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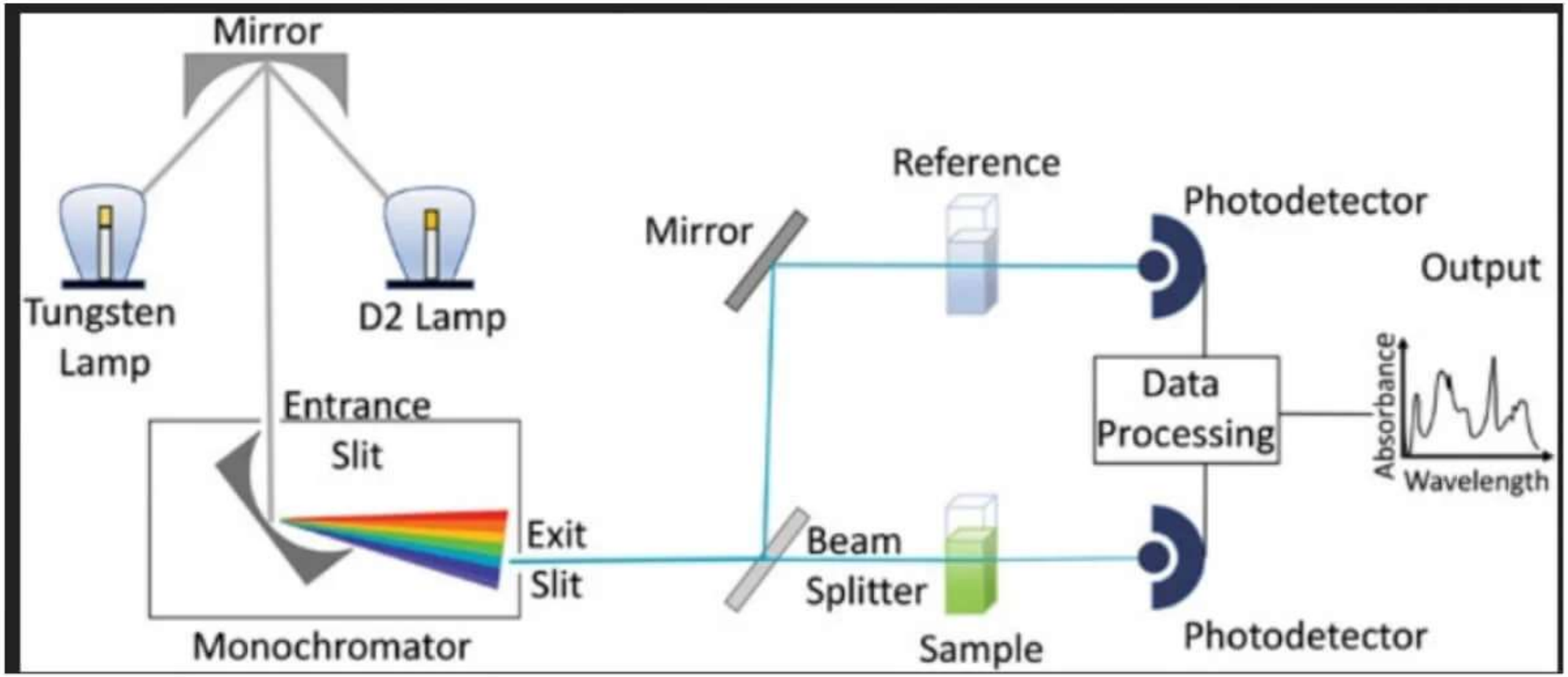
DEPARTMENTOF PHARMACEUTICAL ANALYSIS

SUBJECT NAME- BP701T ,INSTRUMENTALMETHODS OF ANALYSIS

IV Year/VII Semester

UNIT I , UV-VIS SPECTROSCOPY

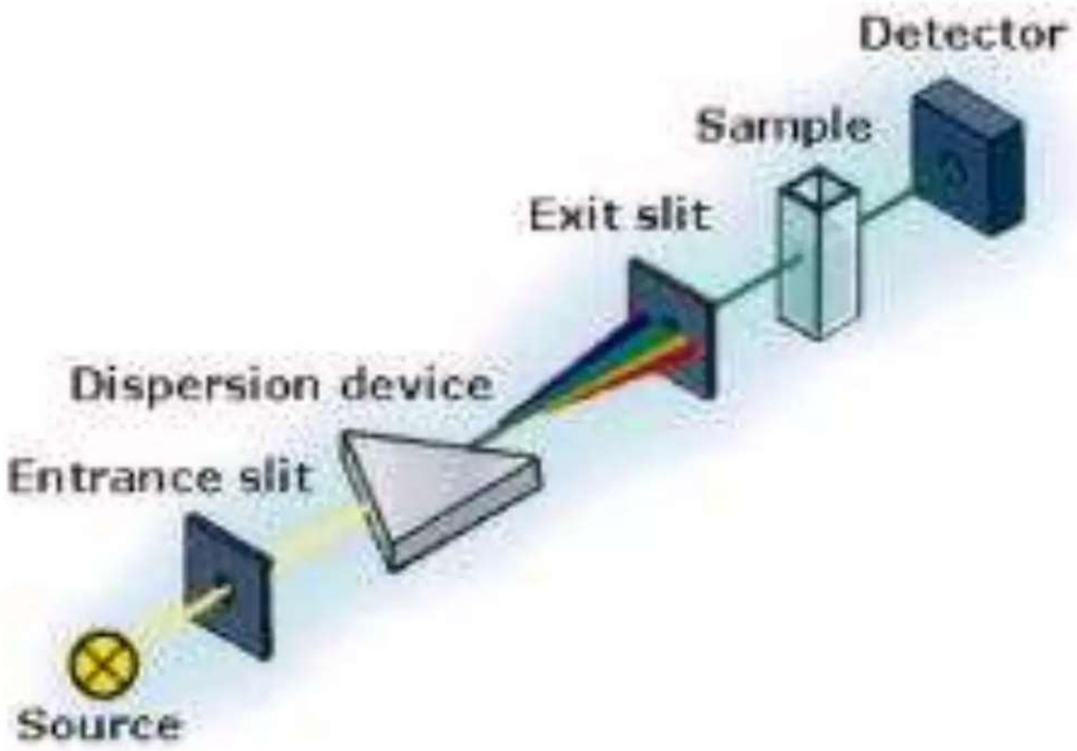
UV Spectroscopy



INSTRUMENTATION

COMPONENTS OF SPECTROPHOTOMETER

- Source
- Wavelength selectors or Dispersing devices
- Sample compartment
- Detector
- Recorder


Detector
Sample
Exit slit
Dispersion device
Entrance slit
Source

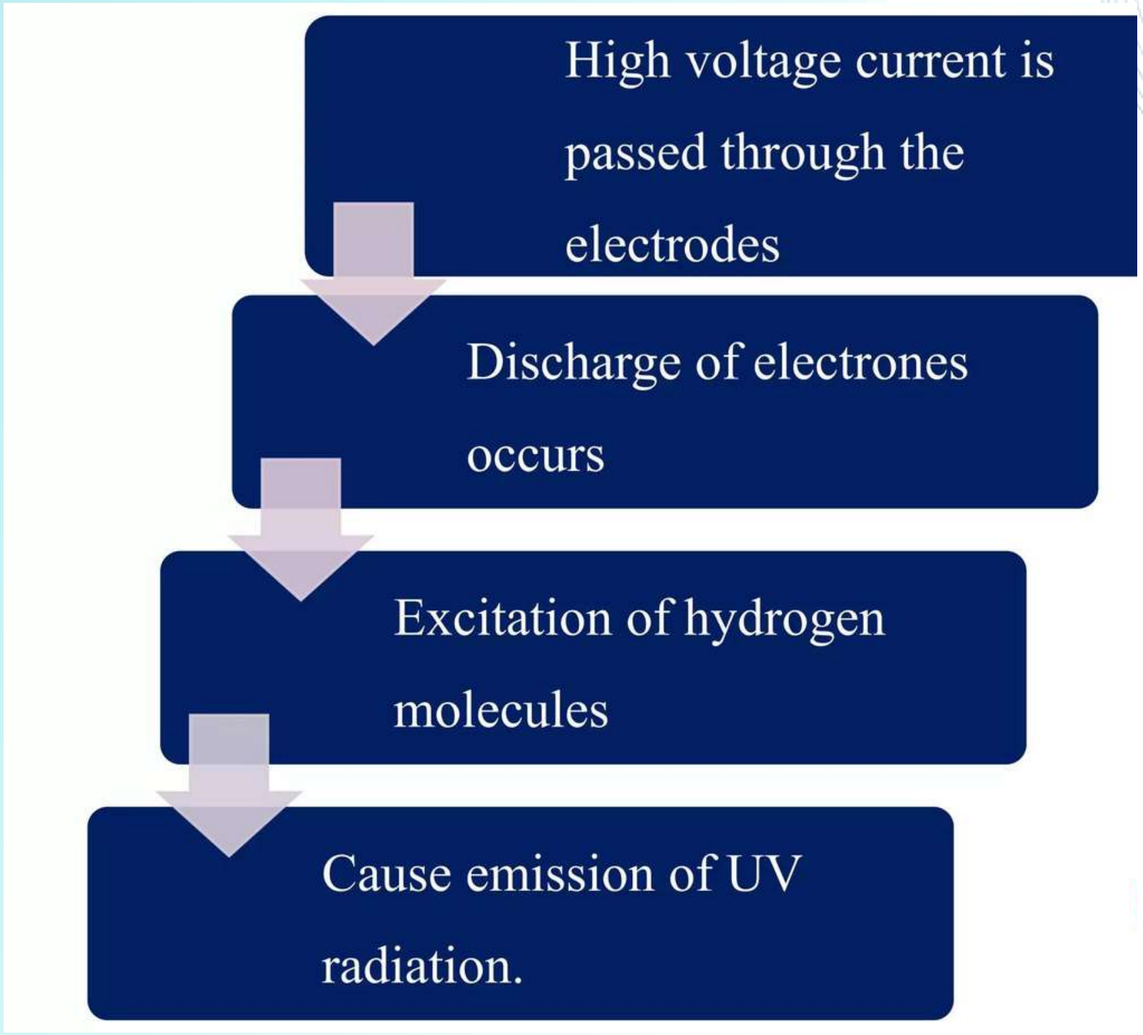
1. LIGHT SOURCE

Requirements:

- It should be stable.
- It should provide continuous radiation.
- It must be of the sufficient intensity for the transmitted energy to be detected at the end of the optical path.

1. HYDROGEN DISCHARGE LAMPS

- In these lamps a pair of electrodes is enclosed in a glass tube filled with hydrogen gas under relatively high pressure.



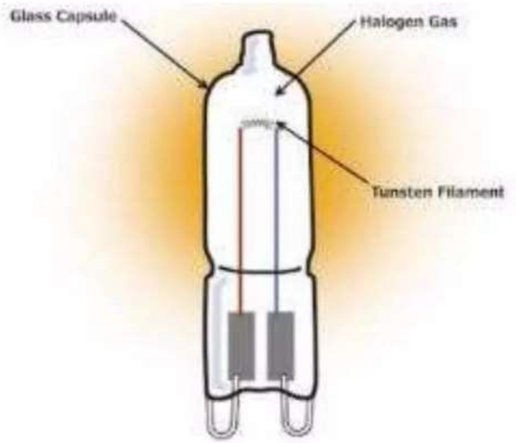
- It is a continuous source.
- Covers a range 160-375nm.
- Stable,robust and widely used.

2. DEUTERIUM LAMPS

- Similar to hydrogen discharge lamp.
- Deuterium is filled in place of hydrogen.
- The intensity of radiation emitted is 3 to 5 times intensity of a hydrogen lamp.
- More expensive than hydrogen lamp.
- Used when high intensity is required.

3. TUNGSTEN LAMP

- Similar in its functioning to an electric bulb.
- It provides a supply of radiation in the wavelength range of 320-2500nm.
- Continuous source of light.
- When tungston filament is heated to incandescence an electric current,the light is produced.
- The glass bulb enclosing the filament contains a low pressure of inert gas,usually argon.
- Small amounts of halogen like iodine is added to improve the intensity (Tungston-Iodine lamp).



2 WAVELENGTH SELECTORS

- Converts polychromatic light to monochromatic light.
- Filters and Monochromators are used for this purposes.

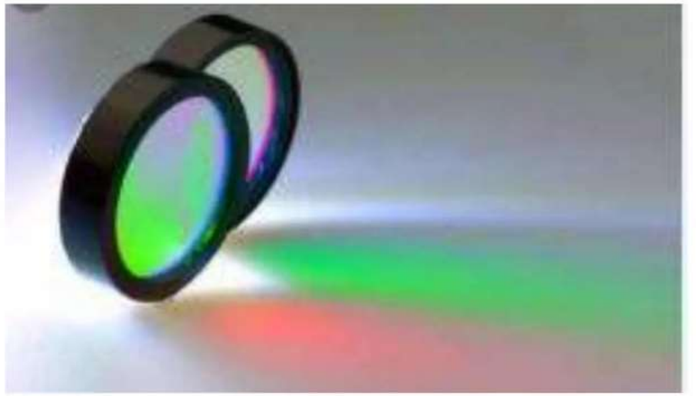
FILTERS

- It is frequently necessary to filter or remove wide bands of radiation from a signal.
- Filters isolate a wider band than the monochromators.

1. ABSORPTION FILTERS

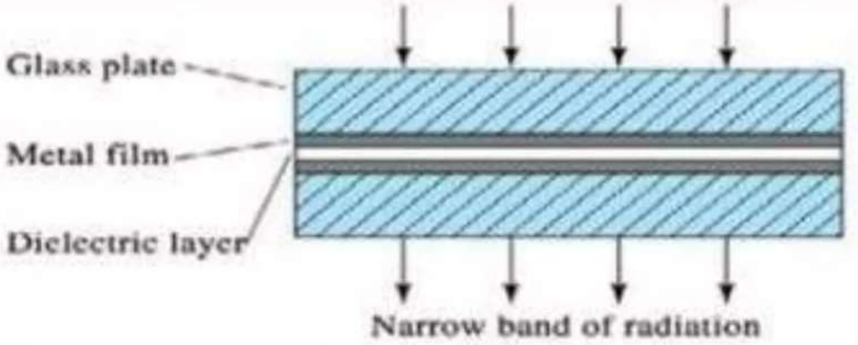
- These filters have a bandwidth that ranges from 30-250µm.
- The absorption filters consists of coloured glass or a dye suspended in gelatin and sandwitched between the two glass plates.

- The coloured glass filter has the advantage of greater thermal stability.
- Each instrument is provided with a set of 12 filters to cover the range from 390-700µm.
- A narrow spectral band can be obtained by coupling cut off filters with other filters but this combination decreases the intensity of light.



2. INTERFERENCE FILTERS

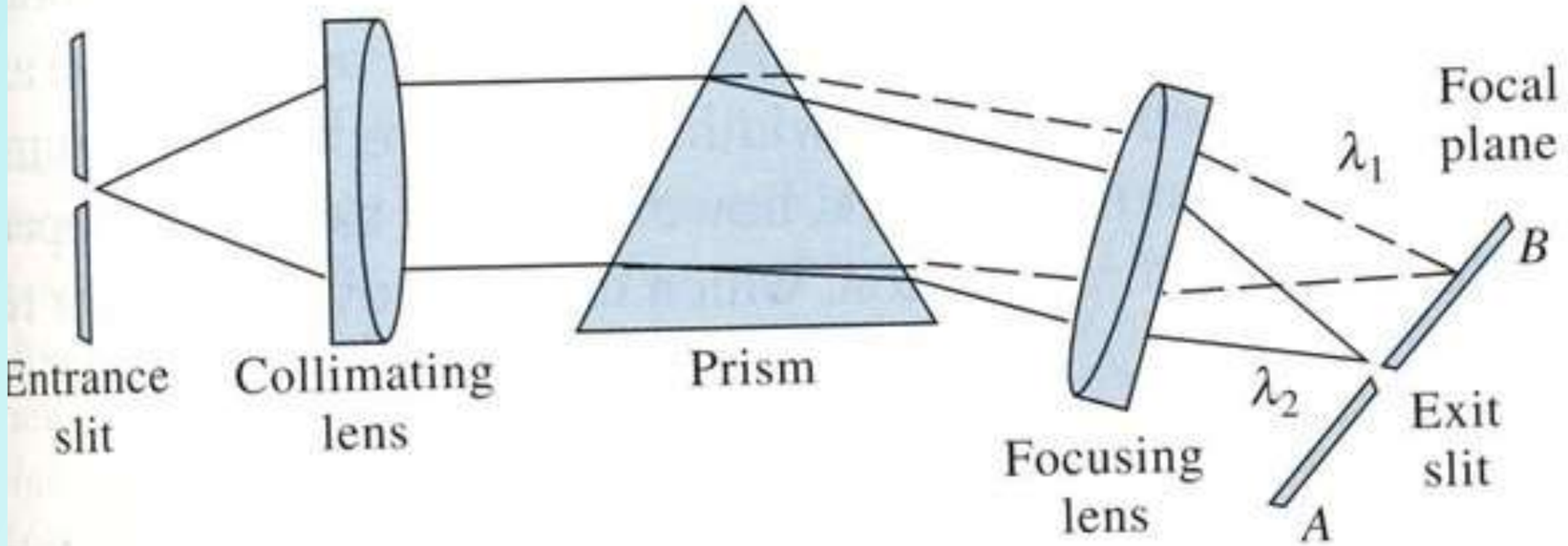
- Based on interference phenomenon at desired wavelength thus permitting rejection of unwanted radiation by selective reflection and producing narrow band.
- It consists of a dielectric layer(eg:CaF_2) between 2 parallel silver films which is sandwitched by glassplate.



Schematic cross section of an interference filter

3. MONOCHROMATORS

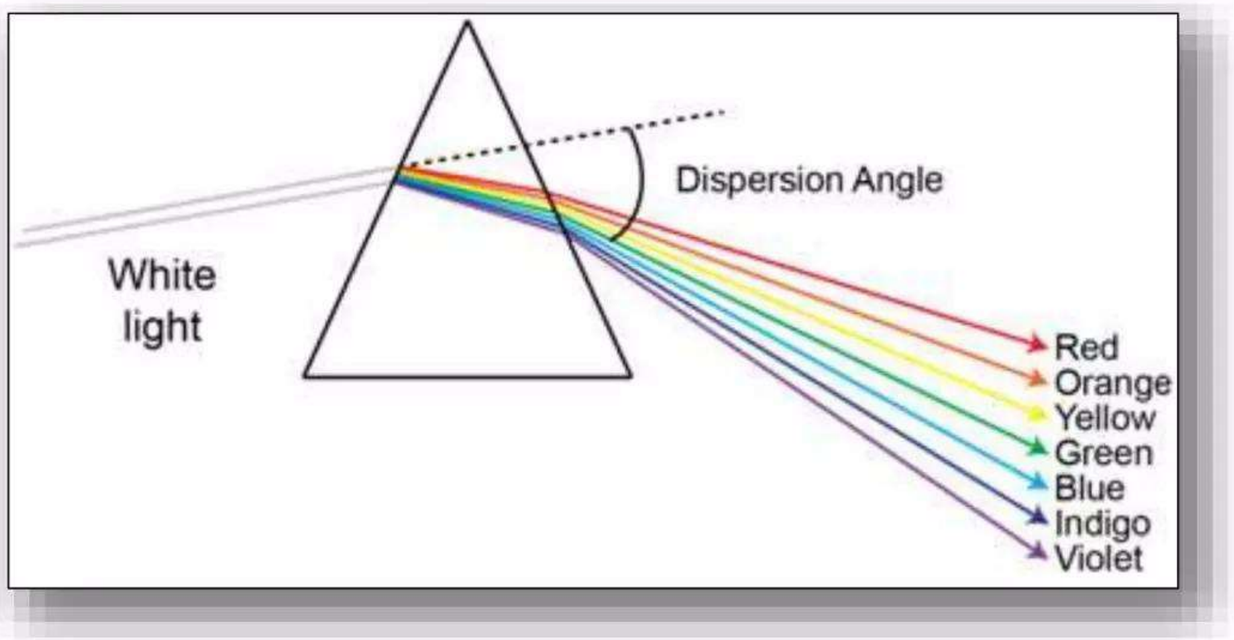
- It is used to disperse the heterochromic radiation into its component wavelength and to permit the isolation of desired portion of the spectrum.
- It consists of an entrance slit, an exit slit and a dispersing device either a prism or grating.
- Materials of construction should be selected with care to suit the range in which it has to work.
- For eg:
  - Quartz for ultraviolet.
  - Normal glass for visual range.
  - Alkali halides for IR region.
- Gratings are cheaper than prism.



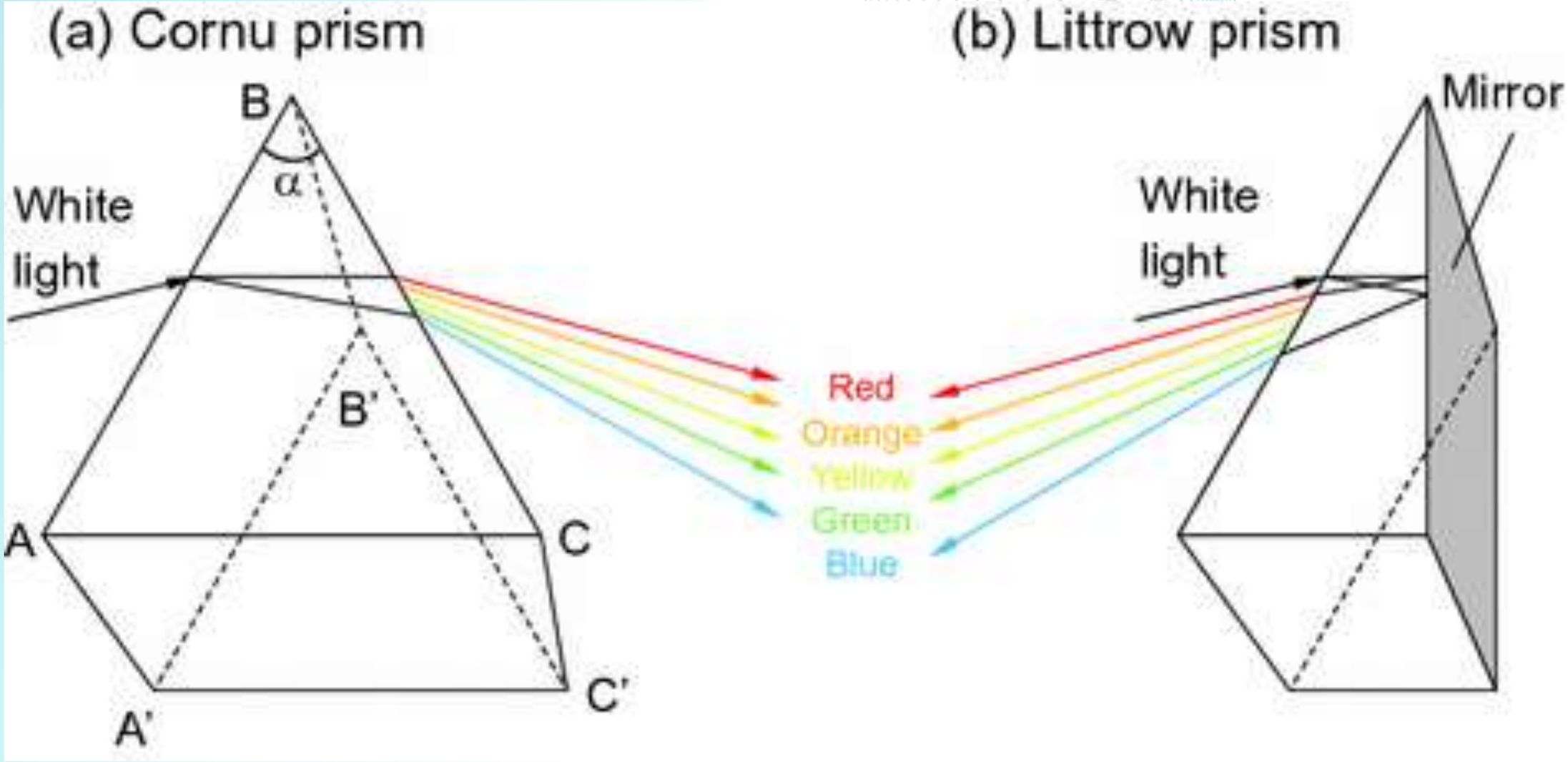
A Bunsen monochromator

1- Prism Monochromator

- ✓When electromagnetic radiation passes through a prism, it is refracted because the index of refraction of the prism material is different from that of air.
- ✓Shorter wavelengths are refracted more than longer wavelengths.
- ✓By rotation of the prism, different wavelengths of the spectrum can be made to pass through an exit slit and through the sample.



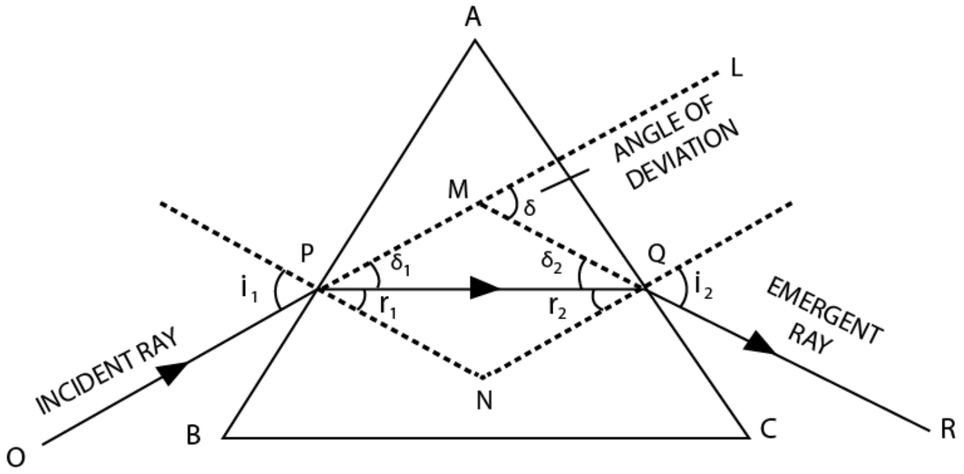
Prism monochromators are of 2 types.



CORNU TYPE:

It has an optical angle of 60° and it is adjusted such that on rotation the emerging light is allowed to fall on exit slit.

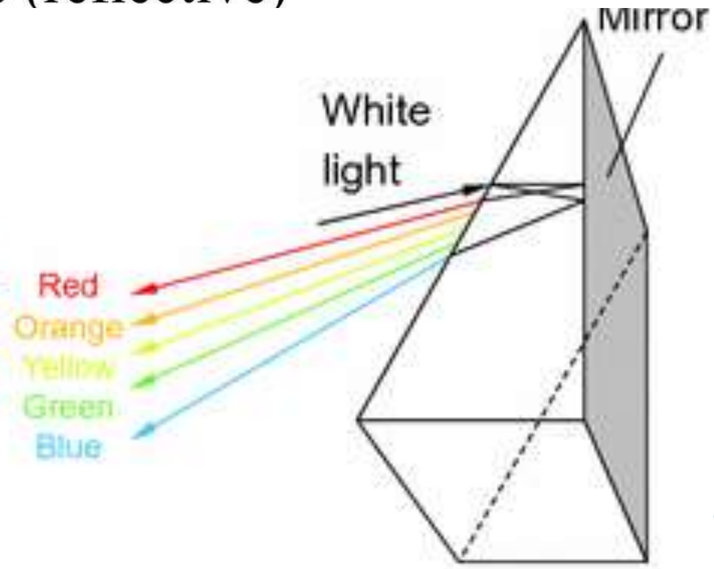
Cornu type (refractive)



LITTROW TYPE:

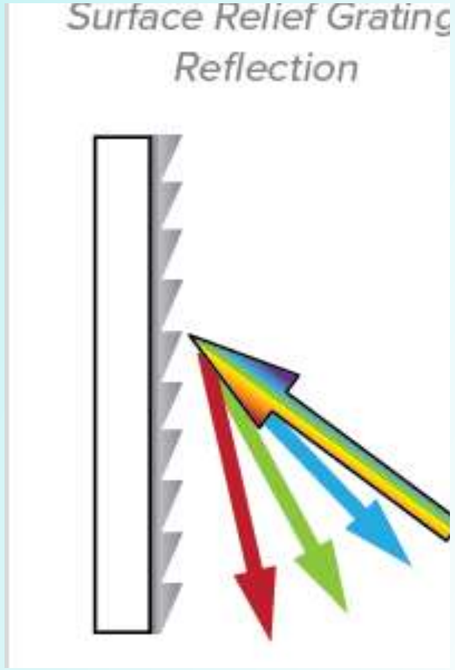
- It has optical angle 30° and its one surface is aluminized with reflected light back to pass through prism and to emerge on the same side of the light source i.e. Light doesn't pass through the prism on other side.

Littrow type (reflective)



2.Grating Monochromators

- **Gratings are rulings made on glass,Quartz or alkyl halides.**
- **Depending upon the instruments the rulings differ**
- **for UV-VIS , number of rulings per mm are more than 3600**

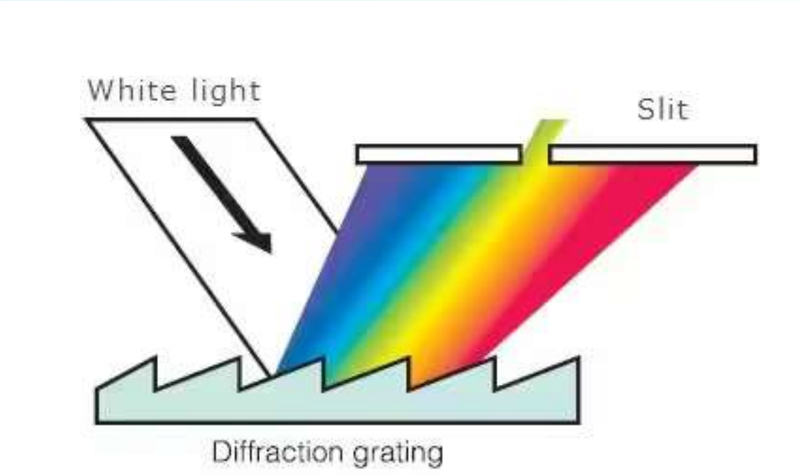


- Gratings are of 2 types.

1. Diffraction grating
2. Transmission grating

1.Diffraction Gratings

- Divides(disperses, spreads) light composed of lots of different wavelengths(e.g., white light) into light components by wavelength.
- The light from all the slits is reinforced in the same way to produce "diffracted light."



$$\sin\alpha - \sin\beta = Nm\lambda$$

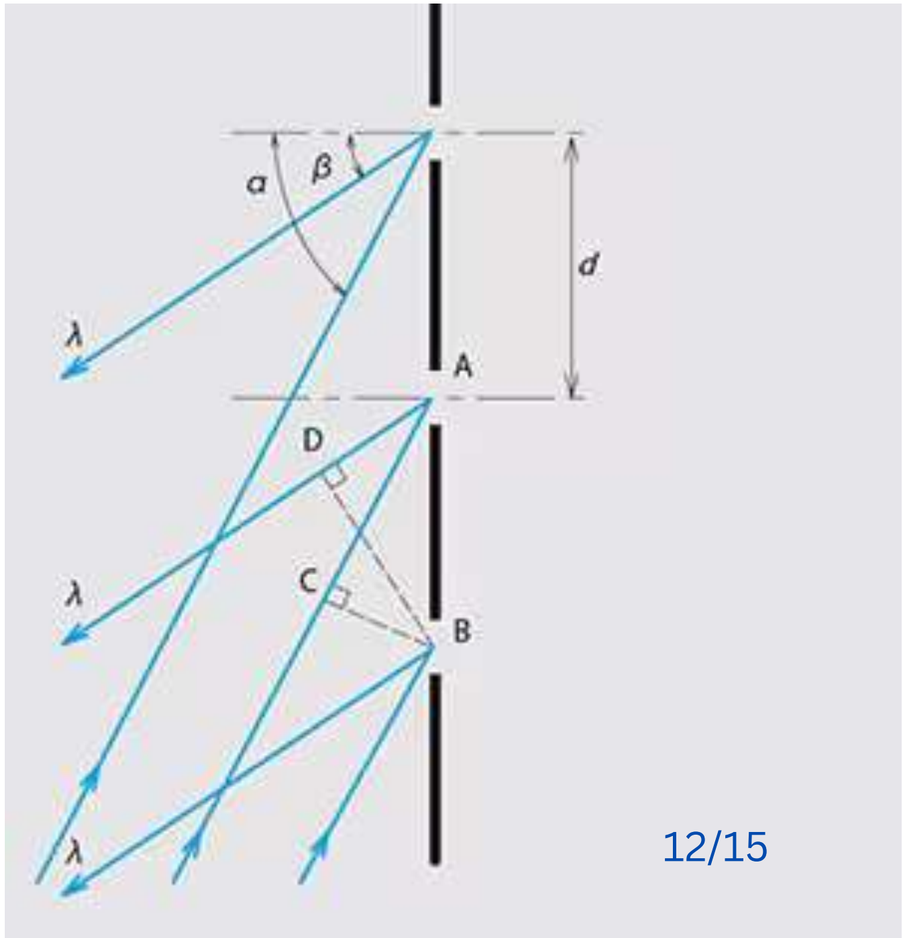
N : Number of slits per mm

m : Order of diffraction (m = 0, ± 1, ± 2,...)

λ : Wavelength

α-incident angle

-reflected angle



2. TRANSMISSION GRATING

- It is similar to diffraction grating but refracton takes place instead of reflection.
- Refraction produces reinforcement.This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

$$\sin\alpha + \sin\beta = Nm\lambda$$

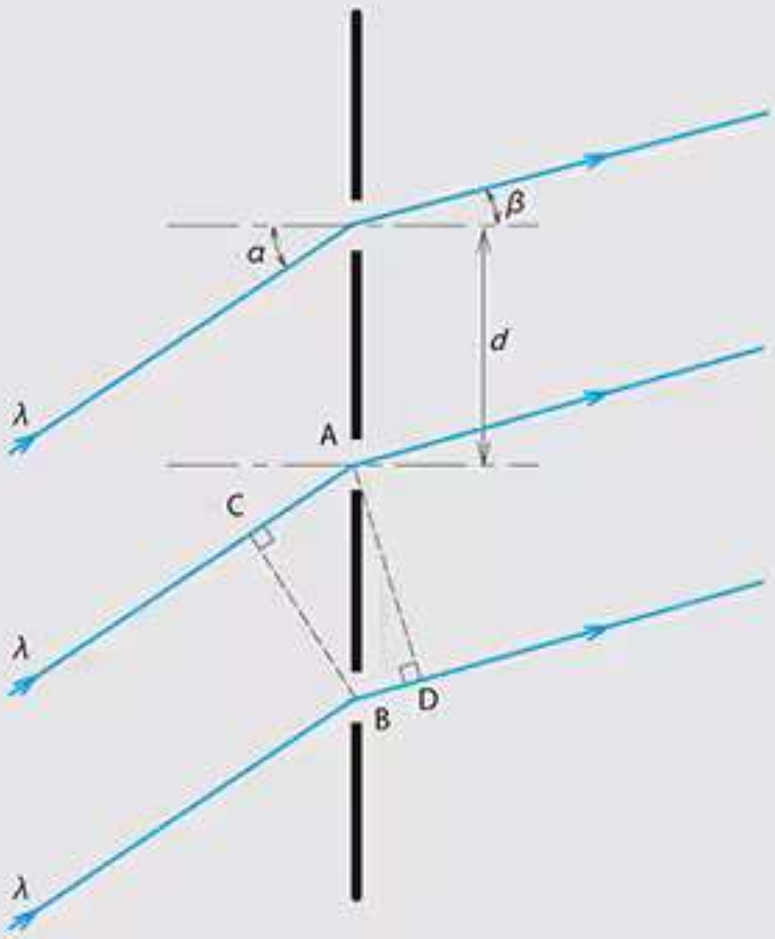
N : Number of slits per mm

m : Order of diffraction (m = 0, ± 1, ± 2,...)

λ : Wavelength

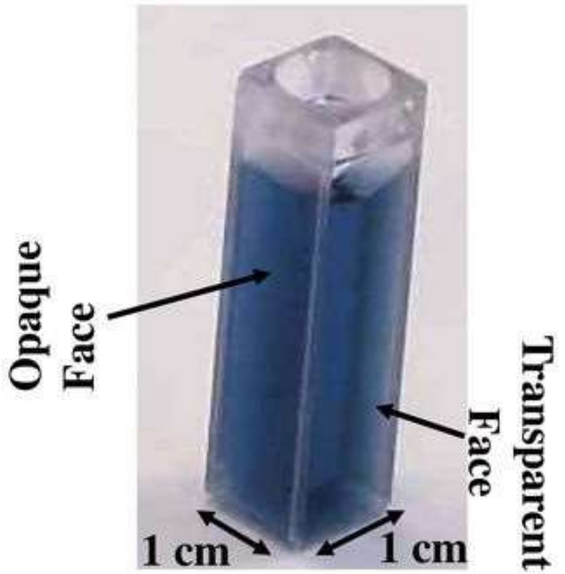
α -incident angle

-diffracted angle

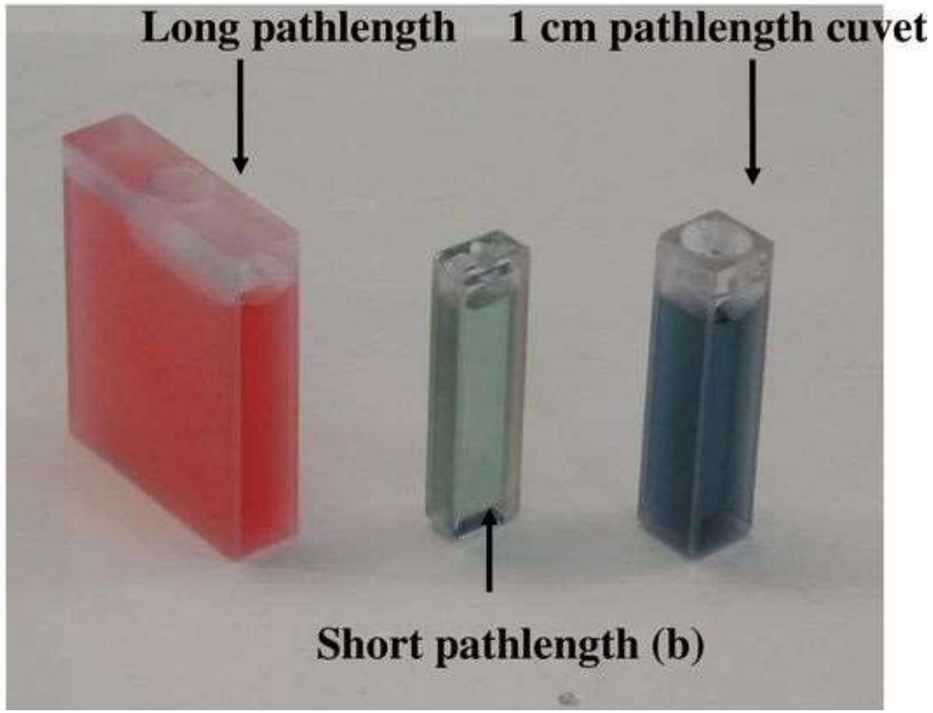


4. Sample container (Cuvette)

- For Visible and UV spectroscopy, a liquid sample is usually contained in a cell called a cuvette.
- Glass is suitable for visible but not for UV spectroscopy because it absorbs UV radiation.
- Quartz can be used in UV as well as in visible spectroscopy



Holding capacity 1.5 mL to 3.5 mL



5. DETECTORS

- Device which converts light energy into electrical signals, that are displayed on readout devices.
- The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample.

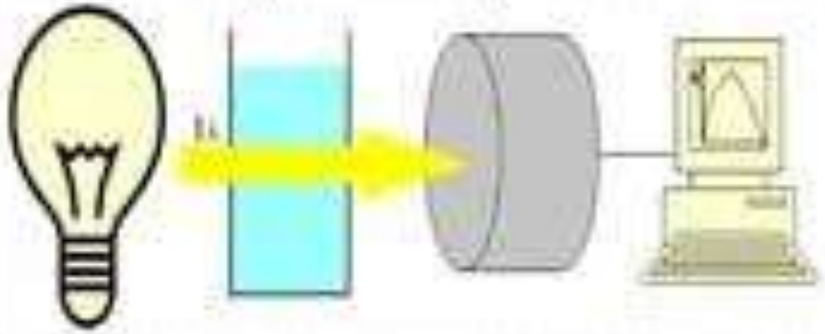
1. Barrier layer cell/Photovoltaic cell
2. Phototubes/Photoemissive tube
3. Photomultiplier tube

Requirements of ideal detectors:

- It should give quantitative response.
- It should have high sensitivity and low noise level.
- It should have a short response time.
- It should provide signal or response quantitative wide spectrum of radiation received.

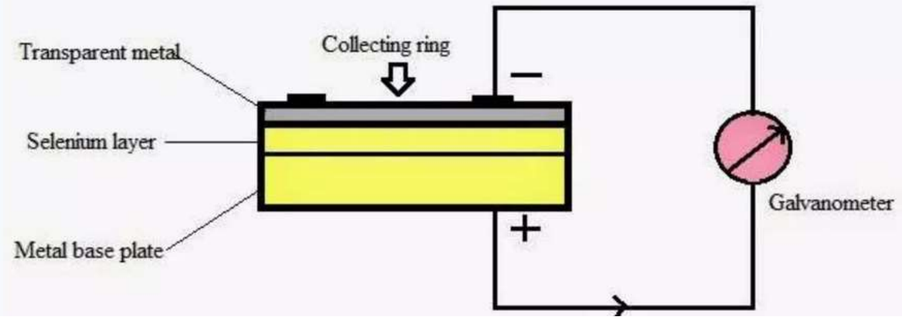
READOUT DEVICE

- The data from a detector are displayed by a readout device, such as an analogue meter, a light beam reflected on a scale, or a digital display , or LCD .
- The output can also be transmitted to a computer or printer.



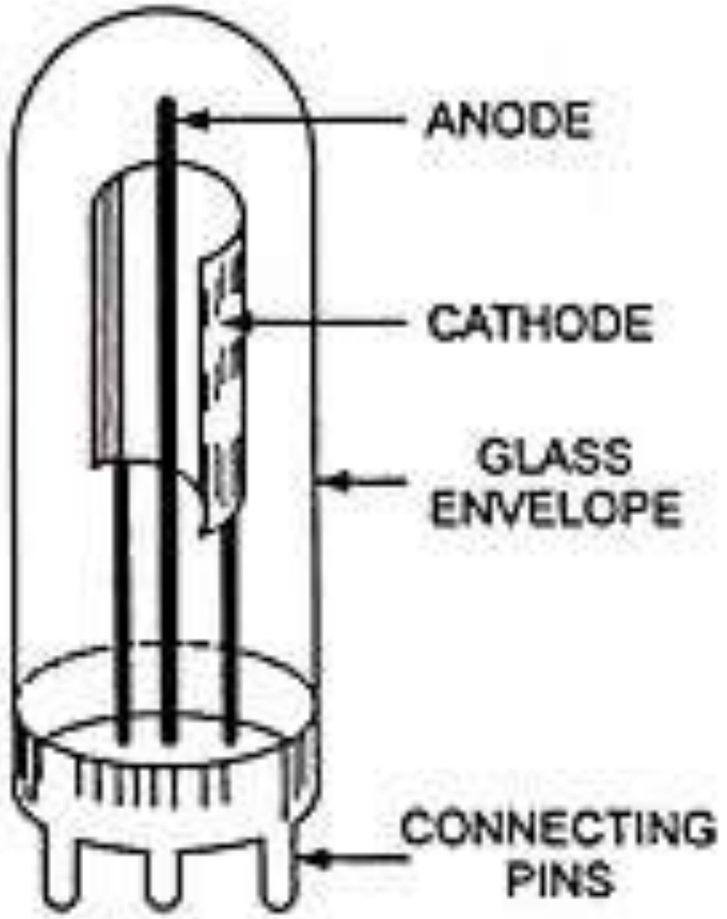
1. *Barrier layer cell/Photovoltaic cell*

- The detector has a thin film metallic layer coated with silver or gold and acts as an electrode.
- It also has a metal base plate which acts as another electrode.
- These two layers are separated by a semiconductor layer of selenium.
- When light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer.
- This creates a potential difference between two electrodes & causes the flow of current.
- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.



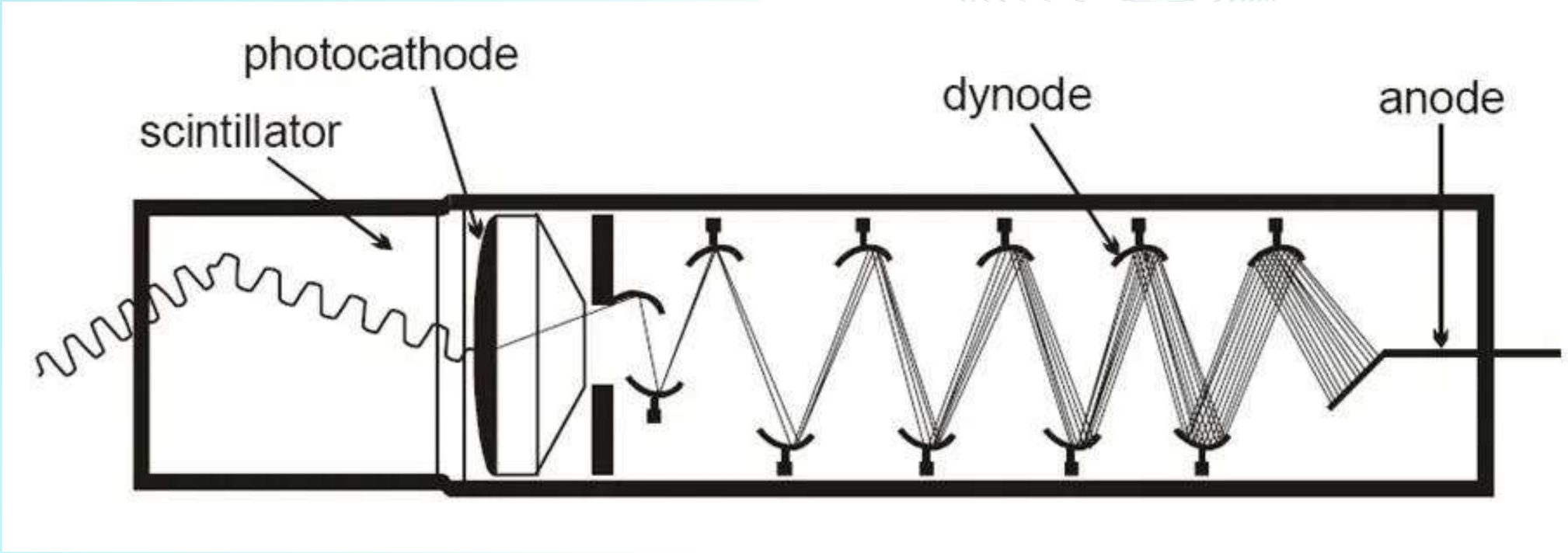
PHOTOTUBES/ PHOTOEMISSIVE TUBES

- Consists of a evacuated glass tube with a photocathode and collector anode.
- The surface of photocathode is coated with a layer of elements like cesium,siver oxide or mixture of them.
- When radiant energy falls on photosensitive cathode, electrones are attracted to anode causing current to flow.



PHOTOMULTIPLIER TUBES

- The principle employed in this detector is that,multiplication of photoelectrones by secondary emission of electrons.
- In a vaccum tube a primary photo-cathode is fixed which receives radiation from the sample.
- Some eight to 10 dynodes are fixed each with increasing potential of 75-100V higher than preceeding


photocathode
scintillator
dynode
anode

READOUT DEVICE

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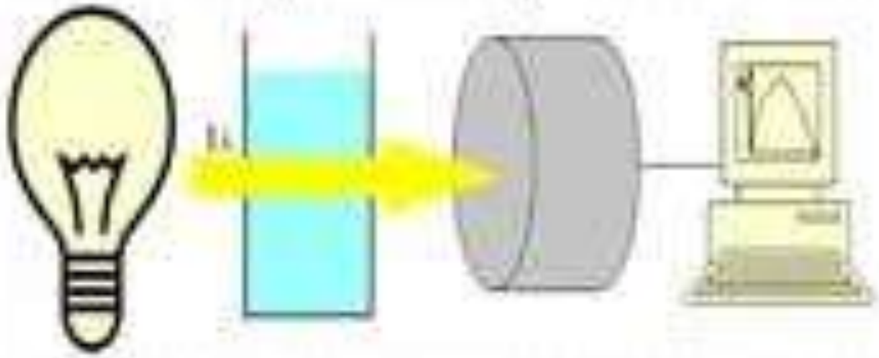
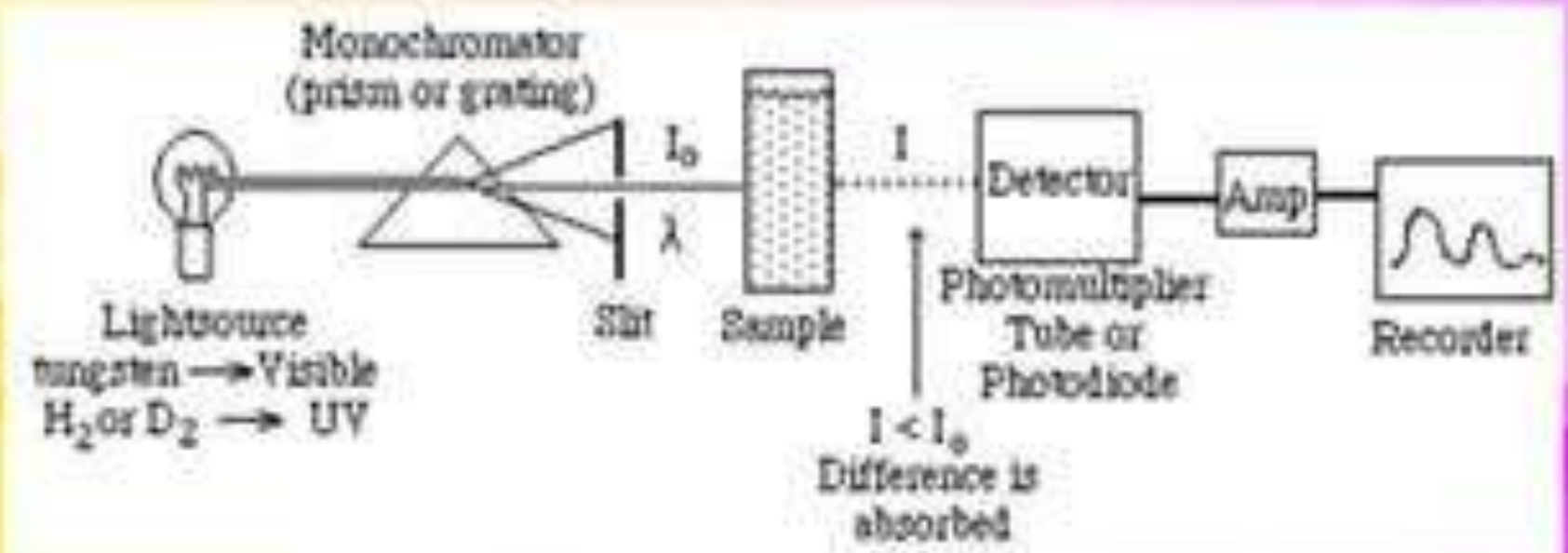


Single beam UV-Spectrophotometer

- ✓ Light from the source is carried through lens and/or through aperture to pass through a suitable filter.
- ✓ The type of filter to be used is governed by the colour of the solution.
- ✓ The sample solution to be analysed is placed in cuvettes.



Double Beam UV-Spectrophotometer

Double beam instrument is the one in which two beams are formed in the space by a U shaped mirror called as beam splitter or beam chopper .

Chopper is a device consisting of a circular disc. One third of the disc is opaque and one third is transparent, remaining one third is mirrored. It splits the monochromatic beam of light into two beams of equal intensities.