



BALAJI COLLEGE OF PHARMACY

UV VIS SPECTROSCOPY

PHARM D III YEAR

PREPARED BY

B. VISHNU VANDANA M.pharm

Dept of pharmaceutical analysis

Balaji college of pharmacy



SPECTROSCOPY

Spectroscopy is the **measurement** and **interpretation** of **Electromagnetic radiation** either **absorbed** or **emitted** when the molecules or atoms or ion in sample move from **one energy state** to **another energy state** .

EMR – made up of discrete particles (**PHOTONS**)

- **wave characteristic nature**
- **particle characteristic nature**

it can travel through vacume also!

Energy of EMR

$$E=hf$$

E=energy of radiation

H= planks constant (6.624×10^{-34})

Objectives

- **Spectrophotometer**
 - Components of optical instruments
 1. Sources
 2. Wavelength selectors (filters, monochromators)
 3. Sample containers
 4. Detectors
 5. Readout devices
- **Single and double beam instruments**
- **Applications of Spectrophotometry**
 - Spectrophotometry is more suited for quantitative analysis rather than qualitative one

SOURCES OF LIGHT

Hydrogen Discharge lamp :

- More stable
- Radiation will be provided from 120 to 350nm.
- The hydrogen gas will be stored under high pressure.

Deuterium Lamp ;

- Similar to HDL.
- Deuterium is filled in place of hydrogen.
- Provides 3 – 5times intense light but expensive.

Xenon Discharge Lamp :

- Xenon at 10 – 30 atm pressure.
- 2 tungsten electrodes.
- Intensity > HDL

Mercuric Arc Lamp :

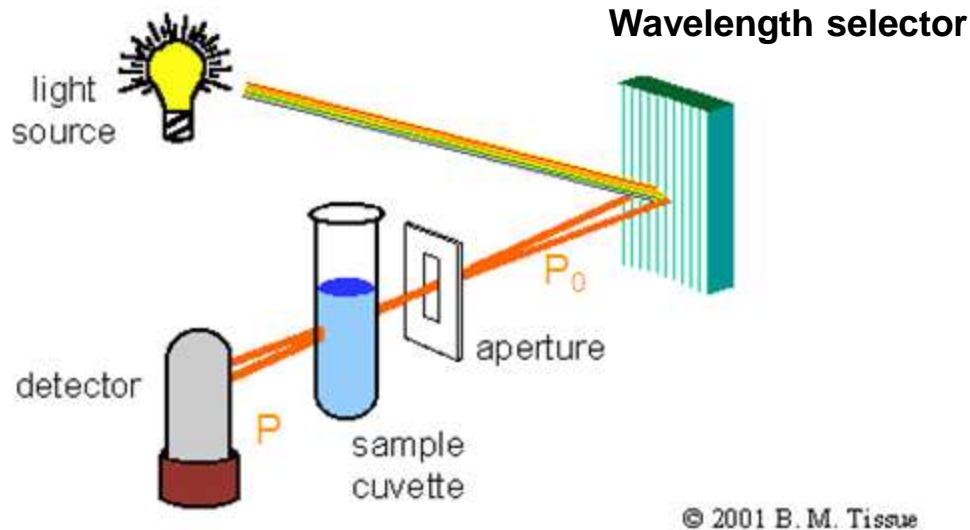
- Contains mercury vapours- offers sharp bands.
- Spectrum is not continuous so rarely used.

Tungsten lamp – lamp consists of tungsten filament in vacuum bulb.it offers sufficient intensity.

Carbon arc lamp – Very high intensity

- It provides entire range of visible spectrum.

Instrumentation (Spectrophotometers)



A single beam spectrophotometer

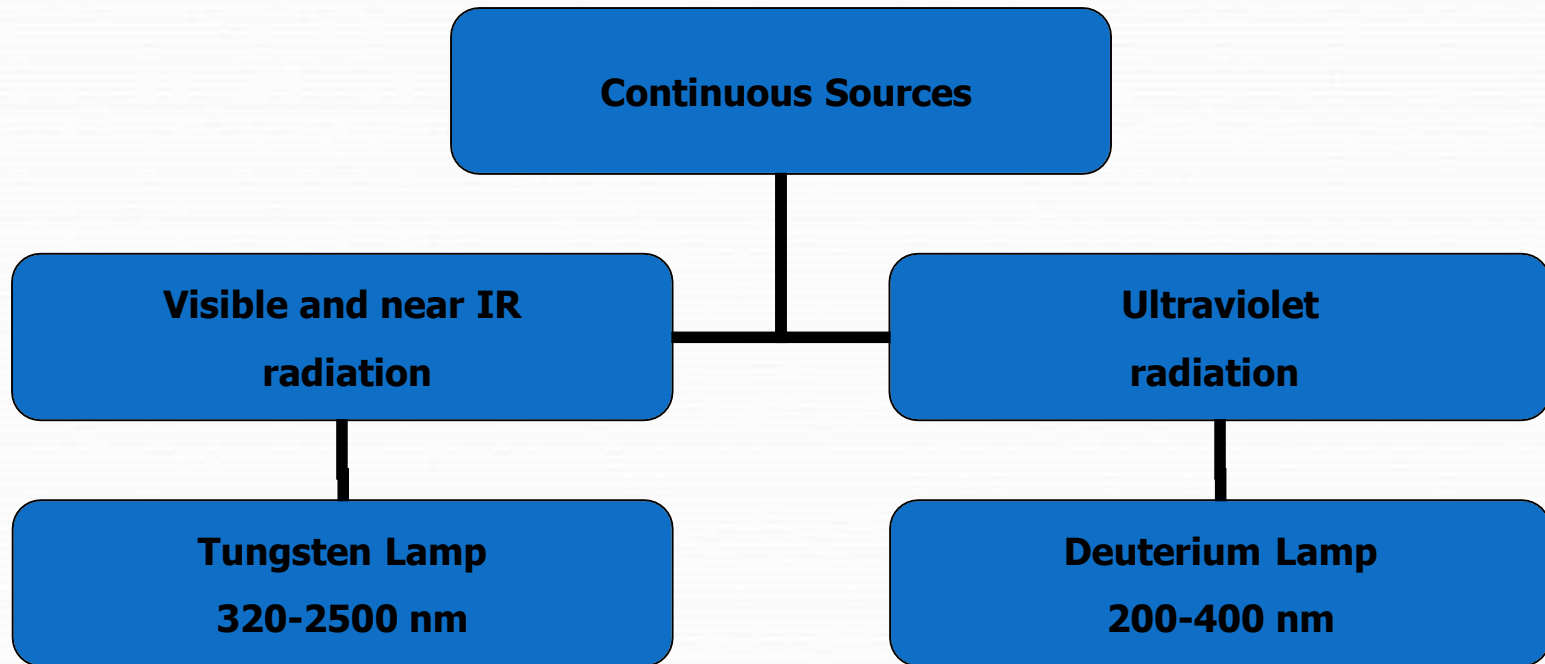
The above essential features of a spectrophotometer shows that polychromatic light from a **source** separated into narrow band of wavelength (nearly monochromatic light) by a **wavelength selector**, passed through the **sample compartment** and the transmitted intensity, P , after the sample is measured by a **detector**

In a **single beam instrument**, the light beam follows **a single path** from the source, to the monochromator, to the sample cell and finally to the detector

1- Sources of light

Sources used in UV-Vis Spectrophotometers are continuous sources.

- Continuous sources emit radiation of all wavelengths within the spectral region for which they are to be used.
- Sources of radiation should also be stable and of high intensity.

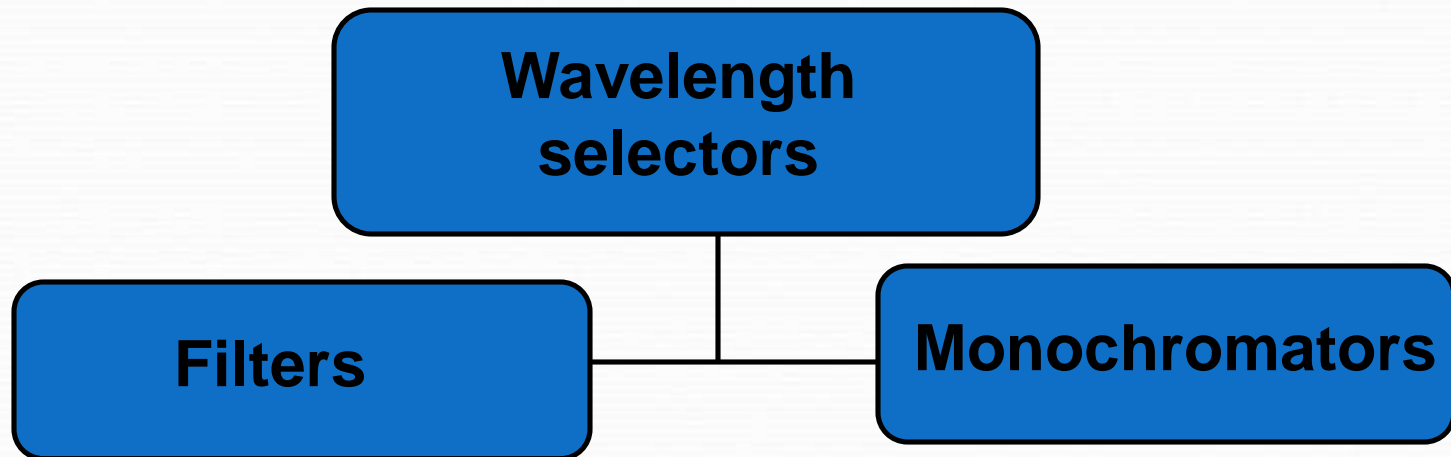


2. Wavelength Selectors

Ideally the output of a wavelength selector would be a radiation of a single wavelength.

No real wavelength selector is ideal, usually a band of radiation is obtained.

The narrower this bandwidth is, the better performance of the instrument.



- Filters – 1. Absorption
2. Interference
- Monochromators – 1. Prism type – a) Dispersive
b) Littrow type
2. Grating type – a) Diffraction
b) Transmission

Filters

ABSORPTION FILTERS INTERFERENCE FILTERS

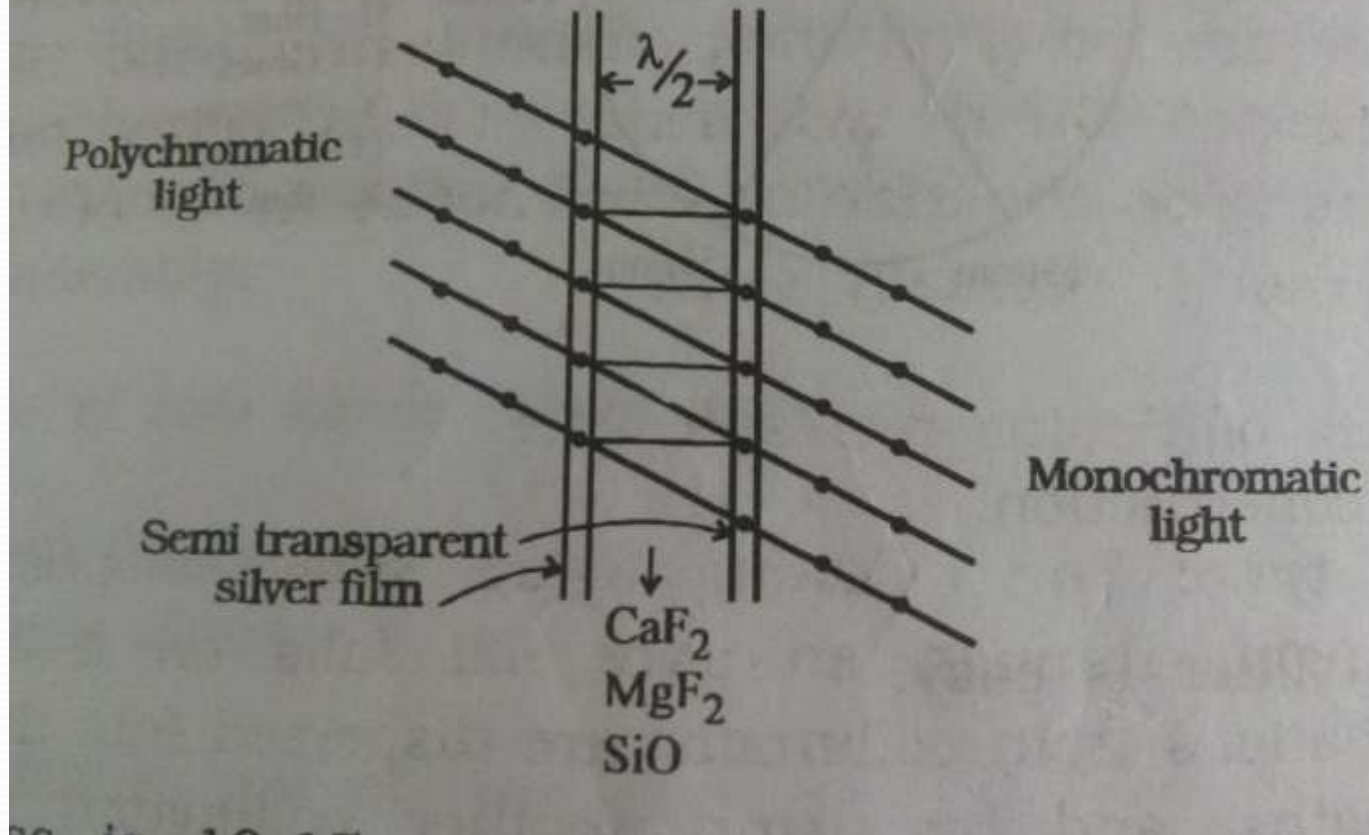
Selection of absorption filter is done according to the following procedure:

- ▶ Draw a filter wheel.

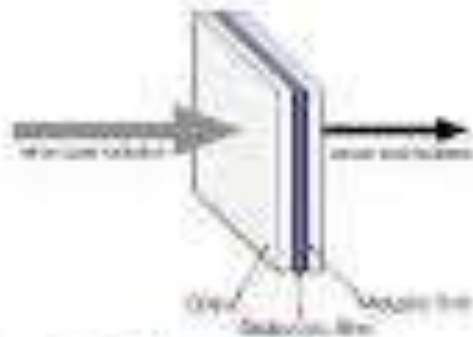


- ▶ Write the color VIBGYOR in clockwise or anticlockwise manner, omitting Indigo.

**Fig 1.5. INTERFERENCE FILTER
(FABRY-PEROT)**

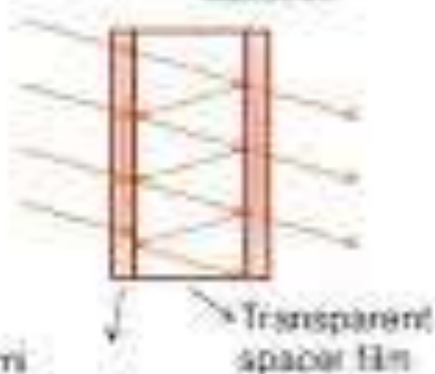


Optical Filters: Interference Filter



Advantages:

- 1) Allow a much narrower band of wavelength to pass and are similar to monochromator in selectivity.
- 2) Simpler and less expensive.
- 3) Can be used with high intensity light sources.
- 4) Continuous selection is possible by using wedge filter.



Semi-transparent silver film

Transparent spacer film

INTERFERENCE FILTER

Interference filter has dielectric spacer film is made up of CaF₂ MgF₂ b/w two parallel reflecting silver films

The thickness of dielectric spacer film can be $\frac{1}{2} \lambda$ (1st order), $2\frac{1}{2} \lambda$ (2nd order), $3\frac{1}{2} \lambda$ (3rd order), $4\frac{1}{2} \lambda$ (4th order).

The mechanism is radiation reflected by 2nd film and incoming radiation undergoes constructive interference to give monochromatic radiation

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

λ = wavelength of light obtained

n = dielectric constant of layer material

b = layer thickness

m = order no (0,1,2,3,4.....etc))

i- Filters

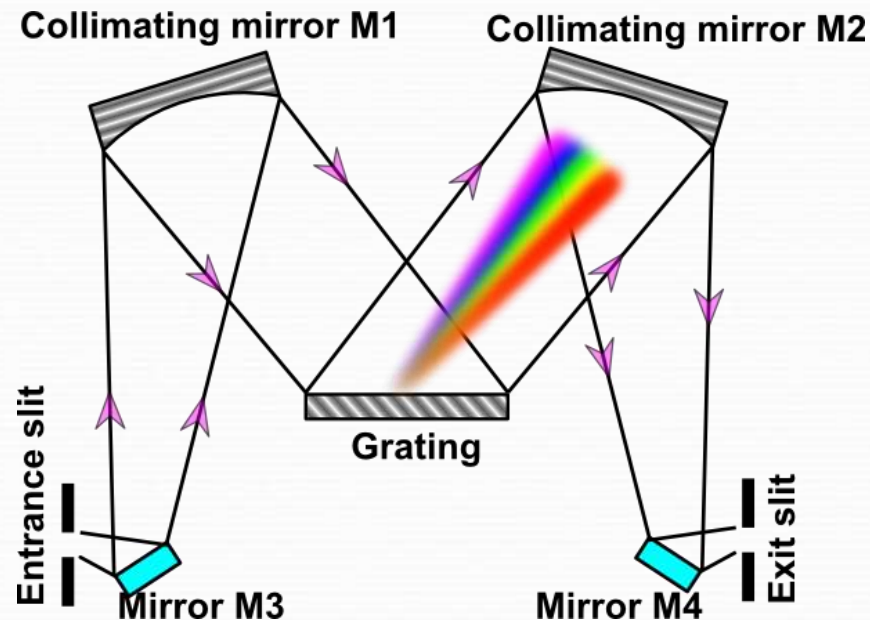
- Filters permit certain bands of wavelength (bandwidth of ~ 50 nm) to pass through.
- The simplest kind of filter is absorption filters, the most common of this type of filters is colored glass filters.
- They are used in the visible region.
- The colored glass absorbs a broad portion of the spectrum (complementary color) and transmits other portions (its color).

Disadvantage

- They are not very good wavelength selectors and can't be used in instruments utilized in research.
- This is because they allow the passage of a broad bandwidth which gives a chance for deviations from Beer's law.
- They absorb a significant fraction of the desired radiation.

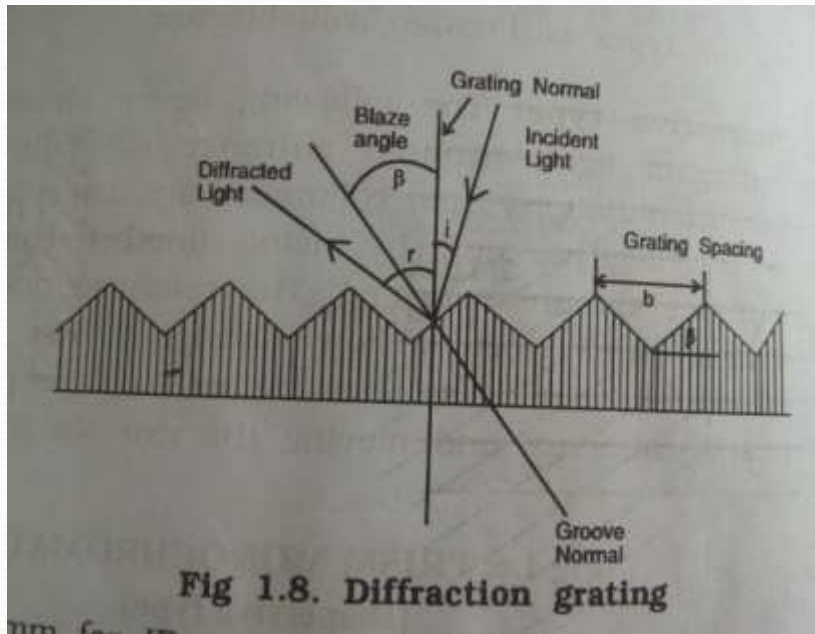
ii- Monochromators

- They are used for spectral scanning (varying the wavelength of radiation over a considerable range).
- They can be used for UV/Vis region.
- All monochromators are similar in mechanical construction.
- All monochromators employ slits, mirrors, lenses, gratings or prisms.

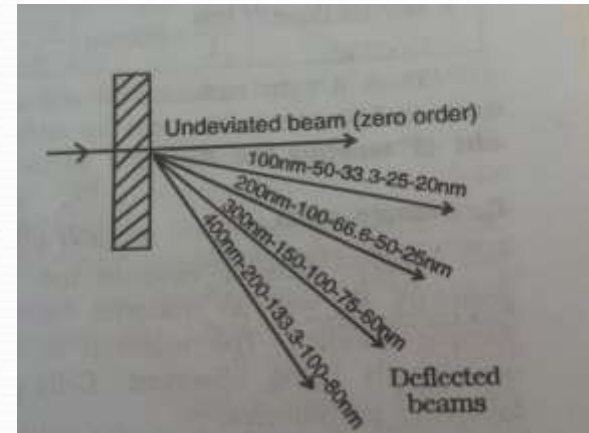
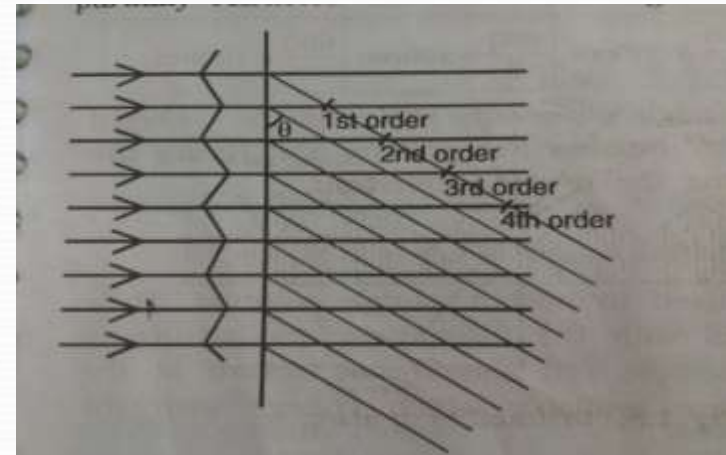


Grating kind monochromators

Diffraction grating



Transmission grating



Diffraction grating resulting radiation can be governed by equation

$$m\lambda = b(\sin i \pm \sin r)$$

m = order (0, 1, 2, 3, 4,etc)

λ = wavelength of light

i = angle of incidence

r = angle of reflection

Transmission grating resulting radiation produced can be determined by equation

$$\lambda = \frac{d \sin \theta}{m}$$

λ = wavelength of light

d = lines per cm

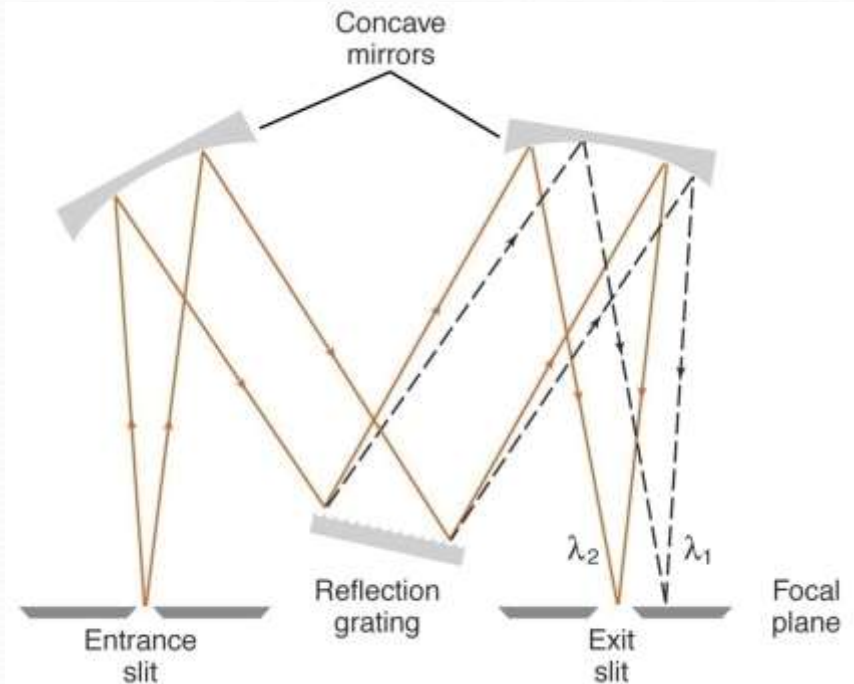
m = order (0, 1, 2, 3, 4,etc)

θ = angle of deflection

1-Grating monochromators

Reflection grating

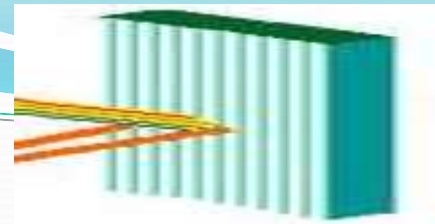
- ☛ Polychromatic radiation from the entrance slit is collimated (made into beam of parallel rays) by a concave mirrors
- ☛ These rays fall on a reflection grating, whereupon **different wavelengths are reflected at different angles.**
- ☛ The orientation of the reflection grating directs only one narrow band wavelengths, λ_2 , to the exit slit of the monochromator
- ☛ Rotation of the grating allows different wavelengths, λ_1 , to pass through the exit slit



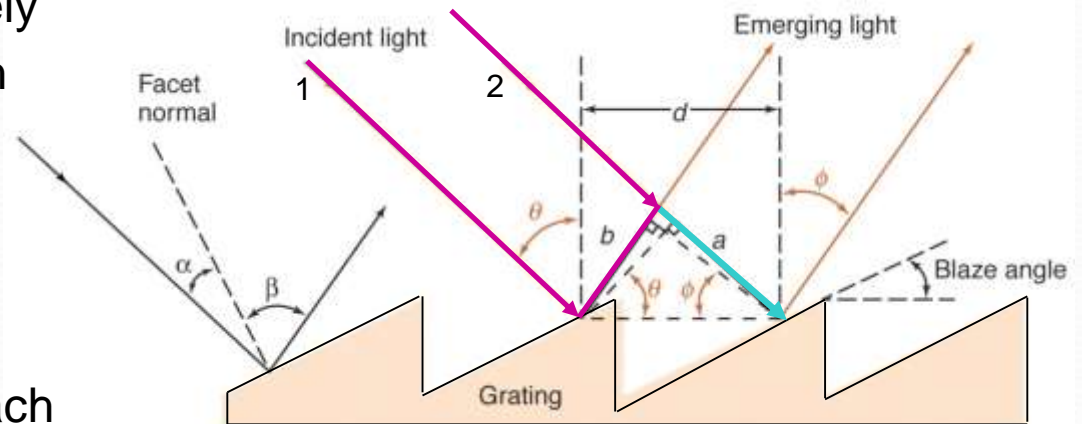
The reflection grating monochromator

Device consists of **entrance** and **exit** slits, **mirrors**, and a **grating** to disperse the light

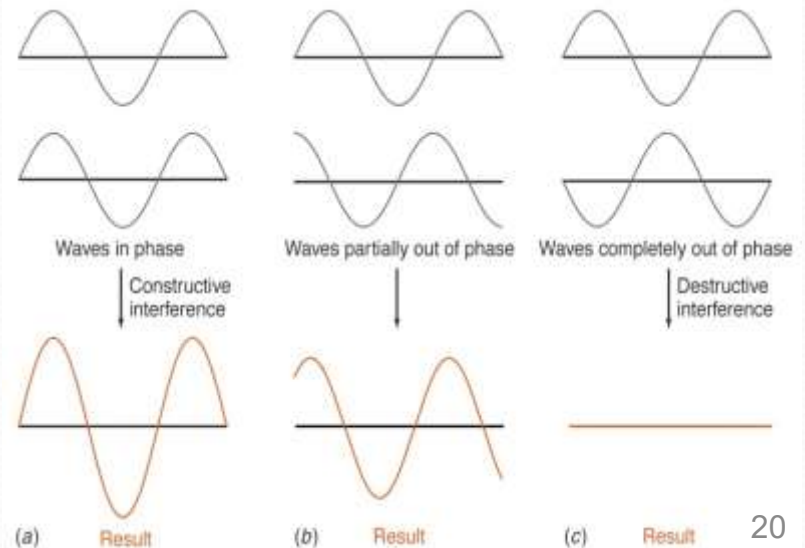
Echelle Reflection Grating



1. The reflection grating is ruled with a series of closely spaced, parallel grooves with repeated distance d .
2. The grating is covered with Al to make it **reflective**.
3. When polychromatic light is reflected from the grating, each groove behaves as **a new point source** of radiation.
4. When adjacent light rays are in phase, they reinforce one another (**constructive interference**).
5. When adjacent light rays are not in phase, they partially or completely canceled one another (**destructive interference**).



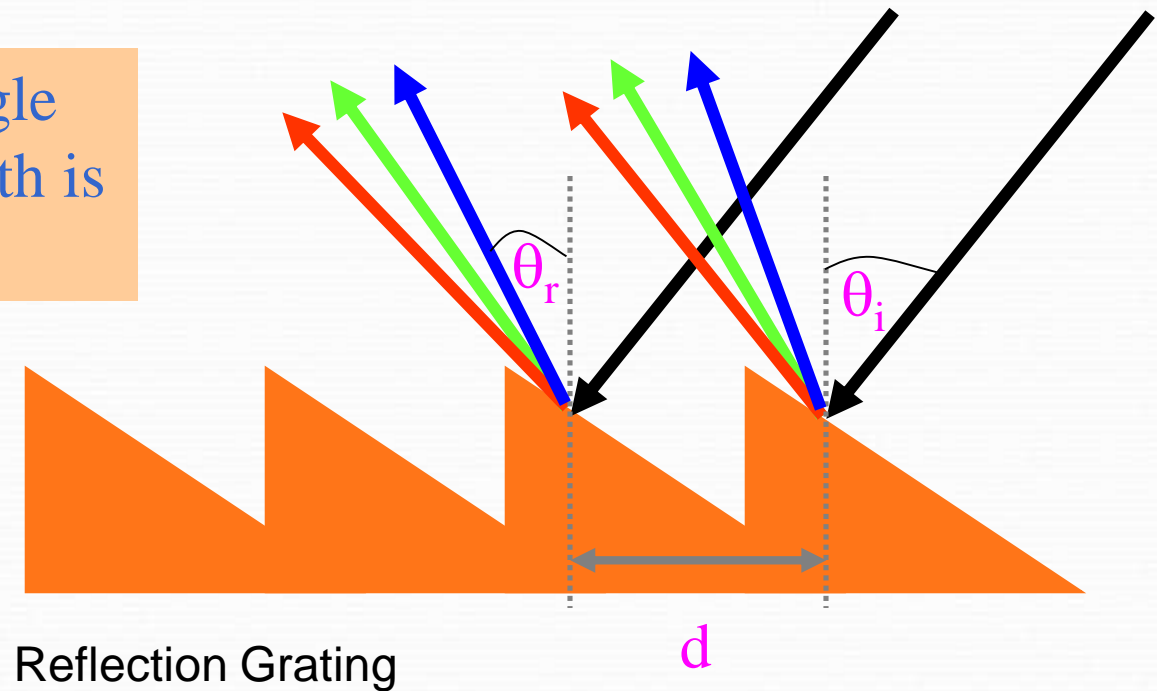
Reflection followed by either constructive or destructive interferences



Echelle Grating equation

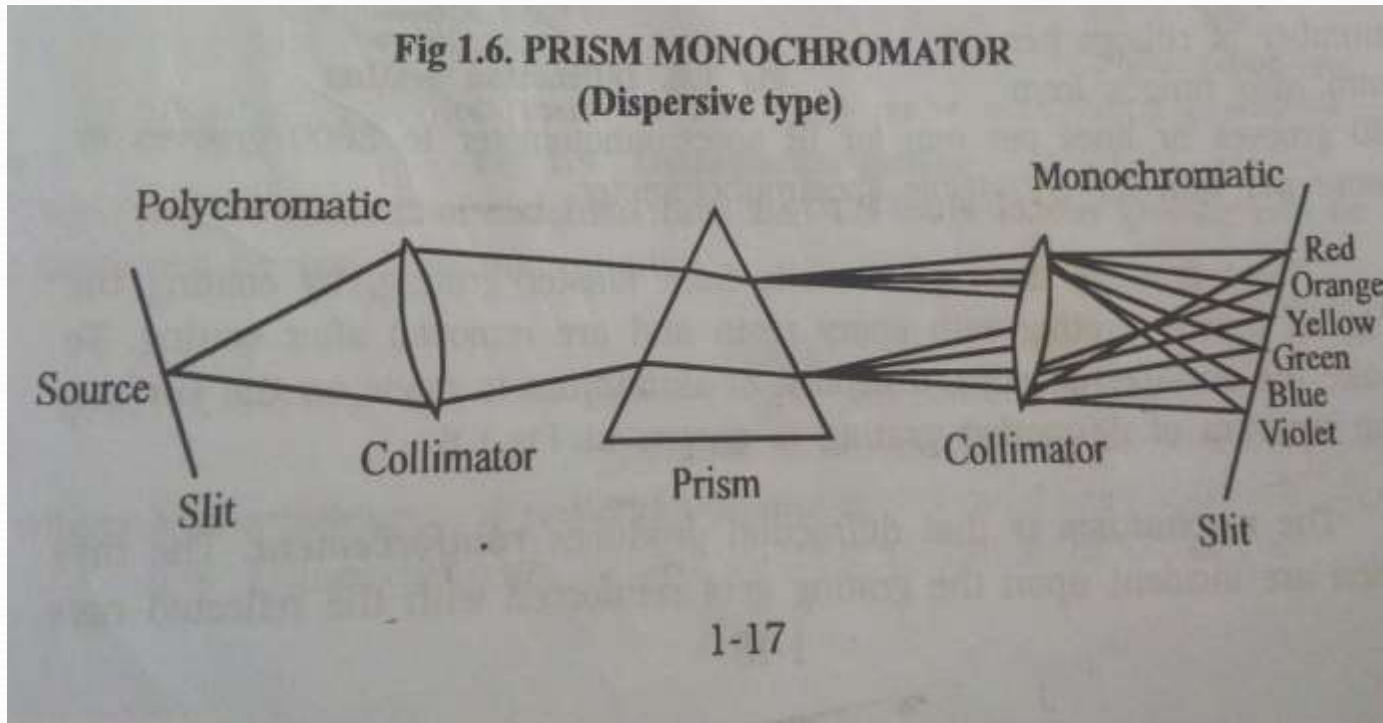
- $n \lambda = d (\sin \theta_i + \sin \theta_r)$ where $n = 1, 2, 3, \dots$
- Since incident angle $\theta_i = \text{constant}$; therefore $\lambda \propto \theta_r$

For each reflection angle θ_r , a certain wavelength is observed

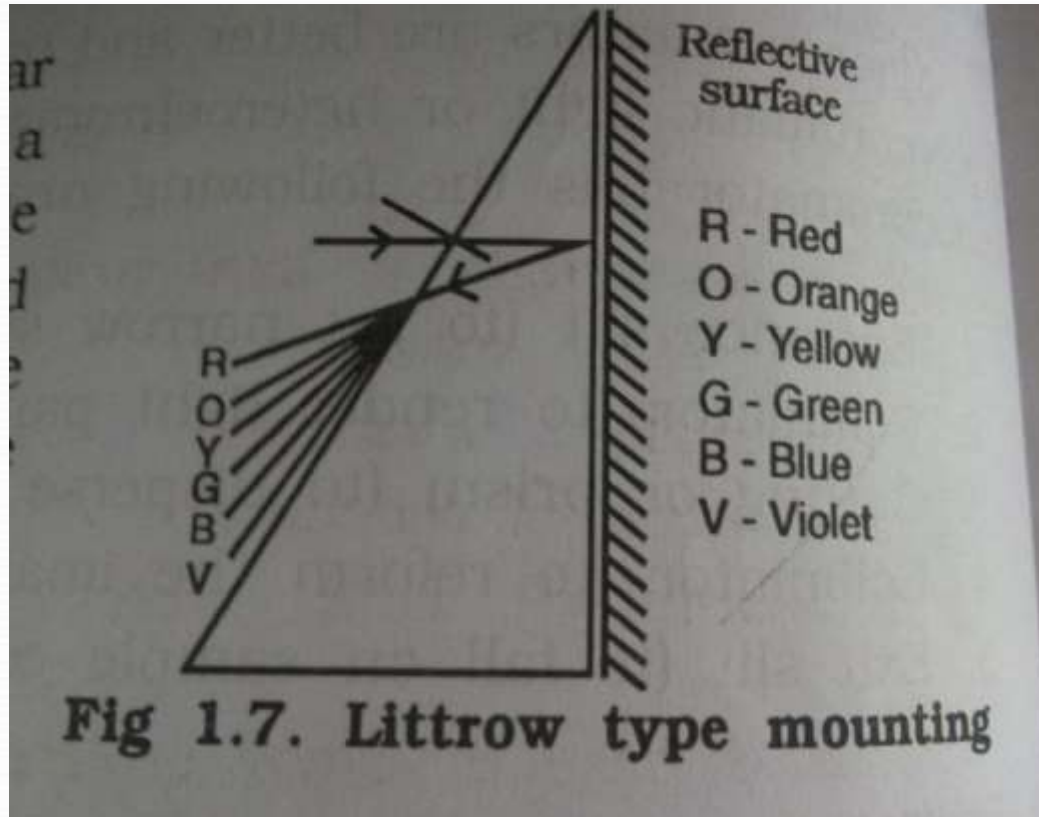


Note: For more detail see Skoog text book p. 159-160

Refractive prisms

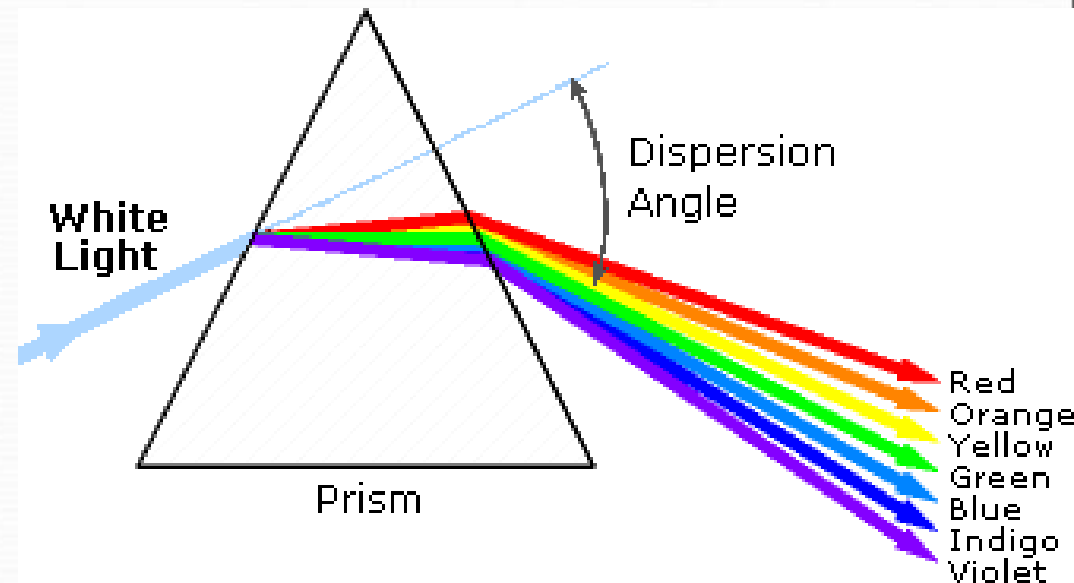
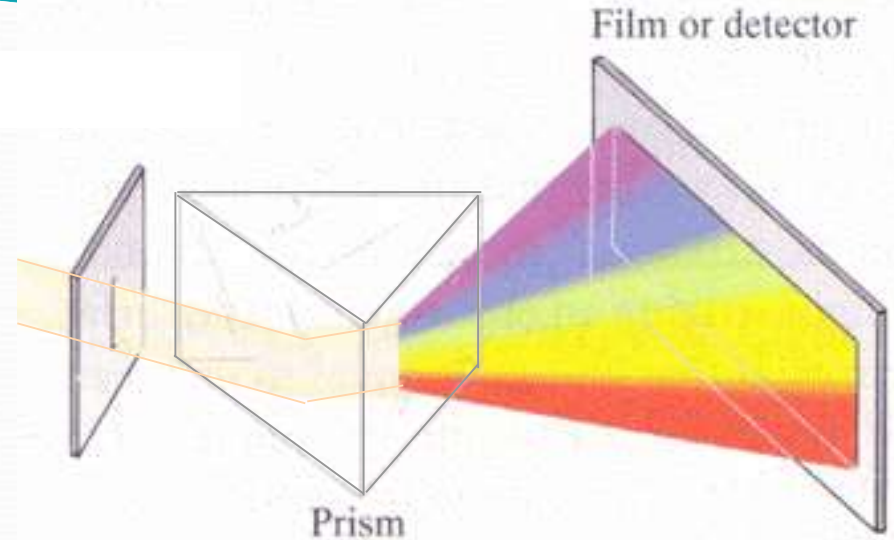


Reflective type or littrow type



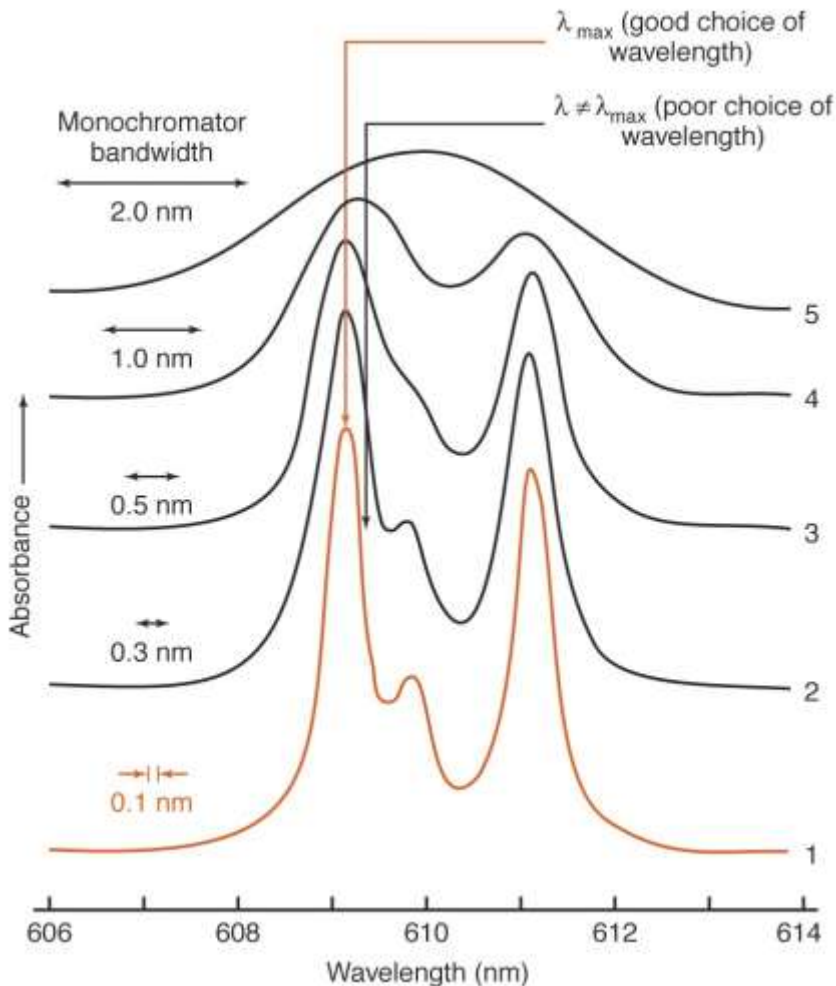
2- Prism monochromators

- 👉 Dispersion by prism depends on **refraction of light which is wavelength dependent**
- 👉 Violet color with higher energy (shorter wavelength) are diffracted or bent most
- 👉 While red light with lower energy (longer wavelength) are diffracted or bent least
- 👉 As a result, the polychromatic white light is dispersed to its individual colors.



What are the advantages and disadvantages of decreasing monochromator slit width?

Bandwidth Choice



The size of the monochromator exit slit determines the width of radiation (**bandwidth**) emitted from the monochromator.

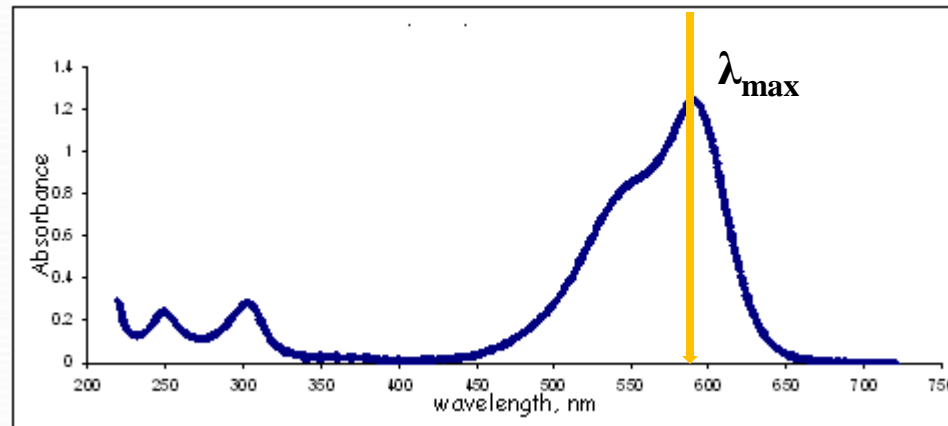
A **wider** slit width gives **higher sensitivity** because higher radiation intensity passes to the sample but on the other hand, **narrow** slit width gives **better resolution** for the spectrum.

In general, the choice of slit width to use in an experiment must be made by **compromising** these factors. Still, we can overcome the problem of low sensitivity of the small slit by **increasing the sensitivity of the detector**.

Selection of wavelength

Absorbance measurements are always carried out at **fixed wavelength** (using monochromatic light). When a wavelength is chosen for **quantitative analysis**, three factors should be considered

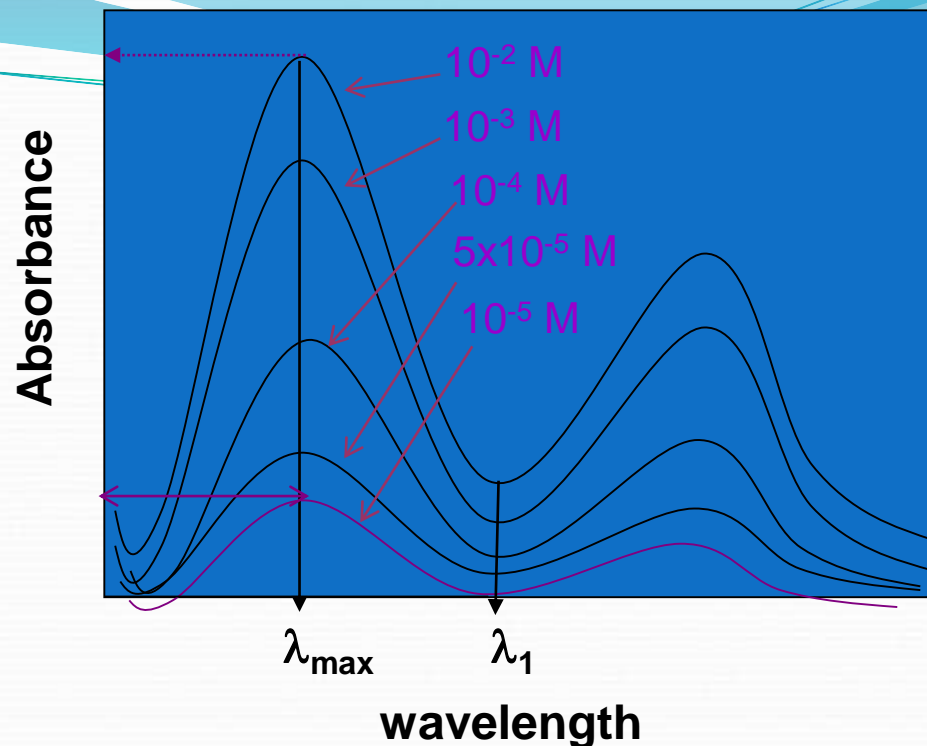
1. Wavelength should be chosen to give the **highest possible sensitivity**. This can be achieved **by selecting λ_{\max}** or in general the wavelengths at which the absorptivity is relatively high.



λ_{\max} - wavelength where maximum absorbance occurs

By performing the analysis at such wavelengths, it will be sure that the lowest sample concentration can be measured with fair accuracy.

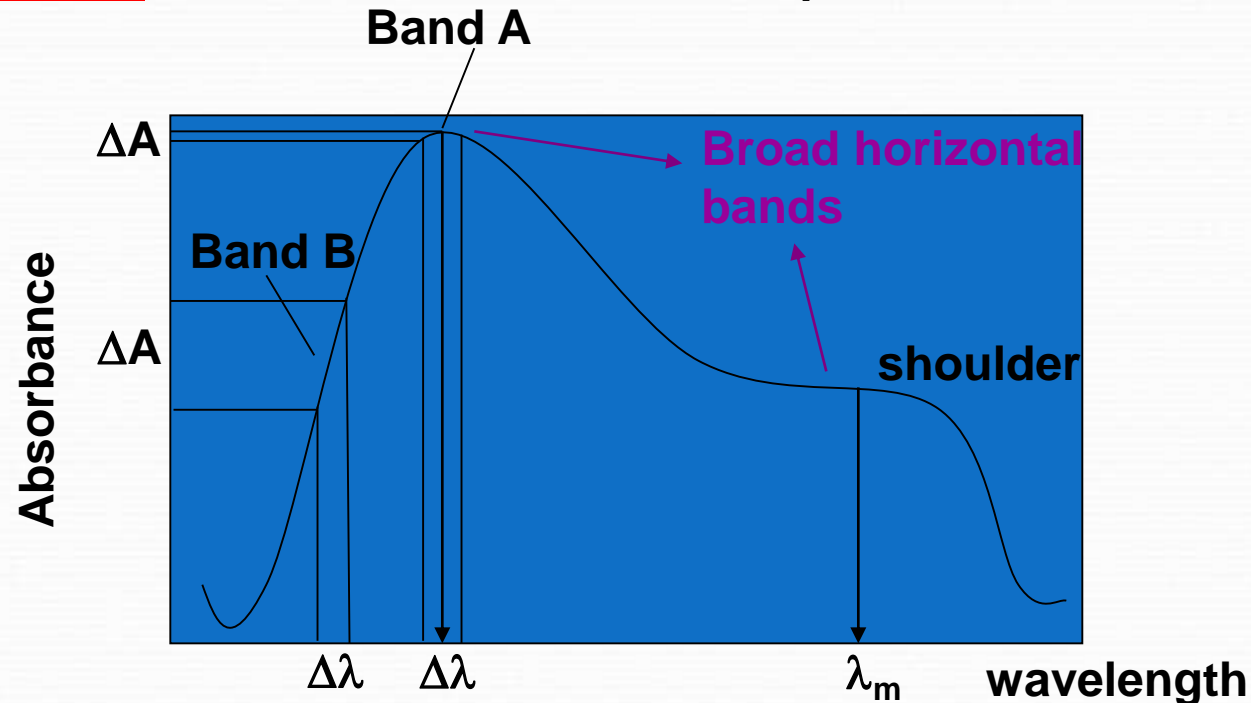
For example, the lowest sample concentration (10^{-5} M) can be measured with good accuracy at λ_{\max} , while at other wavelength (λ_1), it may not be detected at all.



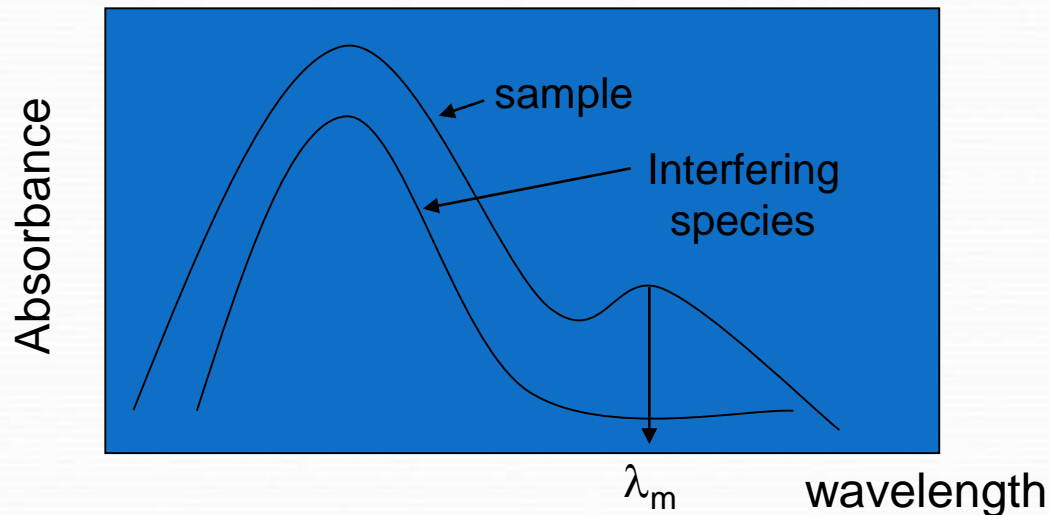
2. It is preferable to choose the wavelength at which the absorbance will not significantly change if the wavelength is slightly changed, i.e., $\Delta A / \Delta \lambda$ is minimum.

At a wavelength corresponding to broad horizontal band on the spectrum (band A), the radiation is mainly absorbed to the same extent ($\Delta A / \Delta \lambda \sim \text{zero}$).

However on a steep portion of the spectrum (band B), the absorbance will change greatly if the wavelength is changed ($\Delta A / \Delta \lambda$ is large). Thus on repeating the absorbance measurements, you might get different readings and the precision of the measurements will be poor.

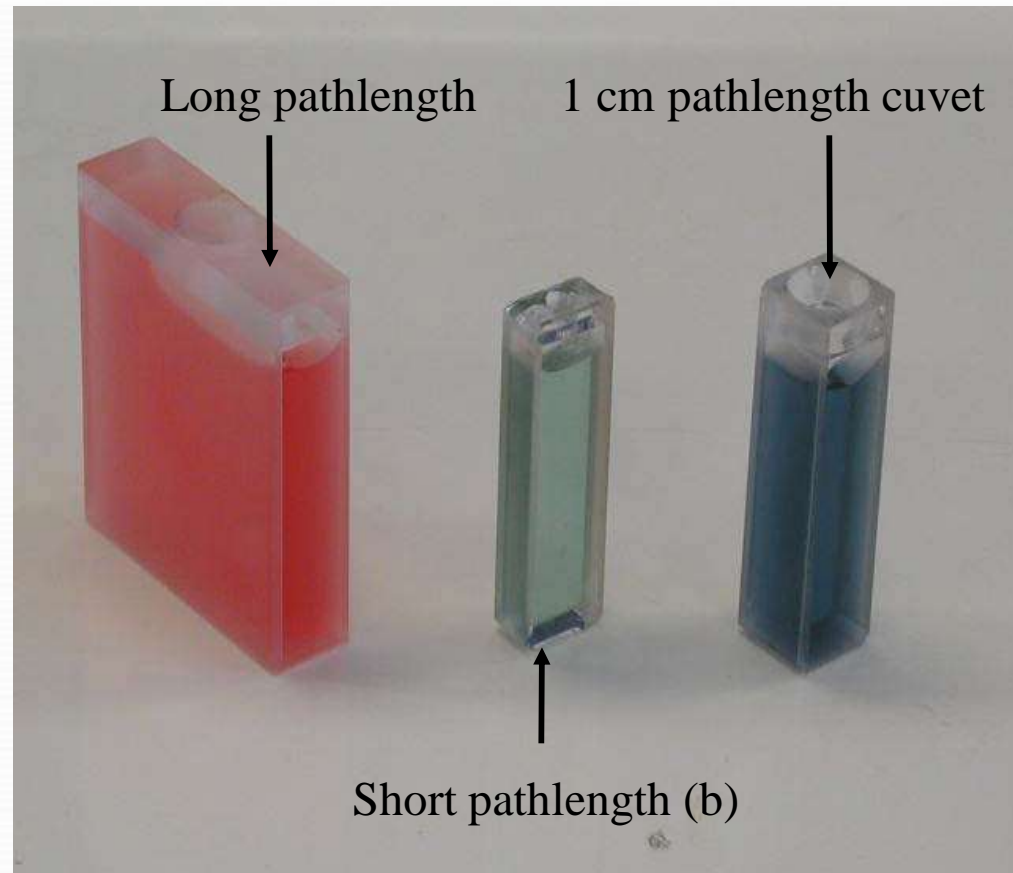
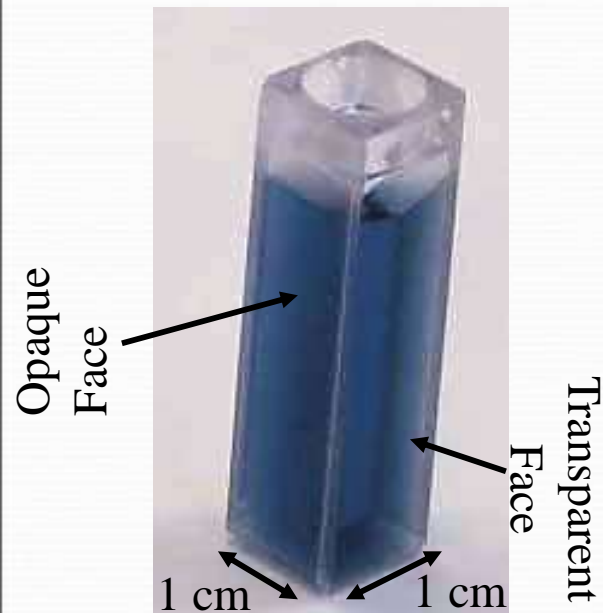


3- If the solution contains more than absorbing species, the wavelength should be chosen, whenever possible, in region at which the other species does not absorb radiation or its absorbance is minimum. By this way, the second species does not interfere in the determination.



3- Sample compartment (cells)

- 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

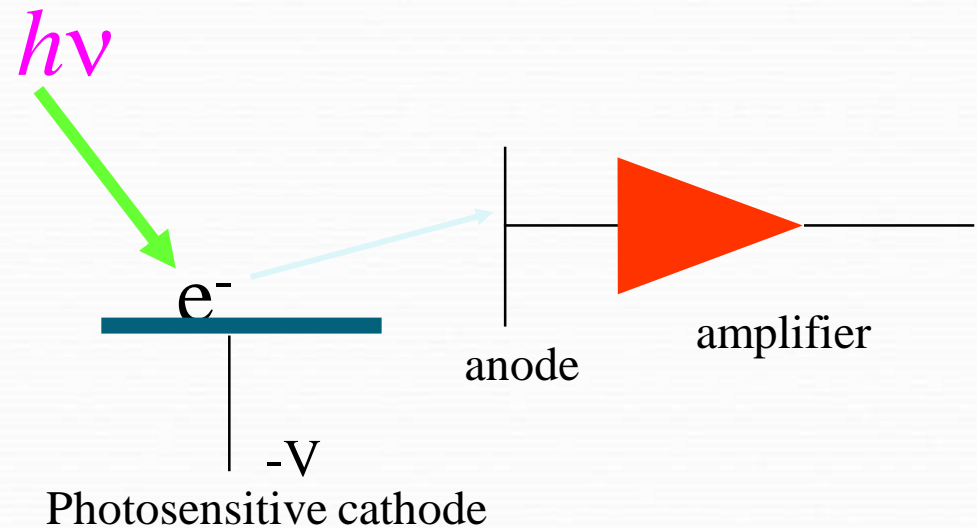


4- Detectors

- 👁 The detectors are devices that **convert radiant energy into electrical signal**.
- 👁 A Detector should be **sensitive**, and has a **fast response** over a considerable range of wavelengths.
- 👁 In addition, the electrical signal produced by the detector must be **directly proportional** to the transmitted intensity (**linear response**).

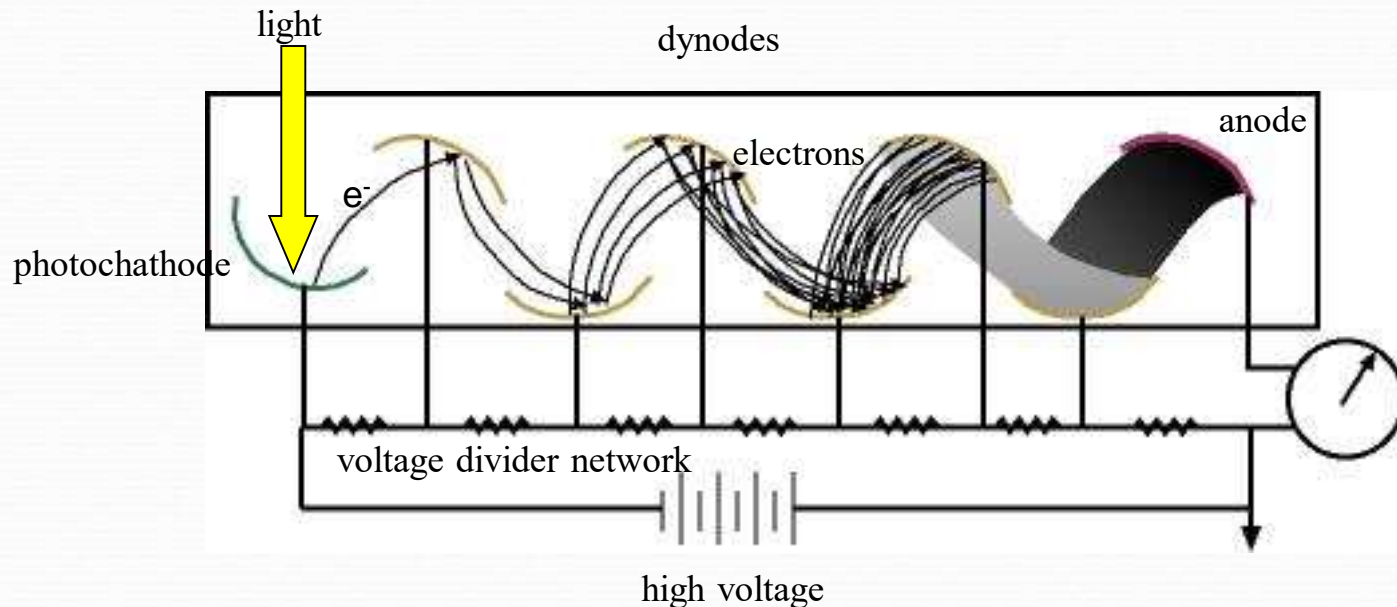
i- Phototube

- ⊙ Phototube emits electrons from a **photosensitive, negatively charged cathode** when struck by visible or UV radiation
- ⊙ The electrons flow through vacuum to **an anode** to produce current which is proportional to radiation intensity.

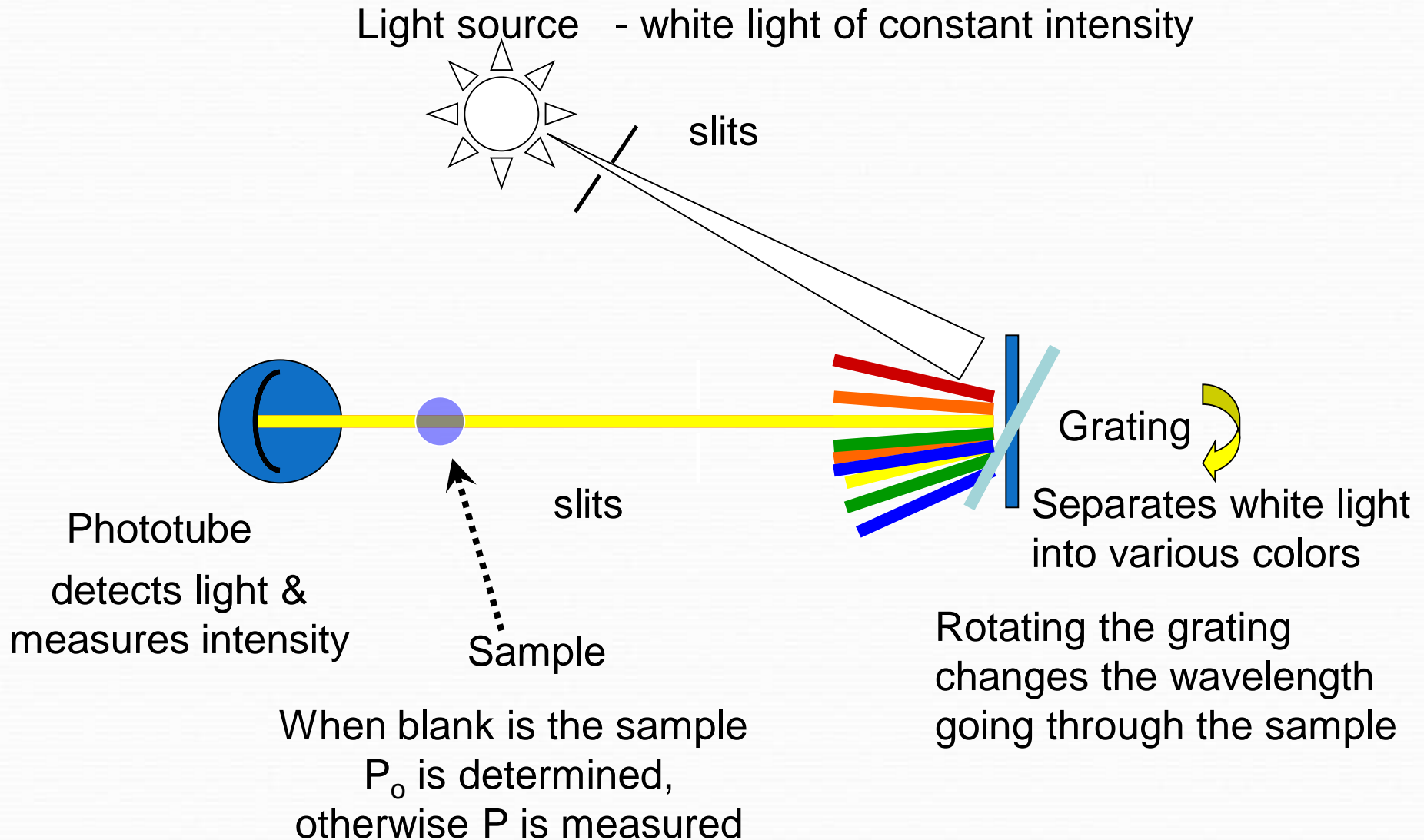


ii. Photomultiplier tube

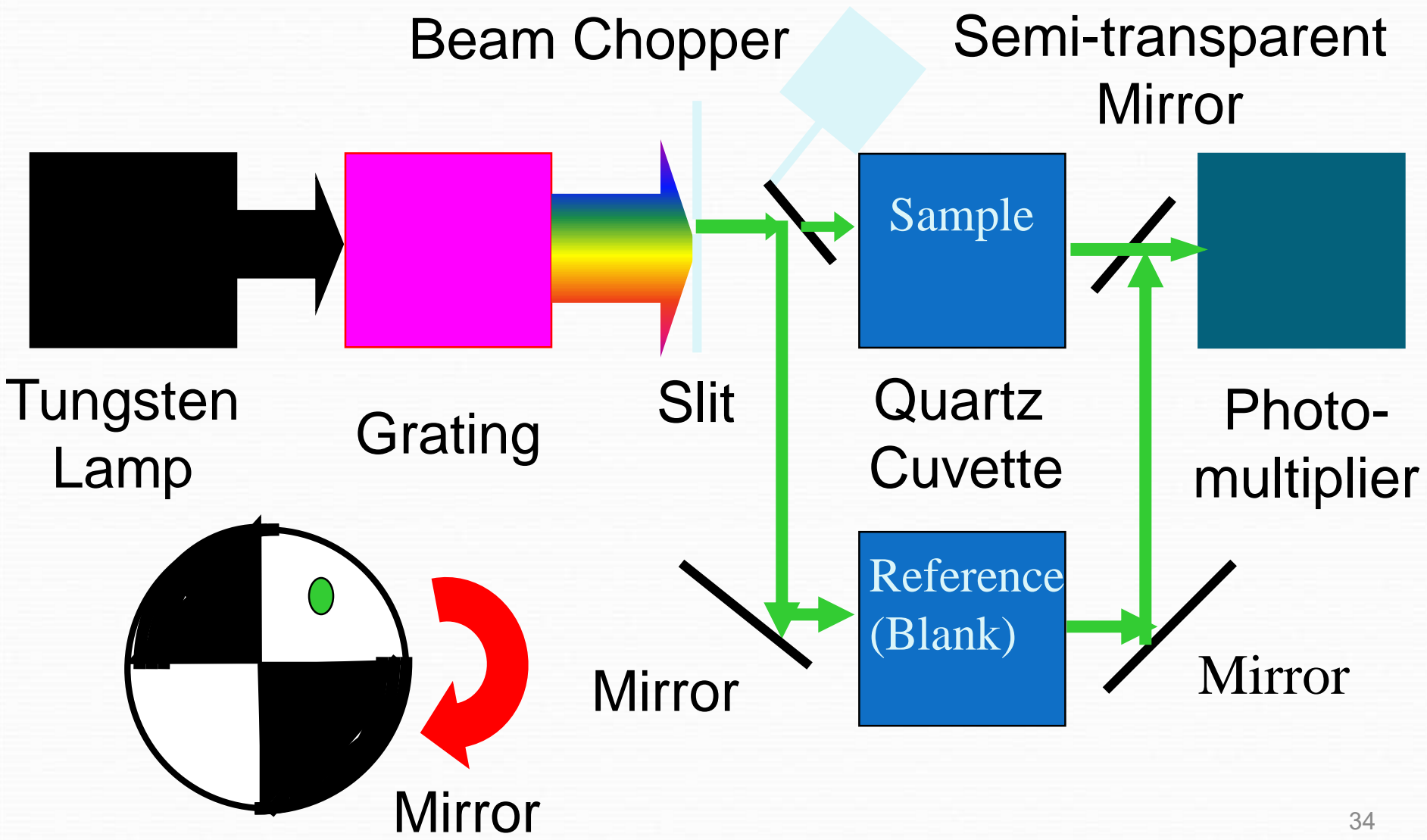
- It is a very sensitive device in which electrons emitted from the photosensitive cathode strike a second surface called **dynode** which is positive with respect to the original cathode.
- Electrons are thus accelerated and can knock out more than one electrons from the dynode.
- If the above process is repeated several times, so more than 10^6 electrons are finally collected for each photon striking the first cathode.



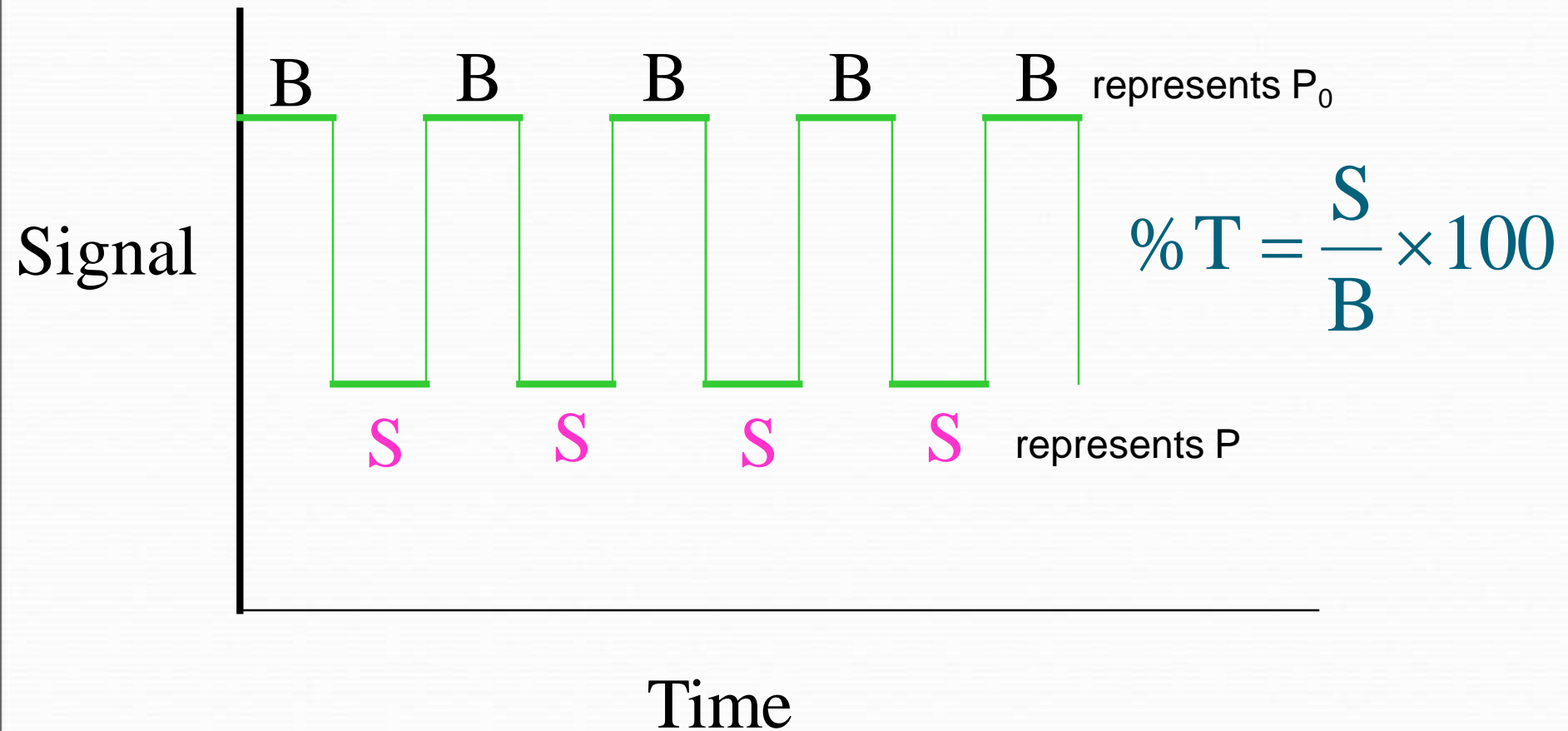
The components of a single beam spectrophotometer

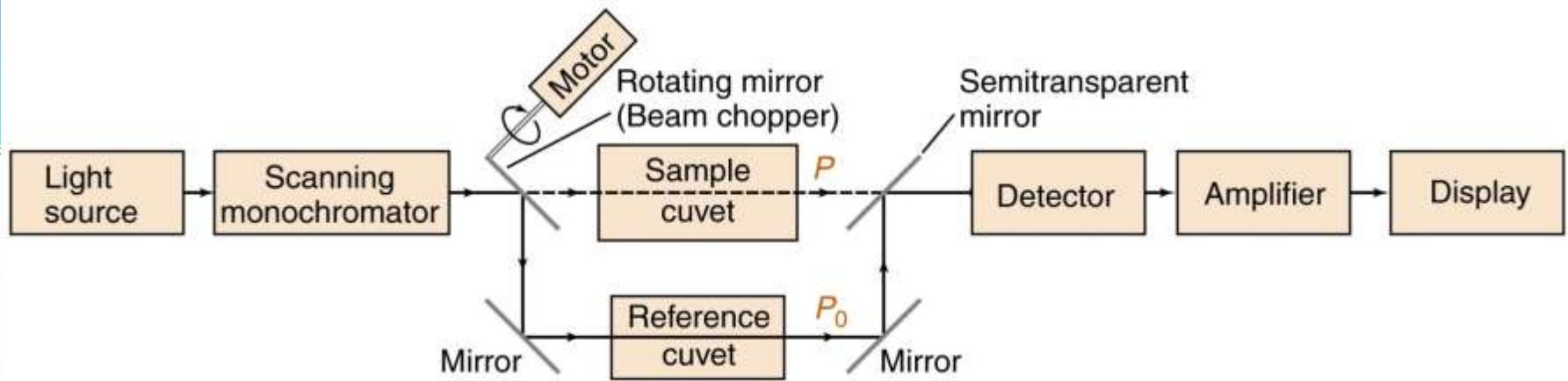


Double Beam Spectrophotometer



Double Beam Spectrophotometer





Schematic diagram of a double beam scanning spectrophotometer

- ★ In double beam arrangement, the light alternately passes through **the sample and reference** (blank), directed by rotating half-sector mirror (chopper) into and out of the light path.
- ★ When light passes through the sample, the detector measures the **P**. When the chopper diverts the beam through the blank solution, the detector measures **P₀**.
- ★ The beam is chopped several times per second and the electronic circuit automatically compares **P and P₀** to calculate absorbance and Transmittance.

Advantages of double beam instruments over single beam instruments

Single beam spectrophotometer is inconvenient because

1. The sample and blank must be placed alternately in the light path.
2. For measurements at multiple wavelengths, the blank must be run at each wavelength.

In double beam instruments

1. The absorption in the sample is automatically corrected for the absorption occurring in the blank, since the readout of the instrument is log the difference between the sample beam and the blank beam.
2. Automatic correction for changes of the source intensity and changes in the detector response with time or wavelength because the two beams are compared and measured at the same time.
3. Automatic scanning and continuous recording of spectrum (absorbance versus wavelength).

Applications of Ultraviolet/Visible Molecular Absorption Spectrophotometry

- 🔔 Molecular spectroscopy based upon UV-Vis radiation is used for **identification and estimation** of inorganic, organic and biomedical species.
- 🔔 Molecular UV-Vis absorption spectrophotometry is employed primarily for **quantitative analysis.**
- 🔔 UV/Vis spectrophotometry is probably more widely used in **chemical and clinical** laboratories throughout the world than any other single method.



The important characteristics of Spectrophotometric methods

1. *Wide applicability* to both organic and inorganic systems
2. *High sensitivity* of 10^{-6} - 10^{-4} M
3. *Moderate to high selectivity*.
4. **Good accuracy** the relative error encountered in concentration lie in the range from **1% to 3%**
5. **Ease and convenience** of data acquisition

Resources and references

- Textbook: Principles of instrumental analysis, Skoog et al., 5th edition, chapter 7, 13.
- Quantitative chemical analysis, Daniel C. Harris, 6th edition , chapter 20.
- Lecture slides partially adopted from Dr. Raafat Aly slides.
- Useful links

http://www.youtube.com/watch?v=pxC6F7bK8CU&feature=player_detailpage

<http://bio-animations.blogspot.com/2008/04/double-beam-uvvis-spectrophotometer.html>