(UNIT-I) UV VISIBLE SPECTROSCOPY

Points to be covered in this topic

- ❖ Electronic transitions,
- ❖ Chromophores,
- ❖ Auxochromes,
- ❖ Spectral shifts,
- ❖ Solvent effect on absorption spectra,
- ❖ Beer and Lambert's law, Derivation and deviations.

❖ Instrumentation

➤ Sources of radiation,

➤ wavelength selectors, sample cells,

➤ detectors – Photo tube,

➤ Photomultiplier tube,

➤ Photo voltaic cell

➤ Silicon Photodiode.

❖ Applications

➤ Spectrophotometric titrations

➤ Single component and multi component

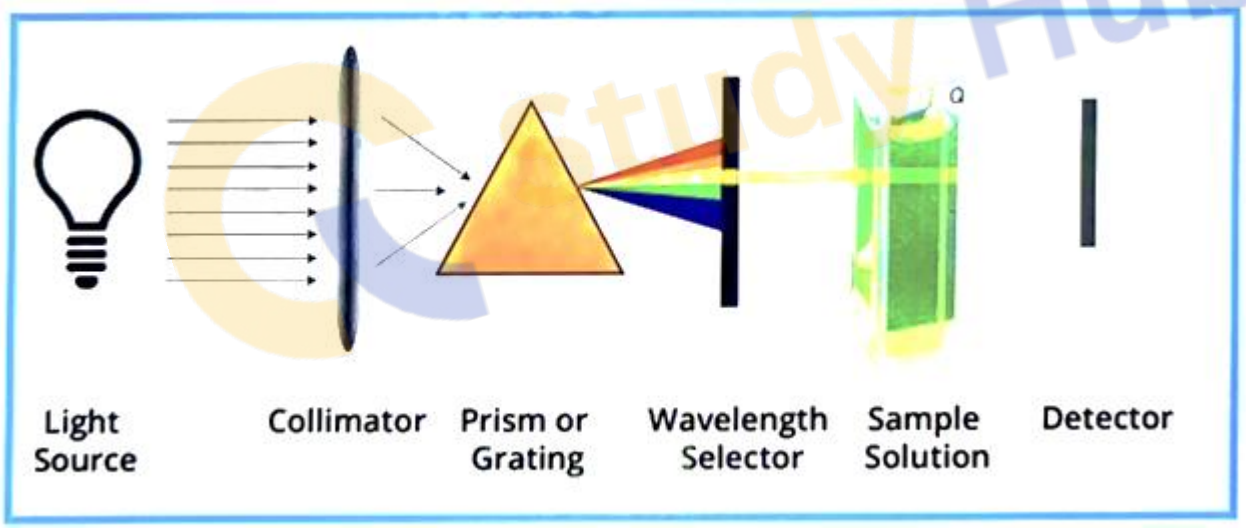
❖ INTRODUCTION

● Ultraviolet and visible spectroscopy deals with the recording of the **absorption of radiations** in the UV and visible regions of the **electromagnetic spectrum**.

● The UV region extends from **10–400 nm**. It is sub-divided into the near UV (quartz) region (**200–400 nm**) and the far or vacuum UV region (**10–200 nm**).
The visible region extends from **400–800 nm**.

● **Absorption of electromagnetic radiations** in the UV and visible regions induces the excitation of an electron from a lower to higher molecular orbital (**electronic energy level**). Since UV and visible spectroscopy involves electronic transitions, it is often called **electronic spectroscopy**.

● Organic chemists use UV and visible spectroscopy for detecting the presence and elucidating the nature of **conjugated multiple bonds or aromatic rings**.



❖ ELECTROMAGNETIC RADIATIONS (EMR)

● Electromagnetic radiations (EMR) are the **waves (or their photons)** of electromagnetic field, propagating or radiating through the space with a **specific electromagnetic radiant energy**.

● EMR or EM radiation consists of electromagnetic waves, which are **synchronized oscillations of electric and magnetic fields.**

In vacuum, electromagnetic waves travel at the speed of light.

Speed of light is denoted by **'c'** having approx. value **3×10^8 m/s** or **3×10^{10} cm/s.**

❖ LENGTH OF ELECTROMAGNETIC WAVES

● Distance of **two crest or two trough** is termed as length of electromagnetic waves.

● **Types of Electromagnetic Radiations**

● Electromagnetic radiations are of following types:

● Distance of two crest or two trough is termed as length of electromagnetic waves.

✓ Types of Electromagnetic Radiations

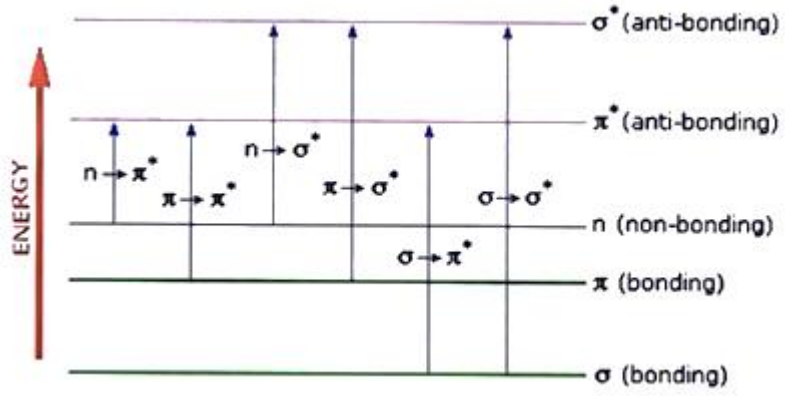
Electromagnetic radiations are of following types

Gamma rays	<0.001 nm
X-rays	0.01-10 nm
Ultraviolet	200-400
Visible light	400-800
Infrared (a) Near IR (b) (b) Mid IR (c) Far	0.8-200 0.8-2.5 2.5-15 μm (Sometimes 2.5-25 μm) 15-200 μm (Sometimes 25-200 μm)
Microwaves	0.01-1
Radio waves	1-107

❖ PRINCIPLE OF UV–VISIBLE SPECTROSCOPY (ELECTRONIC TRANSITION)

● Energy absorbed in UV-visible ranges produces changes in the electronic energy of the molecule resulting from **transition of valence electrons** in the molecule.

● There are distinct types of electrons involved in organic molecules i.e. **σ, π and n electrons.**



❖ TYPES OF ELECTRONS

● **Bonding electrons: σ and π**

● **Non-bonding electrons: n**

● **Antibonding electrons: σ* and π***

σ electrons: These electrons are involved in **saturated bonds**, and known as σ bonds. These bonds are excited by the UV-visible radiation.

Eg. CH_3–CH_3

π electrons:

These electrons are involved in **unsaturated hydrocarbons**.

Eg. CH_2=CH_2

n electrons:

These are the electrons present where **hetero atoms** are present like nitrogen, oxygen, halogen etc.

Eg. $CH_3–CH_2–OH$

- Any molecule has either **n, π, σ or combination** of these bonding and non-bonding electrons absorbs the characteristic wavelength and undergo transition from ground level to excited state.

(energy level diagram showing σ, π, n and their antibonding orbitals)

❖ TYPES OF TRANSITIONS

- **σ ⟶ σ* transition:** This is the **high energy** transition; means it requires higher energy for the transition of electrons from ground state to the excited state.
This transition falls in **vacuum UV region (150 nm).**

- It (**σ ⟶ σ*** transitions) is mainly observed in saturated hydrocarbons.
Eg. methane, ethane etc.

- **n ⟶ σ* Transition:** This is low-energy transition than σ ⟶ σ* transition.

Below is **every word extracted and rewritten exactly in the same structure, formatting style, order, and headings** as in your uploaded screenshots.

- **n ⟶ σ* Transition:** This is low energy transition than σ ⟶ σ* transition means it require lesser energy than σ ⟶ σ* transitions for the transition of electrons from ground state to the excited state.

This transition falls in **near UV region (150–250 nm)**, n ⟶ σ* transitions mainly occur in saturated compounds with heteroatoms (like O/N/S/Halogen) such as alcohols, water etc.

Eg CH_3OH, CH_3I, $CH_3–S–CH_3$ etc.

● **π ⟶ π* Transition:** Less energy is required as compared to **n ⟶ π*** transitions fall in the range of **200–800 nm** based on conjugation.

π ⟶ π* transition occurs in unsaturated compounds having double or triple bonds.

● Eg. Compounds such as aromatic compounds, alkenes, alkynes show this type of transition.

● **n ⟶ π* Transition:** Lowest energy required for the transition from ground state to excited state.

This transition falls nearly **290 nm**, n ⟶ π* transition occurs in unsaturated compounds with heteroatom such as aldehyde, ketone, carbonyl compound etc.

● **The energy required for various transition behaves as following order:**

σ ⟶ σ* > n ⟶ σ* > π ⟶ π* > n ⟶ π*

● **Based on the energy and intensity transition are classified as:**

(a) High energy / Intense transitions:

n ⟶ σ*

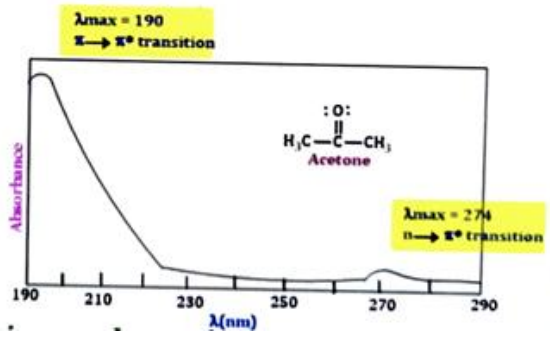
σ ⟶ σ*

● **Low energy / weak transition:**

n ⟶ π*

❖ **Presentation of spectra**

● The ultraviolet–visible spectrum is generally recorded as a **plot of absorbance versus wavelength.**



- **λ_{max}** is the wavelength where maximum absorption occurred.

- **Solvent in UV spectroscopy:**

The choice of the solvent to be used in ultraviolet spectroscopy is quite important; usually solvents that are not conjugated are most suitable for this purpose.

Eg. **Methane (CH_4), ethanol (CH_3OH), water** etc.
but not solvents like toluene as it is the nonconjugated solvents.

❖ CHROMOPHORE

- It is defined as any **isolated covalently bonded group** that shows a characteristic absorption of electromagnetic radiation in the UV or visible region.
A chromophore is the part of a molecule that **absorbs UV or visible light.**

- It is a Greek word (Chroma = Color and Phoros = bearer).

- Compound containing chromophores known as Chromogen Eg. **C=C, C=O**

✓ **Chromophores are generally of two types such as**

(a) Independent Chromophore:

If one chromophore is required to impart the color.

Eg. **Azo group (-N=N-), Nitro group ($-NO_2$)** etc.

(b) Dependent Chromophore:

If more than one chromophore is required to impart the color.
Eg. **acetone** having one ketone group that is colorless whereas **diacetyl** having two ketone groups is having yellow color.

❖ AUXOCHROME

● It is the functional group of atoms with **one or more lone pair of electrons** when attached to chromophore, alters both the wavelength and intensity of absorption.

● If these groups are in **direct conjugation with π-system** of the chromophore, they may increase the wavelength at which the light is absorbed and as a result **intensify the absorption.**

● A feature of auxochrome is the presence of at least one lone pair of electrons which can be viewed as extending the conjugated system by resonance.

There are mainly two types of auxochrome

(a) Acidic – COOH, –OH, $–SO_3H$

(b) Basic – NH_2, –NHR, $–NR_2$

❖ ABSORPTION BANDS

● There are **four types of absorption bands** are classified by the way electronic transition.

(a) K-Bands

(b) R-Bands

(c) B-Bands

(d) E-Bands

Due to which transitions and the compounds in which the bands are observed are explained as

a) K-Bands: This type of bands be seen in the **conjugated compounds** such as dienes, polyenes, enone, aromatic compounds substituted by chromophore like **nitrobenzene**

whereas benzene is the aromatic conjugated system and **$-NO_2$ is the chromophore group.**

Intensity of K band is **greater than** 10^4 **($\varepsilon max > 10^4$).**

K bands occurs due to the **$\pi \longrightarrow \pi^*$** transitions.

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b) R-Bands: R bands occurs due to the **$n \longrightarrow \pi^*$** transitions of single chromophore group and having **at least one lone pair of electrons** on the heteroatom.
R bands are also called as **forbidden bands.**

Intensity of R-band is **less than 100 ($\varepsilon < 100$).**

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c) B-Bands: B bands occurs due to **$\pi \longrightarrow \pi^*$** transition in **aromatic or heteroaromatic compounds.**
Benzene shows absorption peaks between **230–270 nm**, when chromophore group attached to benzene ring, the B-bands are observed at **longer wavelength** than more intense K-bands.

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d) E-Bands: E bands occurs in **electronic transitions in the benzenoid system** of three ethylenic bonds which are closed cyclic conjugation.

These are further characterized as **E_1 and E_2 bands** observed at **184 nm and 204 nm** respectively.

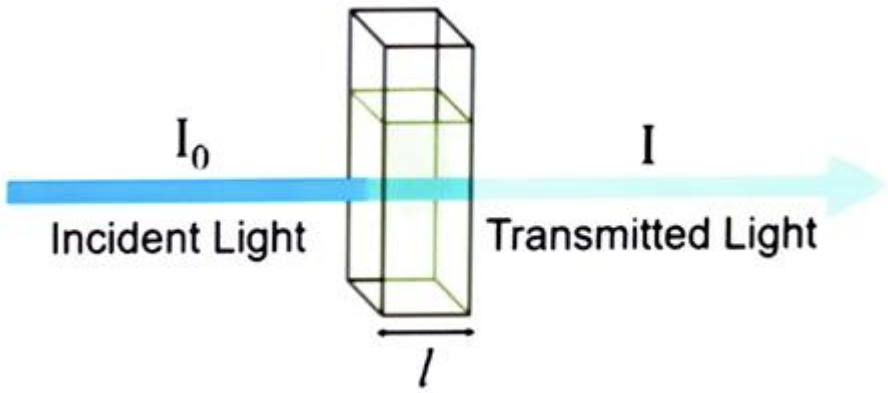
E_1 bands appear at lower wavelength (184 nm) are more intense than E_2 bands as band

E_1 **εmax at 184 nm is 5000** and E_2 band **εmax at 204 nm is 7900.**

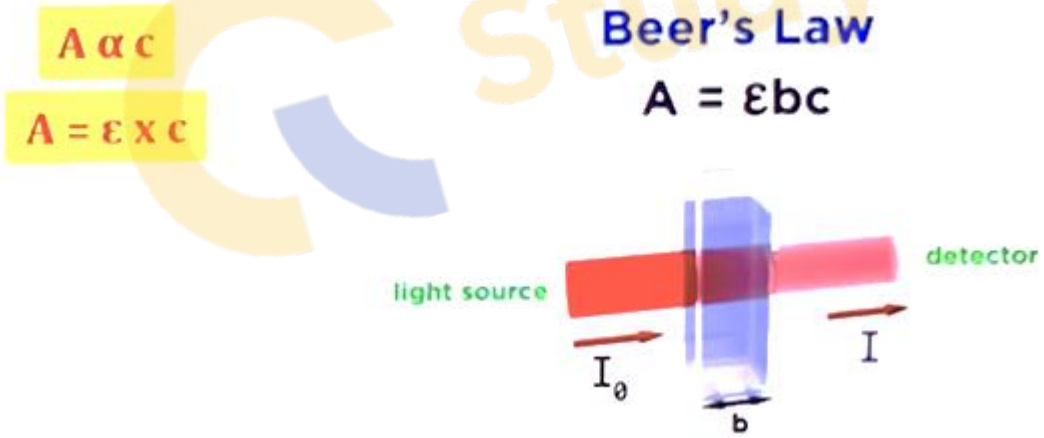
❖ BEER–LAMBERT'S LAW

- Many compounds absorb ultraviolet (UV) or visible (Vis) light.

The diagram below shows a beam of **monochromatic radiation** of radiant power **I_0**, directed at a sample solution, some amount of absorption takes place and **beam leaving the subject is I.**



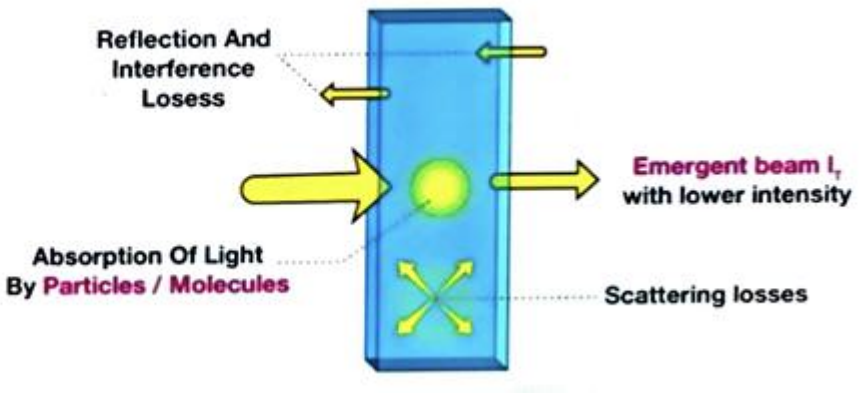
Beer's Law: When a beam of **monochromatic radiation** is passed through the absorbing medium, then the **decrease in the intensity** of the radiation is directly proportional to the **concentration (c)** of the solution.



Lambert's Law: When a beam of **monochromatic radiation** is passed through the absorbing medium, then the **decrease in the intensity** of the radiation is directly proportional to the **thickness / pathlength (l)** of the solution.

$A \alpha l$

$A = \varepsilon x l$



❑ Beer–Lambert's law

● When a beam of **monochromatic radiation** is passed through the absorbing medium, then the **decrease in the intensity of the radiation is directly proportional to the thickness / pathlength (l)** as well as concentration of the solution.

● The Beer-Lambert Law can be expressed in the form of the following equation,

$$A \propto c \times l$$

$$A = \varepsilon \times c \times l$$

A = Absorbance

l = Optical path length, i.e. dimension of the cell or cuvette (cm)

C = Concentration of solution (mol dm^{-3})

ε = Molar extinction coefficient, which is constant for a particular substance at a particular wavelength (L Mol^{-1} cm^{-1})

● If the **absorbance** of a series of sample solutions of **known concentrations** are measured and plotted against their corresponding concentrations, the plot of **absorbance versus concentration should be linear.**

● The ratio I_0 / I_t is termed as **transmittance T**, and the ratio $\log I_0 / I_t$ is termed as **absorbance A.**

$$A = \log I_0 / I_t$$

$$T = I_t / I_0$$

✓ DERIVATION OF BEER–LAMBERT'S LAW

● When a beam of monochromatic radiation is passed through the absorbing medium, then the **decrease in the intensity of the radiation is directly proportional to the thickness / pathlength (l)** as well as concentration of the solution.

● The Beer-Lambert Law can be expressed in the form of the following equation,

$$-\frac{dI}{dl} \propto I \propto C$$

$$-\frac{dI}{dl} = k \times I \times C$$

$$\frac{dI}{dl} = -k \times I \times C$$

$$\frac{dI}{I} = -k \times C \times dl$$

Where,

d = Small change in the intensity or length

I = Intensity of the radiation after passing through

***l* = Thickness of the solution**

k = Proportionality constant (depends on sol.)

c = Conc. of Sol. (in M)

I_o = Initial intensity of the radiation

Inteegrate the int*ensity from* I_0 *to* I *and* int*egrate the length from* 0 *to* l

$$\int_0^I \frac{dI}{I} = -k \times C \times \int_{l=0}^{l=l} \frac{dl}{I}$$

$$[\ln I]_0^I = -k \times C \times [\ln l]_0^l$$

$$\ln I - \ln I_0 = -k \times C \times [\ln l - 0]$$

$$\ln \frac{i}{i_0} = -k \times C \times l$$

$$\ln \frac{i}{i_0} = k \times C \times l$$

$$\int \frac{dx}{x} = \log x$$

When lim *its are applied*

$$\int \frac{dx}{x} = \ln x$$

$$\ln = \log_e = 2.303 \log_{10}$$

$$2.303 \log \frac{I_0}{I} = k \times C \times l$$

$$\frac{1}{c \times l} \log \frac{I_0}{I} = \frac{k}{2.303}$$

$$\frac{1}{c \times l} \log \frac{I_0}{I} = \varepsilon$$

$$\text{og} \frac{I_0}{I} = \varepsilon \times C \times l$$

$$A = \varepsilon \times C \times l$$

- **Important Units:**
  - **Unit of c = Mol/L**
  - **Unit of *l* = cm**
  - **Unit of ε = L $Mol^{-1}cm^{-1}$**
  - **Unit of A = None**

Because A = $\log \frac{I_0}{I}$

❖ Deviations of Beer–Lambert Law

● The linearity of Beer-Lambert law is limited by chemical and instrumental factors.

Causes of nonlinearity include:

(a) The law is not obeyed if the radiation is **not monochromatic**

(b) Deviation in absorptivity coefficients at high concentrations **(>0.01M)** due to electrostatic interaction between molecule and close proximity.

(c) **Scattering of light** due to particulates of the sample.

(d) **Fluorescence or Phosphorescence** of the sample.

(e) Changes in **refractive index** at high analyte concentration.

(f) **Shifts in chemical equilibria** as a function of concentration.

(g) **Chemical deviation** i.e. dissociation or reaction with the solvent.

● Problem 1:

The solution having maximum absorbance of 275 nm.

Molar extinction coefficient is 8400 M^{-1} cm^{-1} and the path length is 1 cm.

Using a spectrophotometer, you find that absorbtion at 275 nm is 0.70.

What is the concentration of guanosine?

Solution:

$A = \varepsilon \times c \times l$

$0.70 = (8400\ M^{-1}\ cm^{-1}) \times (1\ cm) \times c$

$c = 0.70 / (8400\ M^{-1}\ cm^{-1} \times 1\ cm)$

$c = \mathbf{8.33 \times 10^{-5}}$

● **Problem 2:**

There is a substance in a solution (4 g/liter).

The length of cuvette is 2 cm and only 50% of the certain light beam is transmitted.

What is the extinction coefficient?

Solution:

Using Beer–Lambert Law, we compute the absorption coefficient.

$-\log (I_t / I_0) = -\log (0.5 / 1) = A$

$A = \varepsilon \times c \times l$

$A = \varepsilon \times 4 \times 2$

$A = 8\varepsilon$

$8\varepsilon = -\log (0.5 / 1)$

$\varepsilon = 0.0376\ M^{-1}\ cm^{-1}$

❖ INSTRUMENTATION

➤ Introduction

The essential parts of a spectrophotometer are:

(1) Radiation Source:

Both the tungsten and D_2 lamp are present in the UV–visible spectrophotometer. They are stable and efficient.

(2) Wavelength Selector:

It constitutes three essential parts:

(i) **Filter** Absorption and interference filters are mainly used.

(ii) **Monochromator:** It gives the desired wavelength in the entire UV or visible region.

(iii) **Slits:** There are two slits, i.e., entrance slit and exit slit.

(3) Cells or Cuvettes: For holding the sample solution and the pure solvent (reference).

(4) Detector: The most commonly used detectors are photo-emissive cells or phototubes and photomultiplier tubes.

(5) Recording System: Recording is done by recorder pen.

(6) Power Supply

Spectrophotometers are of two types:

1. **Single-beam spectrophotometers**
2. **Double-beam spectrophotometers**

❖ SOURCES OF RADIATION

● The best source of light is the one which is more stable, more intense and which gives range of spectrum from **180–360 nm (up to 400 nm).**

The different sources available are:

1) Hydrogen Discharge Lamp:

- In these lamps, hydrogen gas is stored under relatively **high pressure**.

When an **electric discharge** is passed through the lamp, excited hydrogen molecules will be produced which **emit UV radiations.**

- The **high pressure** in the hydrogen lamps causes the hydrogen to **emit a continuum** rather than a simple hydrogen spectrum.

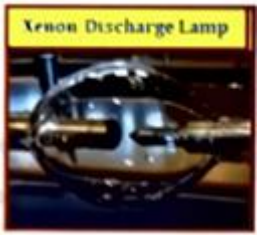
- Hydrogen lamps cover the **range of 3500–1200 Å.**

These lamps are stable, robust, and widely used.

- **If deuterium (D_2) is used instead of hydrogen,**

the emission intensity is increased by as much as a factor of **3 at the short-wavelength end** of the UV range.

2) Deuterium Lamp: It is similar to **hydrogen discharge lamp**, but filled with **deuterium in the place of hydrogen**.
It offers **3–5 times more intensity** than other types.
Deuterium lamps are **more expensive** than hydrogen lamps but are used when **higher intensity is required.**



3) Xenon Discharge Lamp: In this lamp, xenon at **10–30 atmospheric pressure** is filled in and it has two tungsten electrodes.
The intensity is **greater than the hydrogen discharge lamp.**

4) Mercury Arc: This contains **mercury vapour** and offers bands which are sharp.
The **spectrum is not continuous**.
Hence, it is not widely used.

5) Tungsten Lamp: This lamp is similar in its functioning to an electric light bulb.
It is a tungsten filament heated electrically to **white heat**.

It has two shortcomings:

- The intensity of radiation at **short wavelengths (<350 nm) is small.**
- To maintain a constant intensity, the electrical current to the lamp must be carefully controlled.

❑ Wavelength Selectors

Wavelength selectors consist of three parts:

1. **Filters**,
2. **Monochromators**, and
3. **Slits.**

- **Filters**
- Filters provide high radiation throughout, approximately **50–80% efficiency.**

The two types of filters are:

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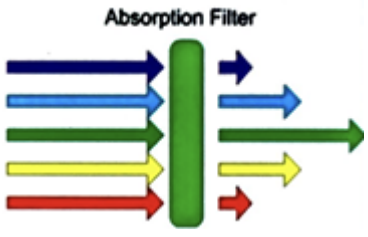
1) Absorption Filters:

- These filters derive their effects from **bulk interactions of radiation** within the material. Absorption filters are produced in a variety of **host materials**, like **gelatin, glass, liquid, and plastic**.



- **Glass filters** are used in automated **chemical analysis equipment** and colorimetry.
- The scattering type depends on **scattering crystals** formed within the glass mass through a **reduction and thermal treatment.**

● **Shorter wavelengths** are scattered and absorbed, while longer wavelengths are unaffected.

Cut-on and cut-off (or sharp-cut) filters are widely used as **blocking filters** to suppress unwanted spectral orders from interference filters and diffraction gratings.

● There are a wide variety of **plastic filters**, of both **sharp cut and intermediate bandwidth types**.

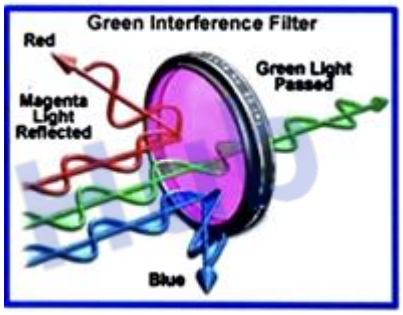
Plastic filters may be produced by bulk colourants introduced into the basic batch or through subsequent dye treatments of **clear base stock**.

Cut-on types, unlike their glass counterparts, **exhibit no fluorescence** in the visible region.

2) Interference Filters:

● These filters, as the name implies, are based on the phenomenon of **optical interference**.

A simple **two-interface (Fabry–Perot) filter** consists of a dielectric spacer film (**CaF_2, MgF_2, or SiO**) sandwiched between two parallel, partially **reflecting metal films**, usually of silver.



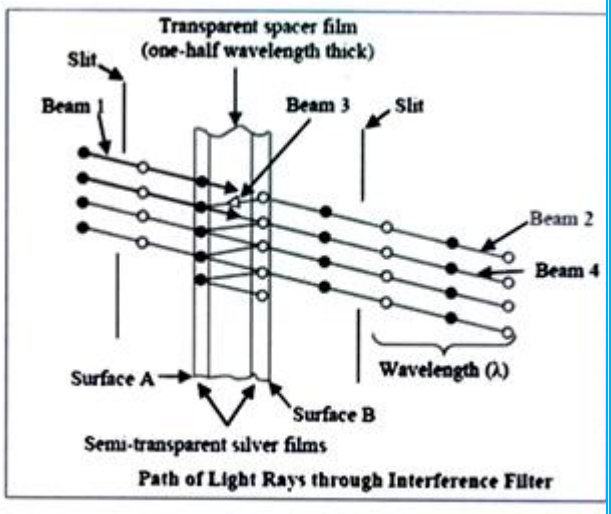
● The **thickness of dielectric film** is controlled to be only **one, two, or three half-waves thick.**

These are referred to as **first-, second-, or third-order filters**, respectively.

A portion of the incident radiation normal to the filter (beam 1) passes through beam 2,

while another portion (beam 3) is reflected back from surface B to surface A.

● Portion of this reflected radiation is again reflected from surface A through the dielectric layer and exits as **beam 4**, parallel (actually coincident) to **beam 2**.



● Thus, the path travelled by **beam 4** is longer than that of **beam 2** by twice the product of the dielectric spacer thickness and its **refractive index**.

● When the layer thickness (b) is half the wavelength of the radiation to be transmitted in the **refractive index (η)** of the dielectric, beams 2 and 4 are in phase and **interfere constructively**.

✓ **The expression for central wavelengths at which full reinforcement occurs is:**

$$\lambda = \frac{2\eta b}{m}$$

Where,

m = Order number.

Since partial reinforcement occurs for other path differences, the filter actually transmits a **band of radiant energy**.

● The angle of incidence of the radiation must be **90°**.

Any phase shifts on reflection are ignored.

● The **bandwidth is 10–15 nm**, Full Width at Half Maximum (FWHM) transmission; the maximum transmission is **usually 40%** with this type of filter.

❑ **Monochromators (Prisms and Gratings)**

● The monochromator is used to disperse the radiation according to the wavelength. The essential elements of a monochromator are an **entrance slit**, a **dispersing element**, and an **exit slit**.

- **The entrance slit sharply defines the incoming beam of heterochromatic radiation.**

- The dispersing element disperses the heterochromatic radiation into its component wavelengths whereas **exit slit allows the nominal wavelength together with a band of wavelengths** on either side of it.

- Position of the dispersing element is always adjusted by **rotating it** to vary the nominal wavelength passing through the exit slit.

- The dispersing element may be a **prism or grating**.
The prisms are generally made of **glass, quartz, or fused silica.**

- Glass has the highest resolving power but it is **not transparent to radiations having wavelength between 2000–3000 Å** because glass absorbs strongly in this region.

- **Quartz and fused silica prisms** are transparent throughout the entire UV range and are widely used in **UV spectrophotometers**.

Fused silica prisms are little more transparent in the **short wavelength region** than quartz prisms, and are used only when very intense radiation is required.

- The **mirrors** in the optical system are front surfaced because **glass starts to absorb in the UV region.**

✓ Characteristics of a Monochromator

1. It allows the largest entrance slit width for the band pass required.
2. It has the **highest dispersion**.
3. The **largest optics** is affordable.
4. Longest focal length is affordable.
5. Highest groove density will accommodate the spectral range.
6. Optics and coatings are appropriate for **specific spectral range**.

7. Entrance optics will optimise end-use.
8. If the instrument is to be used at a **single wavelength in a non-scanning mode**, it must be possible to adjust the exit slit to match the size of the entrance slit image.

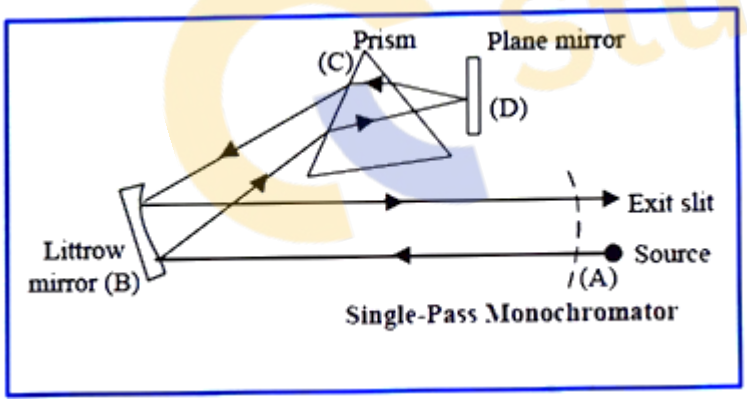
❑ Types of Monochromator

1) Prism Monochromator:

● The sample is kept at or near the **focus of the beam**, just before the entrance slit **(A)** to the monochromator.

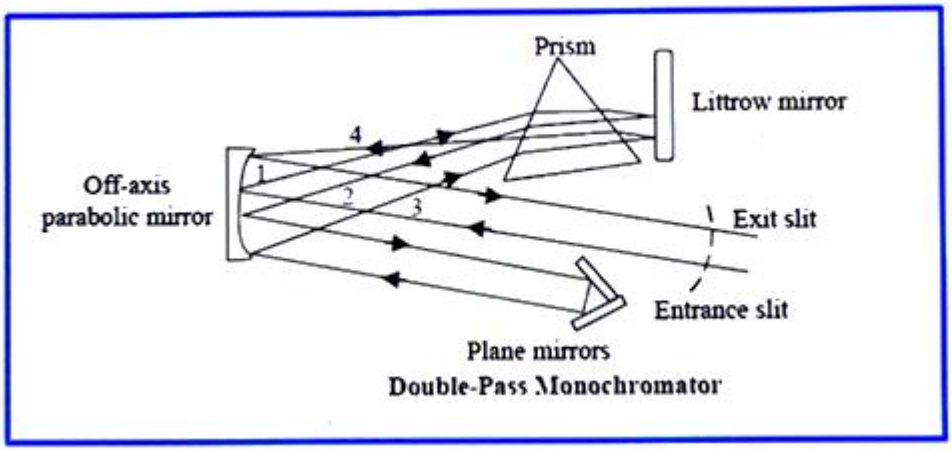
● **The radiation from the source after passing through the sample and entrance slit, strikes the off-axis parabolic Littrow mirror (B), which renders the radiation parallel and sends it to the prism (C).**

The dispersed radiation after reflecting from a **plane mirror (D)** returns through the prism a second time and focuses onto the exit slit of the monochromator, through which it **finally passes into the detector section.**



● **In double-pass monochromator, there occurs a total of four passes of radiations, i.e., (1), (2), (3) and (4) through the prism.**

The double-pass monochromator produces **more resolution** than the monochromator in the radiation, before it finally passes on to the detector.



2) Grating Monochromator:

- Gratings provide an alternative means of **producing monochromatic light**.

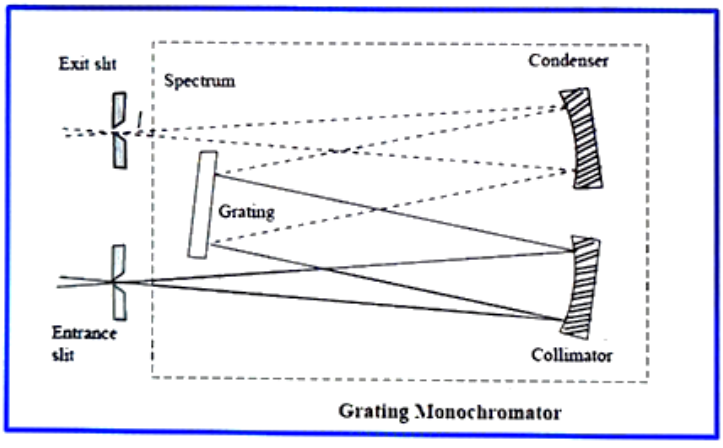
A diffraction grating consists of a series of **parallel grooves (lines)** on a **reflecting surface** that is produced by taking a replica from a master carefully prepared using a machine, or from one which is **holographically generated**.

- The grooves can be considered as **separate mirrors** from which the reflected light interacts with the light reflected from neighbouring grooves to **produce interference**, and so to select preferentially the wavelength that is reflected when the angle of the grating to the **incident beam** is changed.

- When **parallel radiation** illuminates a reflecting diffraction grating, the multiple reflections from the mirror grooves **will overlap and interfere with each other**.

- If the reflected waves are **in phase**, interference is said to be **constructive** and the reflected light is **not affected**.

If the reflected waves are **out of phase**, there is **destructive interference** and light of the wavelength at which such interference occurs will **not be propagated.**



- **The relationship that determines the wavelength of reflected light is expressed by:**

$$n\lambda = 2d\sin\,\theta$$

Where, n = Order

d = Separation of the reflecting surfaces (or lines)

θ = Angle of incidence of the radiation

- **Grating monochromator possesses the following advantages over prism monochromator:**

i) Grating can be made with materials **not attacked by moisture** like aluminium.
ii) Grating monochromators can be used over considerable **wavelength ranges**.
iii) Grating offers **better resolution and energy transfer**.
iv) Grating has **constant bandwidth** due to linear dispersion.
v) Grating requires **less complicated wavelength drive mechanism**.
vi) **Stray light** is limited to imperfections at the grating surface.

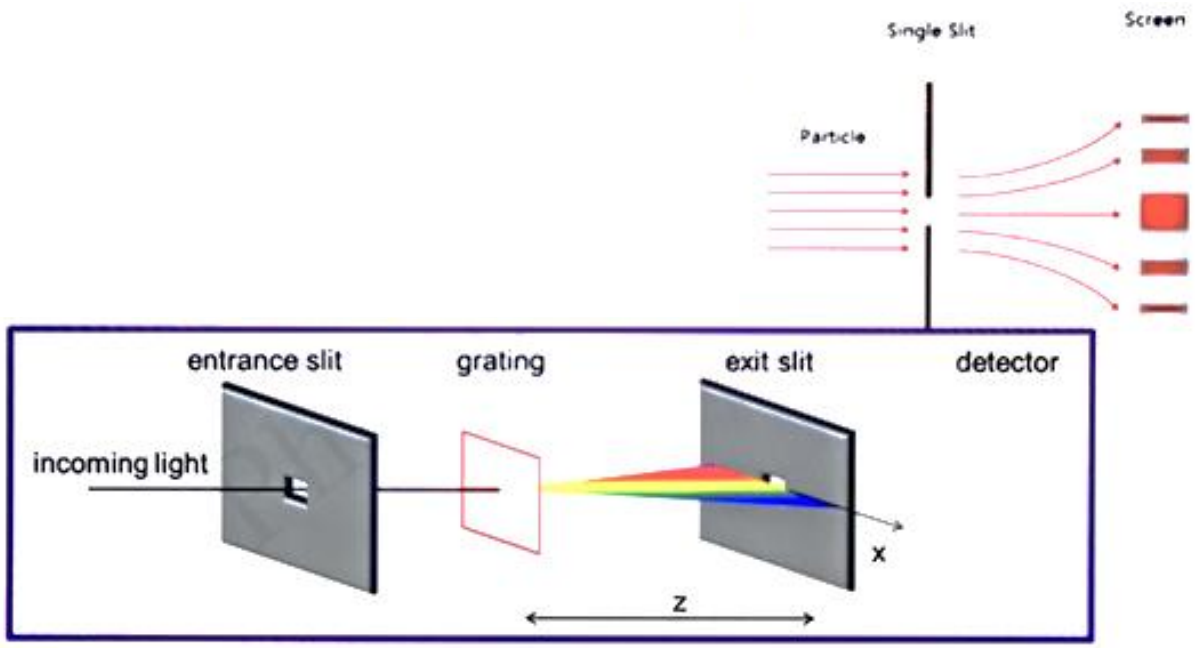
❑ SLITS

- There are **two slits**, i.e., **entrance slit** and **exit slit**.

The main function of the **entrance slit** is to provide a narrow source of light so that there should be **no overlapping of monochromatic images.**

- From this, the **exit slit** selects a narrow band of **dispersed spectrum** for observation by the detector.

- In practical spectrophotometry, the monochromator module is **not capable** of isolating a **single wavelength** of radiation.



● From the **continuous spectrum** emitted by the source ⟶ a definite band of radiation is **passed** by the monochromator.

This finite band arises from the slit distributions.

The entrance or aperture of a monochromator is a **long, narrow slit** whose width is generally adjustable.

● Inside the monochromator, the rays diverge from the entrance slit and **illuminate the collimator mirror**, which renders the rays parallelly and focuses them on the dispersing element.

Leaving the collimator, the parallel set of rays is a **broadened version of the entrance slit.**

❑ Sample Cells or Cuvettes

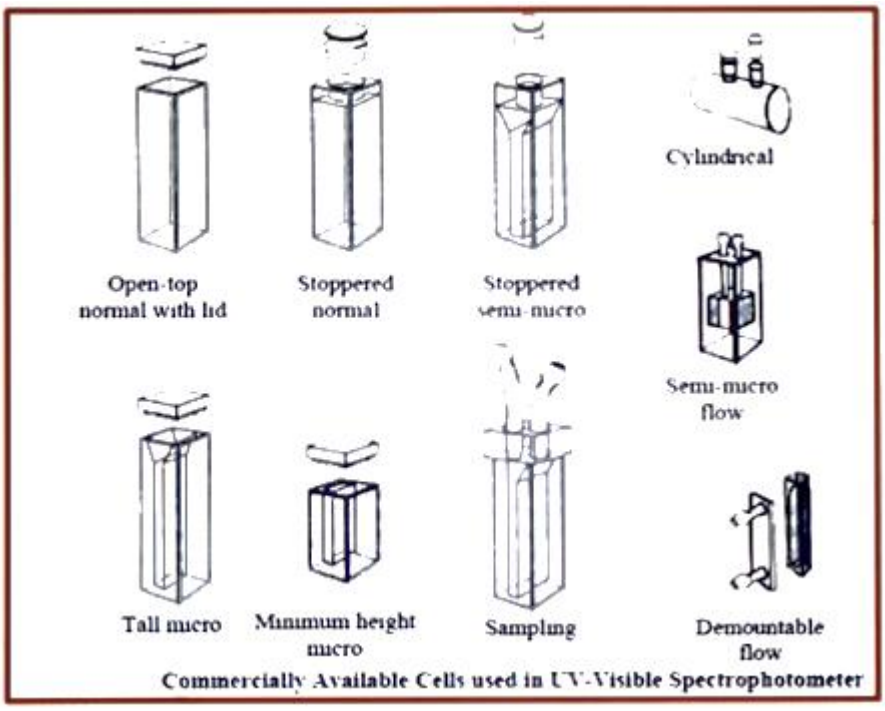
● Sample containers, which are usually called **cells or cuvettes**, must have windows that are transparent in the spectral region of interest.

● **Quartz or fused silica** is required for the **UV region (wavelengths less than 350 nm)** and may be used in the **visible region**.

Silicate glass is ordinarily used for **375–2000 nm region** because of its low cost compared

to quartz.

Plastic cells are also used in the **visible region**.



Commercially Available Cells used in UV-Visible Spectrophotometer

- The **best cells** have windows that are **perpendicular to the direction of the beam** in order to minimize reflection losses.

- The most common **cell path length** for studies in the **UV and visible regions is of 1 cm**; matched, calibrated cells of this size are available from several commercial sources. Many other cells with **shorter and longer path lengths** can be purchased.

- For reasons of economy, **cylindrical cells** are sometimes used.

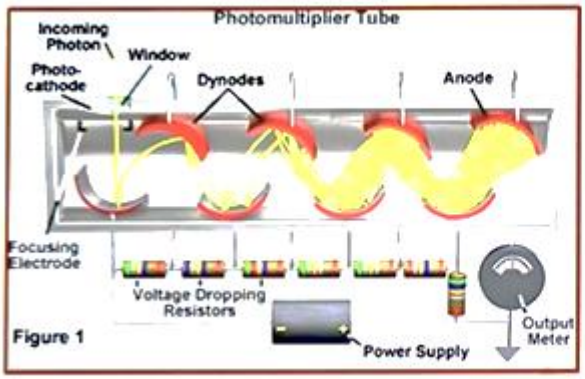
Particular care must be taken to duplicate the position of such cells with respect to the beam; otherwise, variations in path length and **reflection losses at the curved surfaces** can cause significant error.

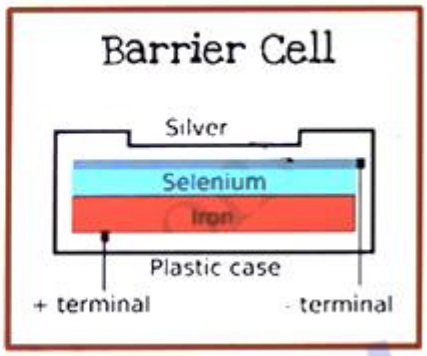
❑ Detectors

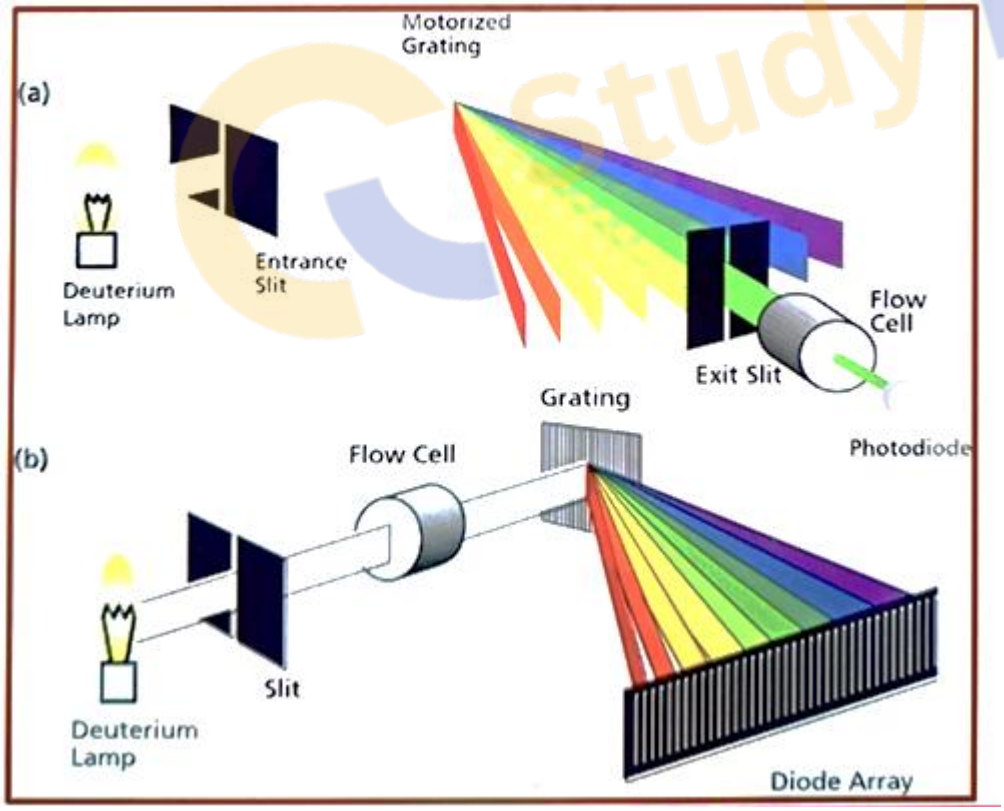
● Detectors used in **UV-visible spectrophotometers** can be called as **photometric detectors**.

The most commonly used detectors are:

1. **Photo tubes or photoemissive cells.**
2. **Photomultiplier Tubes (PMT).**
3. **Photovoltaic cell or barrier-layer cells.**
4. **Photomultiplier Silicon photodiode array detector.**







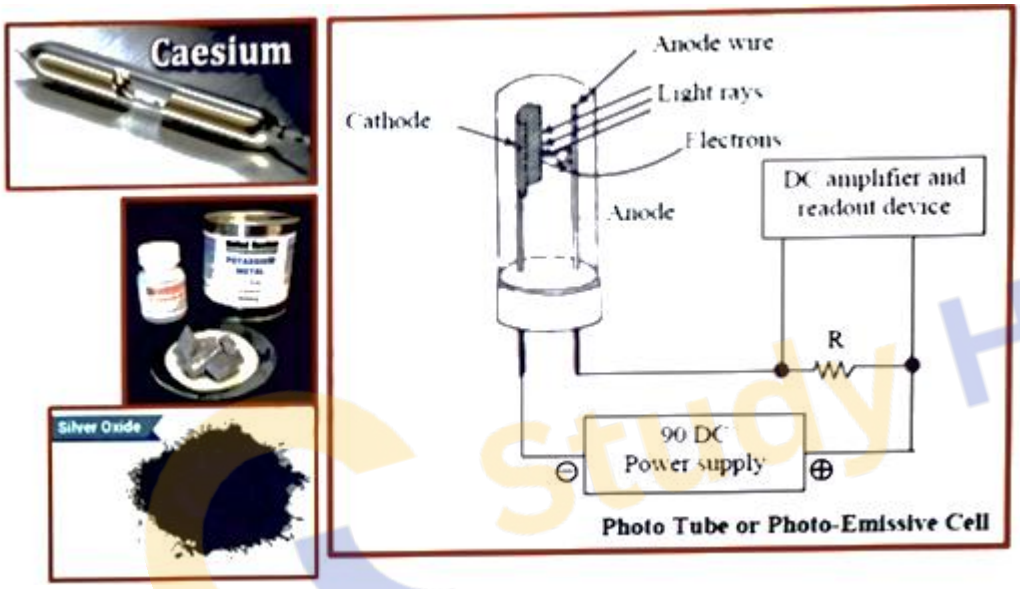
❖ Photo Tubes or Photo-Emissive Cells

● This detector is composed of an **evacuated glass tube**, which consists of a **photocathode and a collector anode**.

The photocathode is coated with elements of **high atomic volume** (like caesium, potassium, or silver oxide), which can liberate electrons when light radiation falls on it.

● This **flow of electrons** towards anode produces a **current proportional to the intensity of light radiation**.

Composite coatings like **caesium/caesium oxide/silver oxide** can also be used, which **increases the sensitivity** and **range of wavelength** in which the detector can be used (**UV region**).



● The signal from the detector can also be amplified using an amplifier circuit.

Phototubes have **better sensitivity** when compared to photovoltaic cells, and hence are more widely used.

❖ Photomultiplier Tubes (PMT)

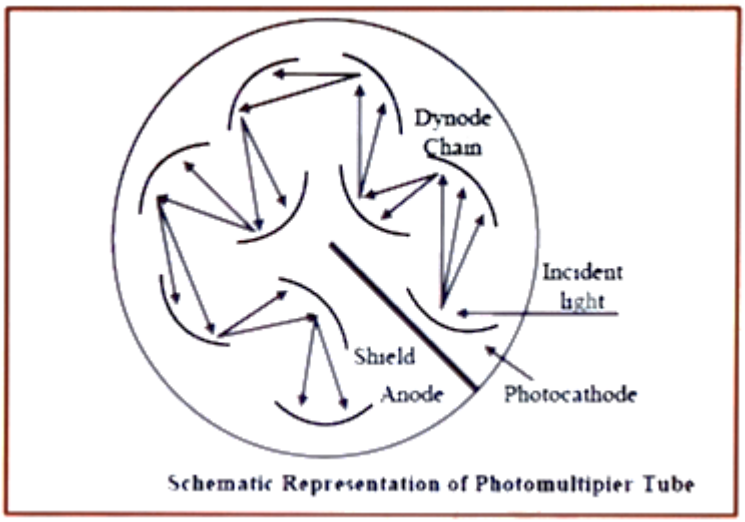
● This type of detector is the **most sensitive of all detectors**, is expensive, and is used in **sophisticated instruments**.

The principle employed in this detector is **multiplication of photoelectrons by secondary emission of electrons**.

This is achieved by using a photocathode and a series of up to **10 anodes (dynodes).**

● Each dynode is maintained at **75–100 V higher** than the preceding one.
At each stage, the electron emission is multiplied by a factor of **4 or 5** due to secondary emission of electrons;

and hence an overall factor of 10^6 is achieved.



Schematic Representation of Photomultipier Tube

❖ Photovoltaic Cell or Barrier-Layer Cells

● This cell is also known as **photronic cell** and operates **without the use of a battery**.
It consists of a **metal base plate** (of iron or aluminium), that acts as an electrode.
On its surface, a thin layer of a **semiconductor metal** **(like selenium)** is deposited.

● Then, the surface of selenium is covered by a **very thin layer of silver or gold** that acts as the second collector electrode.

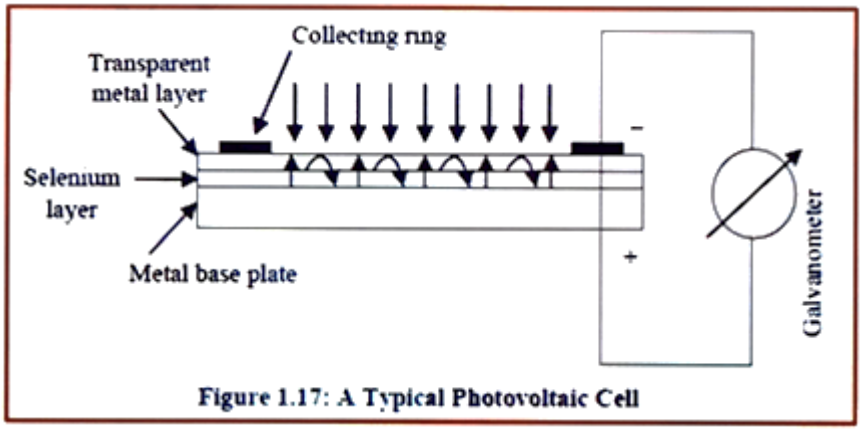


Figure 1.17: A Typical Photovoltaic Cell

● When the radiation is incident upon the surface of selenium, electrons are generated at the **selenium–silver**

interface.

These electrons are collected by the silver.

Accumulation of electrons on the silver surface ⟶ produces an electric current.

● **These cells offer the following advantages:**

✓ Rugged

✓ Require **no external power supply**

✓ Used mostly in the **visible region**

✓ Wavelength response (450–650 nm) is close to that of human eye

● High level of illumination is required to avoid problems of amplifying the output caused by the small resistance of the external circuit.

❖ Silicon Photodiode Array Detector

● The principle involved in silicon photodiode detector is that **upon exposure to light**, the electrical properties of the detector change (**internal photoelectric effect**).
Solar cells have similar structure and work on the same principle.

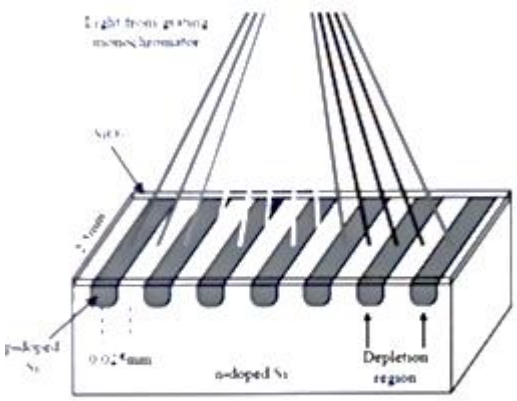
● Silicon photodiodes are advantageous over photomultipliers because of:

✓ Low cost

✓ Low sensitivity in light-receiving surface

✓ No need of special power supply

✓ They obtain photometric data similar to photomultipliers when the light intensity is relatively large.



Diode Array Detector

❑ RECORDING SYSTEM

- The signal from the photomultiplier tube is finally received by the **recording system**.
- The recording is done by a **recorder pen**.
- This type of arrangement is only done in recording UV spectrophotometers.

❑ POWER SUPPLY

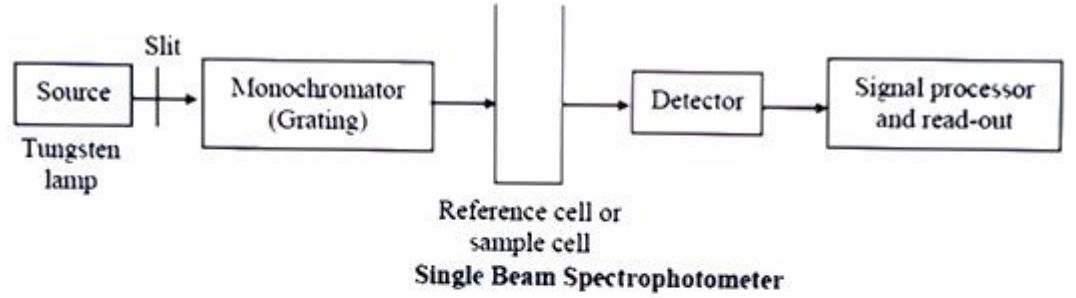
The power supply serves three main functions:

1. **It decreases the line voltage** to the instrument's operating level with a transformer.
2. **It converts AC to DC** with a rectifier if direct current is required by the instrument.
3. **It smoothens out ripples** that may occur in the **line voltage** to deliver a **constant voltage** to the source lamp and instruments.

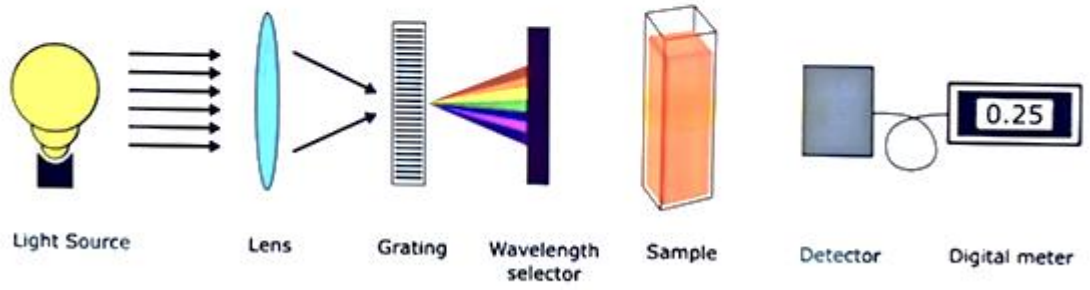
❑ Single beam UV spectrophotometer

- **The steps involved in a single beam UV spectrophotometer are:**

1. **UV radiation is given off by the source.**
2. A convex lens gathers the beam of radiation and focuses it on the **inlet slit**.
3. The **inlet slit permits light** from the source to pass, but **blocks-out stray radiation**.
4. The light then **reaches the monochromator**, which splits it up according to wavelength.
5. The exit slit is positioned to allow light of the **required wavelength** to pass through.
6. Radiation at **all other wavelengths is blocked-out**.
7. The selected radiation passes through the **sample cell** to the detector, which **measures the intensity of the radiation reaching it**.
8. By comparing the intensity of radiation before and after it passes through the sample, it is possible to measure how much radiation is absorbed by the sample at the particular wavelength used.
9. The output of the detector is usually recorded on **graph paper**.



Single Beam Spectrophotometer



Single beam spectrophotometer

● **One problem with the single beam system is that it measures the total amount of light reaching the detector, rather than the percentage absorbed.**

● Light may be lost at reflecting surfaces or may be absorbed by the solvent used to dissolve the sample.
Furthermore, the source intensity may vary with changes in line voltage.

● For example, when the line voltage decreases, the intensity of the light coming from the source may **decrease** unless special precautions are taken. Consequently, the intensity of radiation may be constantly changing.

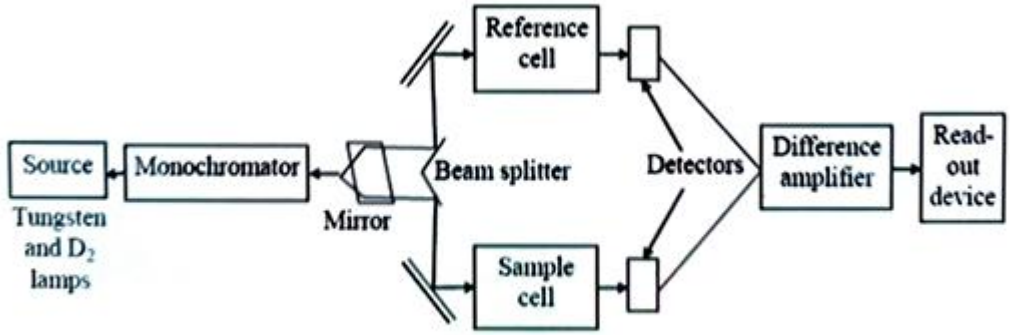
● Another problem is that the **response of the detector varies significantly** with the wavelength of the radiation falling on it.
Even if the light intensity is constant at all wavelengths, if the **wavelength is steadily increased from 200–750 nm**, the signal from the detector starts at a low value, increases to a value that is steady over a wide range, and then decreases once more.
This relationship between the signal from the detector and the wavelength of radiation is called the **response curve**.

❑ Double Beam UV Spectrophotometer

● A **double beam spectrophotometer** is also called a UV-visible spectrophotometer and it utilizes two sources:

(1) A **tungsten lamp** (400–800 nm), and

(2) A **D_2 lamp** (200–400 nm).



Double Beam Spectrophotometer

● **The steps involved in a double beam UV spectrophotometer are:**

1. The radiation from the selected source passes through a fixed slit to the surface of reflection grating (monochromator), and from the diffracted radiation, the desired wavelength is selected.
2. This selected beam of light falls on a **V-shaped mirror**, called a **beam splitter** that splits the radiations into two beams;
   one of which passes through the **reference cell** containing pure solvent and the other simultaneously passes through the **sample solution cell**.
3. The **transmitted light** from the two cells go to the **photoelectric detector alternatively**, and the difference in the absorbance by the solvent and the sample solution is measured electronically with very high accuracy.

● **Although the double beam instruments are more complicated and expensive, they do offer the following advantages:**

1. It is not necessary to continually replace the blank with the sample or to zero adjust at each wavelength as in the single beam units.
2. The ratio of the powers of the sample and reference beams is constantly obtained and used.
   Any error due to variation in the intensity of the **source** and **fluctuation in the detector** is minimized.
3. Because of the previous two factors, the double beam system lends itself to **rapid scanning** over a wide wavelength region and to the use of a recorder or digital readout.

❑ APPLICATIONS

● UV–Vis spectroscopy has been mainly applied for the **detection of functional groups (chromophore)**, the extent of conjugation, detection of polynuclear compounds by comparison, etc.
Some of the important applications are:

❑ Spectrophotometric Titrations

● In a spectrophotometric titration, the **equivalence point is determined** with a spectrophotometer.

● In this technique, the titration vessel is kept **directly in the light path** of the instrument.

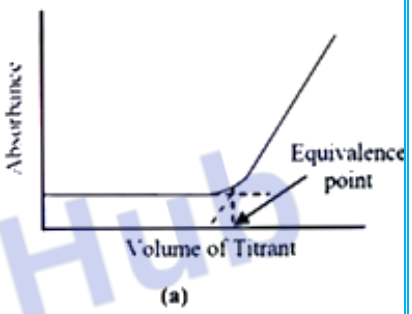
● **Then, the absorbance of the solution is determined after adding titrant, and a plot of absorbance as a function of volume of titrant is prepared.**

● If the titration reaction is complete, the titration curve will consist of **two straight lines intersecting at the equivalence point**, similar to **amperometric and conductometric titrations**.

● On the other hand, if the titration reaction is not complete, there occurs appreciable **curvature** in the equivalence point region but extrapolation of the two linear segments of the titration curve to their intersection gives the equivalence point volume.

1) Curve (a) This curve is characteristic of a case where **only the titrant absorbs**.
Example: Titration of **arsenic(III) with bromate–bromide**, where absorbance readings are taken at the wavelength where bromine absorbs.

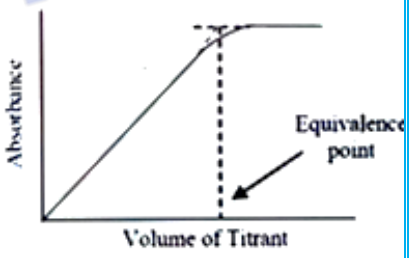
● As long as arsenic(III) remains in the solution, the absorbance will not be changed because the product does not absorb in that region.
As soon as arsenic(III) is consumed by the titrant, the absorbance will increase due to the colour of the titrant (bromine) alone.



(a)

2) Curve (b) This curve is characteristic of a case where **only the product of reaction absorbs**.

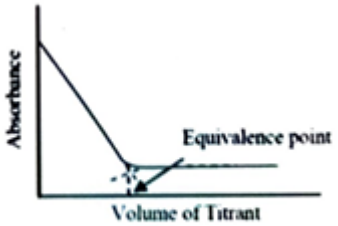
Example: Titration of **Cu(II) with EDTA**, carried out at **745 nm** wavelength. This wavelength is selected because at this position the EDTA–copper complex possesses a much greater absorbance compared to copper solution alone.



(b)

3) Curve (c) This curve is characteristic of a case where **only the substance being titrated absorbs**, while the titrant and the product do not absorb.
Example: Titration of **p-toluidine in butanol with perchloric acid at 290 nm** wavelength.
(diagram: Curve (c))

● **This wavelength is selected because p-toluidine sharply absorbs at this wavelength, whereas the titrant, perchloric acid shows no absorbance in this region.**



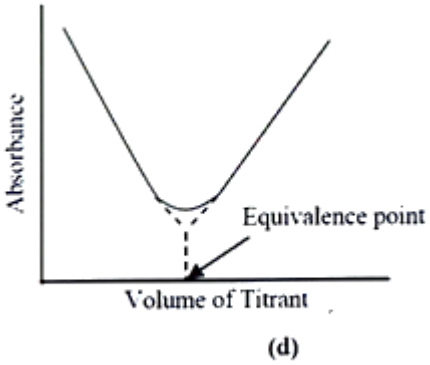
(c)

As soon as the whole quantity of p-toluidine reacts with perchloric acid, the absorbance will become constant after the equivalence point.

4) Curve (d) Curve (d) is obtained when a **coloured analyte is converted into a colourless product by a coloured titrant**.

When titrant is added, the analyte colour starts fading due to the formation of a colourless product.

But after the equivalence point, absorbance again rises due to the colour of the titrant alone.



(d)

➤ Choice of Wavelength

- A number of criteria are considered in the choice of wavelength.

It may be necessary to **balance one against another**:

1. The highest absorption peak will yield the **maximum sensitivity**.
2. A chosen wavelength should yield **minimum interference** from other species that may be in the system.
3. Because of errors that arise from **sharply rising or sharply falling portions** of the absorption curves, these sections should be avoided.
4. Care should be taken to avoid a sharp band if the slit width exceeds the bandwidth.

➤ Apparatus

- In order to carry out spectrophotometric titrations, a **special titration cell** of **5–100 ml capacity** is employed that fits in the cell compartment of the spectrophotometer.

The cell is made up of **perspex sheet**.

As the material perspex is **opaque to UV light**, two openings are made in the cell to accommodate **cellular quartz windows**.

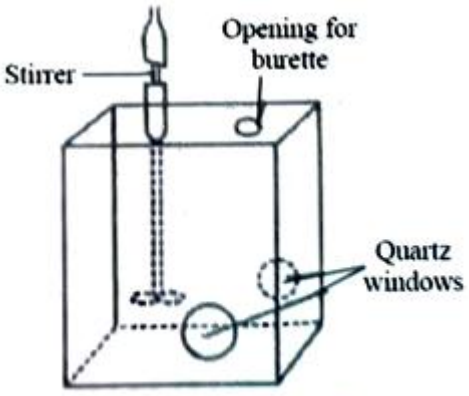


Figure 1.23: Titration Cell

● These windows are inserted in such a way that the beam of **monochromatic light passes through their centres** to the photoelectric cell.
The cell has two small openings, one for the tip of a microburette and the other for a **micro stirrer**.

● Except the quartz windows, the whole cell is covered with black paper to exclude all extraneous light.

➤ Technique

● **The experimental technique is simple and involves the following steps:**

1. The solution to be titrated is taken in the cell.
2. Then, the cell is kept in the light path of a spectrophotometer.
3. The spectrophotometer is **adjusted to the wavelength** at which the experiment is to be carried out and the instrument is **set at zero absorbance** (if the reactant is colourless) or at some other starting value (if the reactant is coloured) that lies within the linearity range of the instrument.
4. After this, a **known volume of titrant** is added to the stirred solution in the cell and the absorbance is read again.
5. This is repeated at several points before the end point and at several points after the end point.
6. Finally, absorbance is plotted against the volume of titrant added.
7. From this graph, the **equivalence point is obtained**.

➤ Dilution Correction

● Since absorbance depends upon concentration, the effect of dilution should be taken into consideration.
This error can be overcome by one of the following methods:

1. Either the titrant is made **much more concentrated** than the solution to be titrated so that there is negligible change in volume, or
2. A simple correction factor is included in the calculation.

$$A = A' \frac{V + v}{V}$$

Where,

A and A' = Corrected and measured absorbance, respectively
V = Original volume of solution
v = Volume of titrant added

- **For best results, appropriate dilution corrections must be made and the above equation becomes:**

$$A_c = (\frac{V_i + V_a}{V_i})(A_m)$$

Where,

A_a = Corrected absorbance

V_i and V_a = Respective initial and added volumes

A_m = Measured absorbance

- **However, a negligible error will result from ignoring dilution corrections if the titrant concentration is much greater than the concentration of the solution being titrated.**

➤ Applications

- **Some typical methods of analysis by spectrophotometric titrations are:**

1) Acid-Base Methods:

Phenols can be titrated with NaOH.
Absorbance occurs due to the formation of **phenolate ion**.

2) Oxidation-Reduction Methods:

Ce(III) can be titrated with Co(III), forming Ce(IV).

3) Complexometric Titrations:

- Bi(III) can be titrated with thiourea.

Copper(II) is added, resulting in the formation of **Cu–EDTA complex**;
or thiourea is added resulting in the disappearance of bismuth–thiourea complex.

- Fe(III) can be titrated with EDTA, resulting in the disappearance of Fe–sulphosalicylic acid complex.

- Cu(II) can be titrated with EDTA, resulting in formation of Cu–EDTA complex.

Copper and bismuth in a mixture can be estimated by just a single titration with EDTA.
Measurements are made at **745 nm**, where copper–EDTA complex absorbs strongly but bismuth complex does not.

4) Precipitation Titrations:

SO_4^{2-} ions can be titrated with Ba(II) ions, resulting in appearance of turbidity.

F^- ions can be titrated with Th(IV) ions.
Reaction of Th(IV) with the indicator **SPADNS results in a precipitation**.

➤ Advantages

- Spectrophotometric titrations have several **advantages over direct spectrophotometric analysis**:

1. It can be applied to a **large number of non-absorbing constituents** as only one absorber is to be present among the reactant, the titrant, or the reaction products.

2. Presence of other absorbing species at the analytical wavelength **does not cause interference** because only the change in absorbance is significant.
3. It can be applied to **highly coloured solutions** that could not be determined by visual indicators.
4. It can be applied to reactions that tend to be **appreciably incomplete** at the equivalence point.
5. It is quite accurate. One can obtain **accuracy and precision** of a few tenths per cent with comparative ease.
6. Unlike normal absorbance procedures, it is not necessary that the titration be performed in the region of **wavelength of maximum absorbance**.
   Thus, **greater choice of wavelengths** is available.
7. Relatively large quantities of other absorbing species at the chosen wavelength are necessary before noticeable interference.
8. **Turbid solutions** may be titrated in selected circumstances.
9. More **dilute solutions** may be employed than in other titrations.
10. A variety of equipment may be employed.

➤ Single Component Analysis Methods

- **The methods involved in the calculation of single component analysis are:**

---

1) Direct Analysis:

Essentially all compounds containing **conjugated double bond or aromatic rings**, and many inorganic species absorb light in the UV-visible regions.
In these techniques, the substance to be determined is dissolved in a suitable solvent and diluted to the required concentration by appropriate dilutions, and then absorbance is measured.

2) Indirect Analysis:

This method involves analysis **after addition of some reagent**.

➤ Indirect Analysis (continued)

● These methods are based on the conversion of analyte by a chemical reagent having different spectral properties.
Chemical derivatisation may be adopted for any of the several reasons:

i) If the analyte **absorbs weakly in the UV-region**.
ii) The interference from irrelevant absorption may be avoided by converting the analyte to a derivative, which **absorbs in the visible region**, where irrelevant absorption is negligible.
iii) This technique can be used to improve the selectivity of the assay in presence of other UV-radiation absorbing substances.
iv) **Cost.**

✓ Methods of Calculating Concentration in Single Component Analysis

1. By using the relationship **A = abc**.
2. By using the formula **Cu = (Au/As) × Cs**.
3. By using the equations **y = mx + c**.
4. By using the **Beer's curve**.

❑ Multicomponent Analysis Methods

● UV spectrophotometric techniques are mainly **used for multicomponent analysis**, thus minimizing the cumbersome task of separating interferents and allowing the determination of an increasing number of analytes, consequently **reducing analysis time and cost**.

- Multicomponent UV spectrophotometric methods are based on **recording and mathematically processing absorption spectra**.

- **The multicomponent analysis methods offer the following advantages:**

1. Avoiding prior separation techniques, e.g., extraction, concentration of constituents, and clean-up steps that might be required.
2. Spectral data are readily acquired with ease.
3. The process is **fast, accurate, and simple**.
4. Wide applicability to both organic and inorganic systems.
5. Typical detection limits of 10^{-4} to 10^{-5} M and moderate to high selectivity.

Different UV spectrophotometric multicomponent analysis methods include:

1. Simultaneous equation method,
2. Difference spectrophotometry,
3. Derivative Spectrophotometry (DS),
4. Absorbance ratio spectra method,
5. Derivative ratio spectra method,
6. Double divisor ratio spectra derivative method,
7. Successive ratio-derivative spectra method,
8. Q-absorbance ratio method,
9. **Isosbestic "isoabsorptive" point method**,
10. Absorptivity factor method,
11. Dual wavelength method,
12. Ratio Subtraction Method (RSM),

13. Mean centering of the ratio spectra,
14. Absorption Factor Method (AFM), and
15. Multivariate chemometric methods.

UNIT-I FLUORIMETRY – (PART 2)

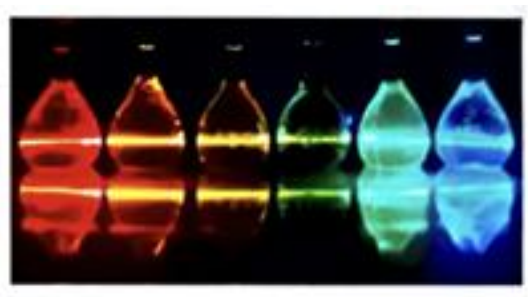
Points to be covered in this topic

- Theory
- Concepts of singlet
- Doublet and triplet electronic states
- Internal and external conversions
- Factors affecting fluorescence
- Quenching
- Instrumentation
- Applications

FLUORIMETRY

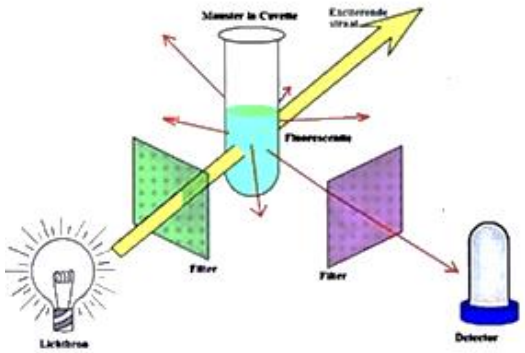
INTRODUCTION

● **Fluorescence:** It is a phenomenon of **emission of radiation** when the molecules are excited by **radiation at certain wavelength**.



● **Fluorimetry:**

It is measurement of fluorescence intensity at a particular wavelength with the help of a **filter fluorimeter** or a **spectrofluorometer**.



❖ PRINCIPLE

● Molecule contains **σ electrons, π electrons and nonbonding (n) electron**.

● The electrons may be present in **bonding molecular orbital**.

It is called **highest occupied molecular orbital (HOMO)**.

It has less energy and is more stable.

● When the molecules absorb radiant energy from a light source, the bonding electrons may be promoted to **anti-bonding molecular orbital (LUMO)**.

It has **more energy and hence less stable**.

➤ **The process of promotion of electrons from HOMO to LUMO with absorption of energy is called *excitation*.**

✓ **Singlet state:**

A state in which **all the electrons in a molecule are paired (↑↓).**

✓ **Doublet state:**

A state in which **unpaired electrons are present ↓ or ↑.**

✓ **Triplet state:**

A state in which **unpaired electrons of same spin** are present (↑ ↑ ↑).

(figure: Singlet ground state ⟶ Singlet excited state ⟶ Triplet excited state)

✓ **Singlet excited state:**

A state in which electrons are **unpaired but of opposite spin like** ↑↓
(unpaired AND opposite spin).

- When light of appropriate wavelength is absorbed by a molecule the electrons are promoted from singlet ground state to singlet excited state.

- Once the molecule is in this excited state, relaxation can occur via several processes by emission of radiation.

The processes can be the following:

1. Collisional deactivation
2. Fluorescence
3. Phosphorescence

(Images: Fluorescence vs Phosphorescence glow)

- **Collisional deactivation:**

Entire energy is lost due to collision deactivation and **no radiation emitted**.

- **Fluorescence:**

Excited singlet state is highly unstable.
Relaxation of electrons from **excited singlet to singlet ground state** with **emission of light**.

- **Phosphorescence:**

At favorable condition like **low temperature** and **absence of oxygen**, there is transition from excited singlet state to triplet state which is called **inner system crossing**.
The emission of radiation when electrons undergo transition from **triplet state to singlet ground state** is called **phosphorescence**.

- **Internal conversion:**

Intermolecular process by which a molecule passes to a **lower energy electronic state without emission** of light.
Overlap of vibrational energy levels in two electronic energy levels.

- **External conversion:**

Deactivation of excited electronic state by **interaction and energy transfer** between the excited molecule and solvent or other solutes.

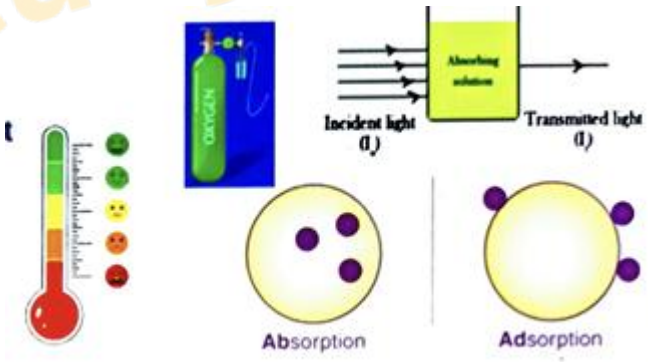
- **Intersystem crossing:**

Process in which spin of an excited electron is reversed and **change in multiplicity results**. Most common when vibrational manifold overlap exists **and when the molecule has a heavy atom substituent** (e.g., Br, I).

(figure: Internal conversion diagram)

❖ FACTORS AFFECTING FLUORESCENCE INTENSITY

1. **Concentration**
2. **Quantum yield of fluorescence**
3. **Intensity of incident light**
4. **Adsorption**
5. **Oxygen**
6. **pH**
7. **Temperature & viscosity**
8. **Photodecomposition**
9. **Quenchers**
10. **Scatter**



1. Concentration

- Fluorescence intensity is **proportional to concentration** only when absorbance is less than 0.02.

- We know, Absorbance $\mathbf{A = \log I_0/I_t}$ OR $\mathbf{A = abc}$

Where:

I_0 = intensity of incident light

I_t = intensity of transmitted light

a = absorptivity constant

b = pathlength

c = concentration

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2. Quantum yield of fluorescence (Φ):

- Φ = number of photons **emitted** / number of photons **absorbed**

- It is always less than **1.0** since some energy is lost by radiationless pathways (Collisional, Intersystem Crossing, Vibrational Relaxation)

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3. Intensity of incident light:

- Increase in intensity of incident light on the sample ⟶ **fluorescence increases**.

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4. Adsorption:

- Adsorption of sample solution on the container walls may lead to a serious problem.

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5. Oxygen:

- Oxidation of fluorescent species to non-fluorescent species **quenches fluorescence**.

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6. pH:

- Alteration of pH has significant effect on fluorescence.
- Example:
  - Aniline in **alkali medium** gives visible fluorescence
  - But in **acidic medium** gives fluorescence in visible region

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7. Temperature and viscosity:

- Temperature ↑ ⟶ collisional deactivation ↑ ⟶ **fluorescence decreases**
- If viscosity ↑ ⟶ collisions ↓ ⟶ **fluorescence increases**

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8. Photochemical decomposition:

- Absorption of intense radiation leads to decomposition of fluorescent substance to **less fluorescent or non-fluorescent** substance.

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9. Quenchers:

- **Quenching** is the *reduction* of fluorescence intensity by the presence of a substance in the sample **other than the fluorescent analyte**.

- **Types of Quenching:**

i) Inner fluorescence effect:

Absorption of UV or emitted light by primary/secondary filters ⟶ **decrease in fluorescence**.

ii) Self-quenching:

At *low* concentration ⟶ linear intensity

At *high* concentration ⟶ intensity decreases due to association of molecules.

iii) Collisional quenching:

Collisions between fluorescent substance & halide ions ⟶ **fluorescence reduction**.

iv) Static quenching:

Occurs due to **complex formation** between fluorescent molecule & others.
Example: caffeine reduces fluorescence of riboflavin.

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10. Scatter

- Scatter is **mainly due to colloidal particles** in solution.
- Scattering of incident light after passing through sample ⟶ **fluorescence decreases**.

❖ INSTRUMENTATION

➤ **Source of light:**

- **Mercury vapor lamp:**
  - High pressure ⟶ intense lines above **350 nm**
  - Low pressure Hg lamp gives additional line at **254 nm** ⟶ used in filter fluorimeter
- **Xenon arc lamp:**
  - More intense than mercury vapor lamp

- Used in spectrofluorometer

● **Tungsten lamp:**

- Used when excitation in the visible region is needed
- Used in low-cost instruments

➤ Filters and Monochromators

● **Filters:**

Optical filters – absorb unwanted light & transmit required wavelength.

In fluorimeters:

✓ **Primary filter:** Absorbs **visible** radiation, transmits **UV**

✓ **Secondary filter:** Absorbs **UV**, transmits **visible**

● **Monochromators:**

Convert polychromatic light into monochromatic.

They isolate a specific range of wavelength.

✓ **Excitation monochromators:** Provide radiation to excite molecules

✓ **Emission monochromators:** Isolate only radiation emitted by fluorescent molecules

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➤ Sample cells

- Used to hold **liquid samples**
- Made of **quartz**
- Shapes: **cylindrical or rectangular**, etc.

❖ Detectors:

➤ Barrier layer / photovoltaic cell:

- It is employed in inexpensive instruments.

For ex: Filter Fluorimeter.

- It consists of a **copper plate coated with a thin layer of cuprous oxide** **(Cu_2O)**.

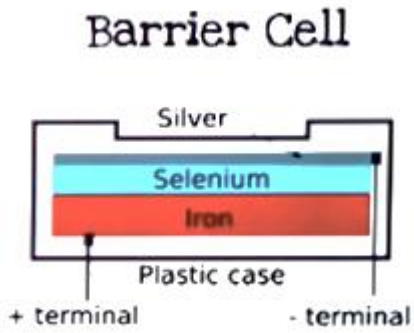
A semitransparent film of **silver** is laid on this plate to provide good contact.

- When external light falls on the oxide layer, the electrons emitted from the oxide layer move into the copper plate.

Then oxide layer becomes positive and copper plate becomes negative.

- Hence an **emf develops** between the oxide layer and copper plate and behaves like a voltaic cell.

So, it is called **photovoltaic cell**.



- A galvanometer is connected externally between silver film and copper plate and the deflection in the galvanometer shows the current flow through it.

- The amount of current is found to be **proportional to the intensity** **of incident light**

➤ Photomultiplier tubes (PMT):

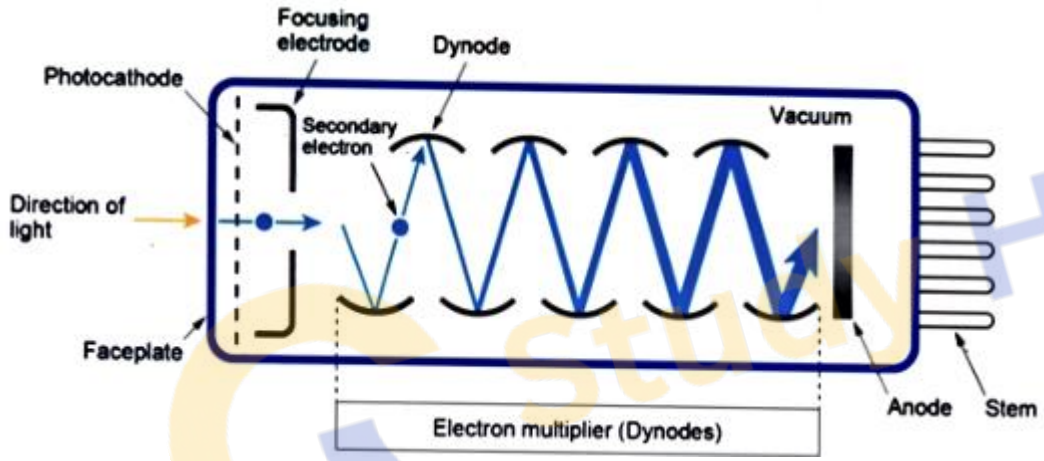
- These are incorporated in **expensive instruments** like spectrofluorometer.
- Its sensitivity is **high** due to measuring **weak intensity of light**.

● The principle employed in this detector is that multiplication of **photoelectrons by secondary emission of electrons**.

● This is achieved by using a **photo cathode** and a series of **anodes (dynodes)**.
Up to **10 dynodes** are used.
Each dynode is maintained at **75–100 V higher** than the preceding one.

● At each stage, the electron emission is multiplied by a factor of **4 to 5**, thus achieving an overall gain of 10^6.

● PMT can detect very weak signals, even **200 times weaker** than could be done using photovoltaic cell. Hence it is useful in fluorescence measurements.



● **PMT should be shielded from stray light** in order to have accurate results.

➤ **Instruments:**

✓ The most common types are:

● **Single beam (filter) fluorimeter**

● **Double beam (filter) fluorimeter**

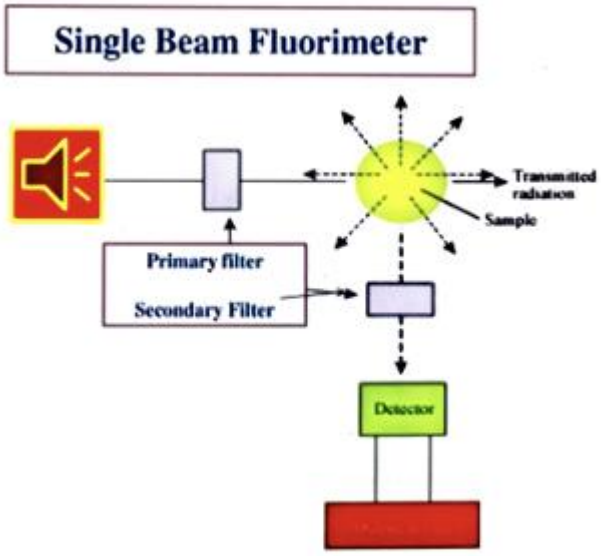
● **Spectrofluorometer (double beam)**

➤ **Single beam (filter) fluorimeter**

- It contains **tungsten lamp** as a source of light and has an optical system consisting of **primary filter**.

- The emitted radiations are measured at **90°** by using a secondary filter and detector.

- Primary filter absorbs **visible radiation** and transmits **UV radiation**, which excites the molecule present in the sample cell.



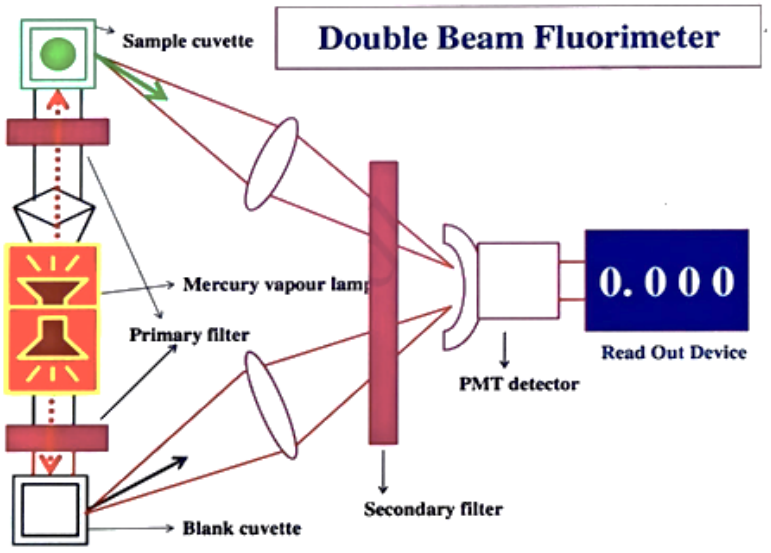
- Instead of 90° (as in colorimetry) the secondary filter has to be highly efficient; otherwise the unabsorbed UV radiation and fluorescent radiation will produce detector response and give false results.

- Single beam instruments are simple in construction, cheaper, and easy to operate.

➤ Double beam fluorimeter

- It is similar to single beam except that the two incident beams from a **single light source** pass through **primary filters** separately and fall on another reference solution.

- Then the **emitted radiations** from the sample or reference sample pass separately through secondary filter and produce the response **combinedly** on a detector.



➤ Spectrofluorometer:

- In this, the **primary filter** in double beam fluorimeter is replaced by **excitation monochromator** and the **secondary filter** is replaced by **emission monochromator**.
- Incident beam is split into **sample and reference beam** by using beam splitter.

❖ APPLICATIONS:

- Fluorometric methods are **not useful in qualitative analysis** and much used in **quantitative analysis**.
- Determination of inorganic substances: Al^{3+}, Li^{+}, Zn^{2+}
- Determination of **thiamine HCl**.
- Determination of **phenytoin**.
- Determination of **indoles, phenols, & phenothiazines**.
- Determination of **naphthol, proteins, plant pigments and steroids**.
- Fluorimetry can be used in detection of impurities in **nanogram level**, better than absorbance spectrophotometer, with special emphasis in determining **components of sample** at the end of chromatographic or capillary column.
- Determination of **ruthenium ions** in presence of other **platinum metals**.
- Determination of **boron** in steel, aluminum in alloys, manganese in steel.
- Determination of **boron in steel** by complex formed with **benzoin**.
- Estimation of **cadmium** with **2-(2 hydroxyphenyl) benzoxazole** in presence of tartrate.
- **Respiratory tract infections**.