:-



Peak at 165 nm is 10 times more intense than peak at 210 nm.

Numerical problem

Numerical problem 4

Transmittance of 3×10^{-4} M solution is 90%. Calculate Absorbance and molar absorptivity. (path length is 1.5 cm)

Given $C = 3 \times 10^{-4}$ M
 $B = 1$ cm, $\%T = 90\%$

Percent transmittance of solution is 90%

$$\%T = T \times 100$$

$$90 = T \times 100$$

$$90/100 = T$$

$$T = 0.90$$

$$\text{Now } A = -\log T$$

$$A = -\log 0.90$$

$$\text{Absorbance} = 0.04575$$

Now from Beer's law

$$A = \epsilon \times b \times C$$

$$0.04575 = \epsilon \times 1 \times 3 \times 10^{-4}$$

$$\epsilon = \frac{0.04575}{1 \times 3 \times 10^{-4}}$$

$$\epsilon = 0.015255 \times 10^4 \text{ L mole}^{-1}\text{cm}^{-1}$$

$$\epsilon = 152.55 \text{ L mole}^{-1}\text{cm}^{-1}$$

Numerical problem

Numerical problem 5

Calculate absorbance, if 80% incident light is transmitted.

Given radiant power of incident light =100
radiant power of transmitted light =80

$$A = \log \frac{P_o}{P_t}$$

$$A = \log \frac{100}{80}$$

$$A = \log 1.25$$

$$A = \log 1.25$$

$$A = 0.09691$$

Numerical problem

Numerical problem 6

An absorbance of 0.40 was obtained after 11.5 ml of titrating agent was added to 70 ml of an initial solution . What was the corrected absorbance of the solution. Find the %error

Dilution correction

$$A_c = \left[\frac{V_i + V_a}{V_i} \right] A_m$$

A_c = corrected absorbance, A_m = measured absorbance, V_i = original volume , V_a = volume of titrant added

$$A_c = \left[\frac{V_i + V_a}{V_i} \right] A_m$$

$$A_c = \left[\frac{70 + 11.5}{70} \right] 0.40$$

$$A_c = \left[\frac{70 + 11.5}{70} \right] 0.40$$

$$A_c = 0.4657$$

$$\% \text{ error} = \frac{11.5}{81.5} \times 100 = 14.11 \%$$

OR

$$\% \text{ error} = \frac{0.4657 - 0.40}{0.4657}$$

$$\% \text{ error} = 14.11 \%$$

Numerical problem

Numerical problem 7

1.5×10^{-4} M Copper sulphate solution shows absorbance of 0.83 at selected wavelength. Calculate concentration of unknown solution of copper sulphate having absorbance 0.23

Numerical problem

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1.5×10^{-4} M Copper sulphate solution shows absorbance of 0.83 at selected wavelength. Calculate concentration of unknown solution of copper sulphate having absorbance 0.23

**Given $A_1 = 0.83$, $C_1 = 1.5 \times 10^{-4}$ M
 $A_2 = 0.23$, $C_2 = ??$**

$$A_1 = abC_1$$

$A_2 = abC_2$ as a and b are constant

$$A_1 = C_1$$

$$A_2 = C_2$$

$$C_2 = \frac{A_2 \times C_1}{A_1}$$

$$C_2 = \frac{0.23 \times 1.5 \times 10^{-4}}{0.83}$$

$$C_2 = 0.4156 \times 10^{-4}$$

$$C_2 = 4.15 \times 10^{-5} \text{ M}$$

Numerical problem

Numerical problem 7

2.5×10^{-5} M potassium dichromate solution shows absorbance of 0.90 at selected wavelength. Calculate concentration of unknown solution of potassium dichromate having absorbance 0.63

**Given $A_1 = 0.90$, $C_1 = 2.5 \times 10^{-5} \text{M}$
 $A_2 = 0.63$, $C_2 = ??$**

$$A_1 = abC_1$$

$A_2 = abC_2$ as a and b are constant

$$A_1 = C_1$$

$$A_2 = C_2$$

$$C_2 = \frac{A_2 \times C_1}{A_1}$$

$$C_2 = \frac{0.63 \times 2.5 \times 10^{-5}}{0.90}$$

$$C_2 = 1.75 \times 10^{-5} \text{ M}$$

Numerical problem

Numerical problem 8

When 2.0×10^{-4} M solution placed in 4 5 cm length cell show absorbance of 0.35. what will be the absorbance of solution if it is placed in 1 cm path length cell.

Given $A_1 = 0.35$, $b_1 = 5$

$A_2 = ??$, $b_2 = 1$

$$A_1 = ab_1C$$

$A_2 = ab_2C$ as a and C are constant

$$A_1 = b_1$$

$$A_2 = b_2$$

$$A_2 = \frac{A_1 \times b_2}{b_1}$$

$$A_2 = \frac{0.35 \times 1}{5}$$

$$A_2 = 0.07$$

- Numerical problem 8

- When 2.0×10^{-4} M solution placed in 5 cm length cell show absorbance of 0.35. what will be the absorbance of solution if it is placed in 1 cm path length cell.