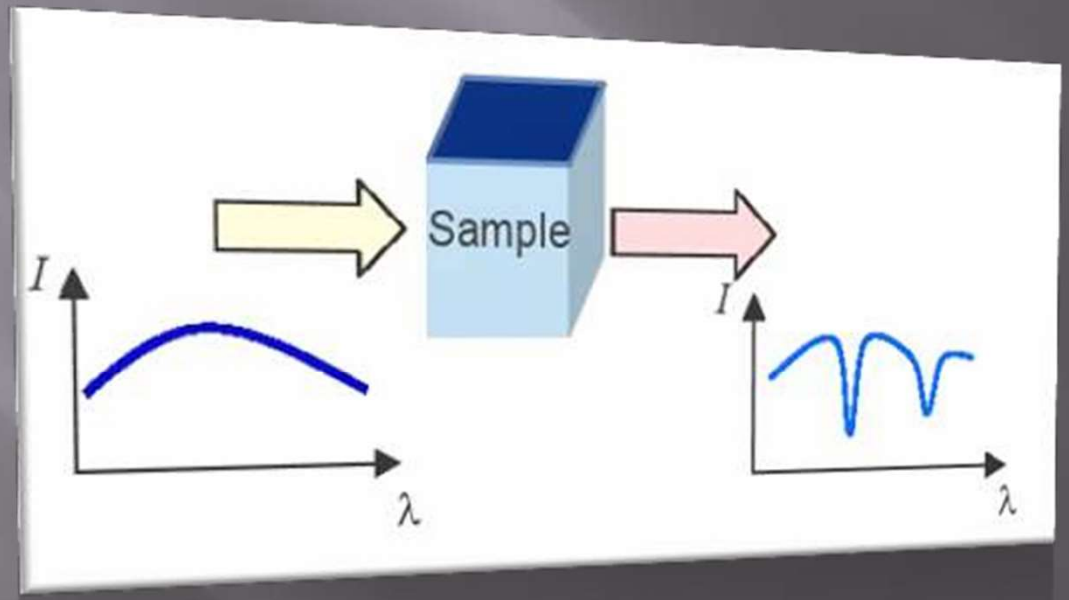
SPECTROPHOTOMETER

Definition

A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
Spectrophotometer techniques are used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer .

- The spectrophotometer technique is to measures light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then the display device .



Purpose (Uses)

1)Measure the concentration ofthe solution

A spectrophotometer optically determines the absorbance or transmission of characteristic wavelengths of radiant energy (light) by a chemical species in solution. Each molecule absorbs light at certain wavelengths in a unique spectral pattern because of the number and arrangement of its characteristic functional groups, such as double bonds between carbon atoms.

According to the Beer-Lambert law, the amount of light absorbed at these wavelengths is directly proportional to the concentration of the chemical species.

2) Identify organic compounds by determining the absorption maximum.

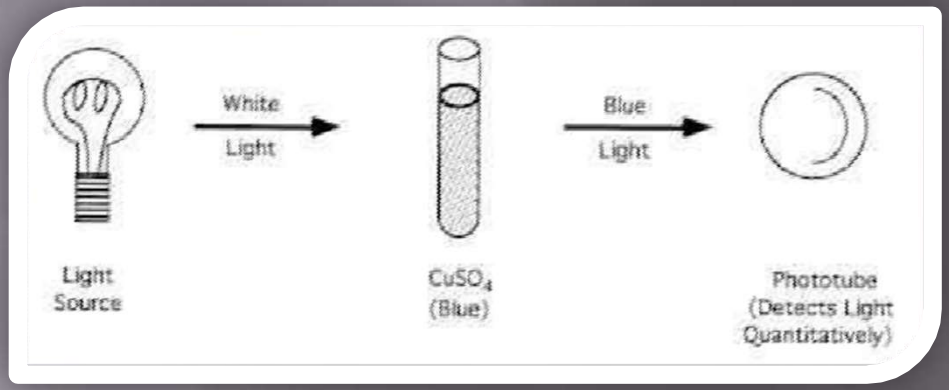
Spectrophotometers are used to identify organic compounds by determining the absorption maxima (which for most compounds and groups of compounds have very distinct fingerprints (that's what the absorption curves and peaks are called).

3) Used for color determination within the spectral range

If one is working in the range of 380 to 700 nm, the spectrophotometers can also be used for color determination within this spectral range

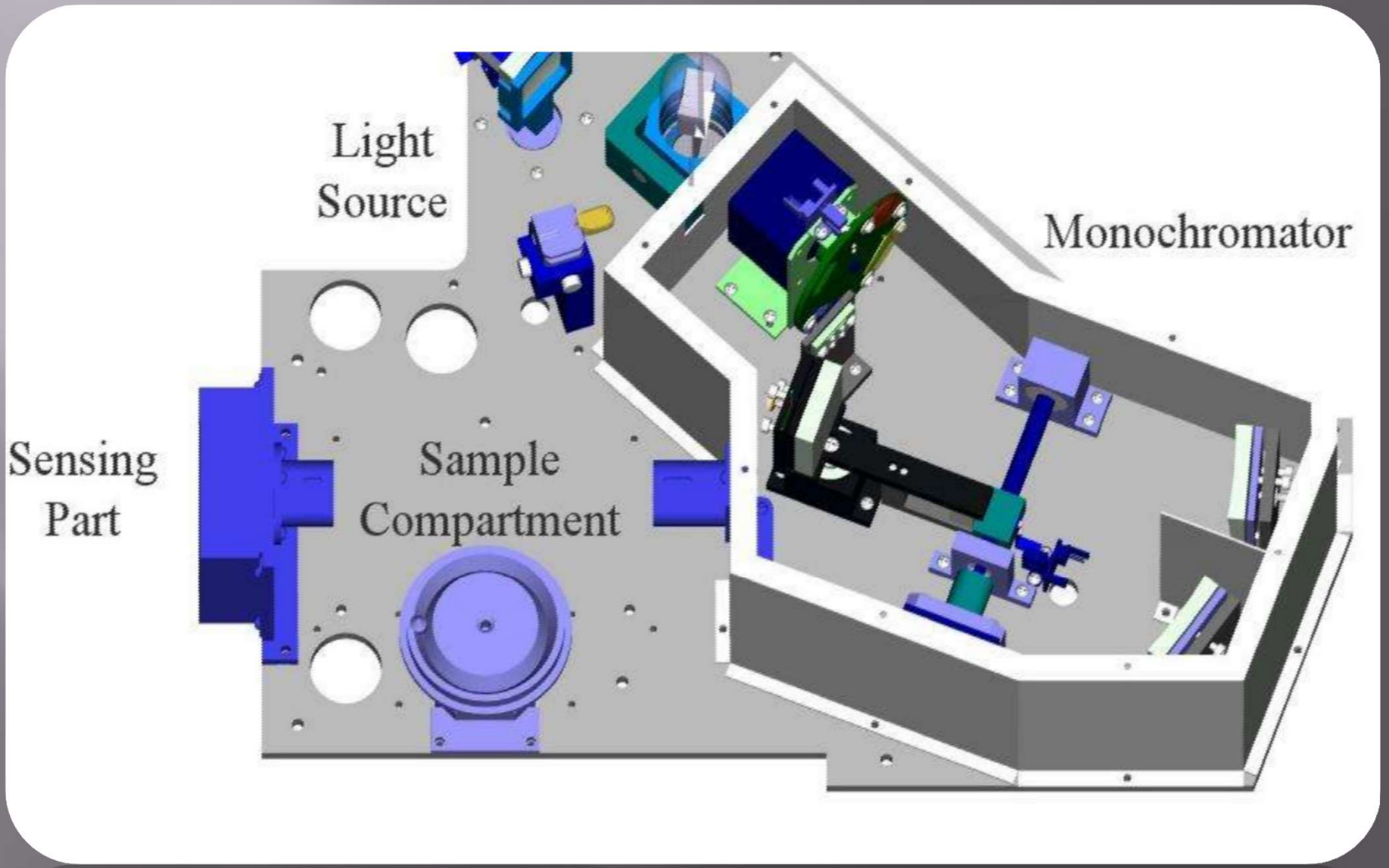
Example

-In the Figure below the red part of the spectrum has been almost completely absorbed by CuSO4 and blue light has been transmitted. Thus, CuSO4 absorbs little blue light and therefore appears blue.



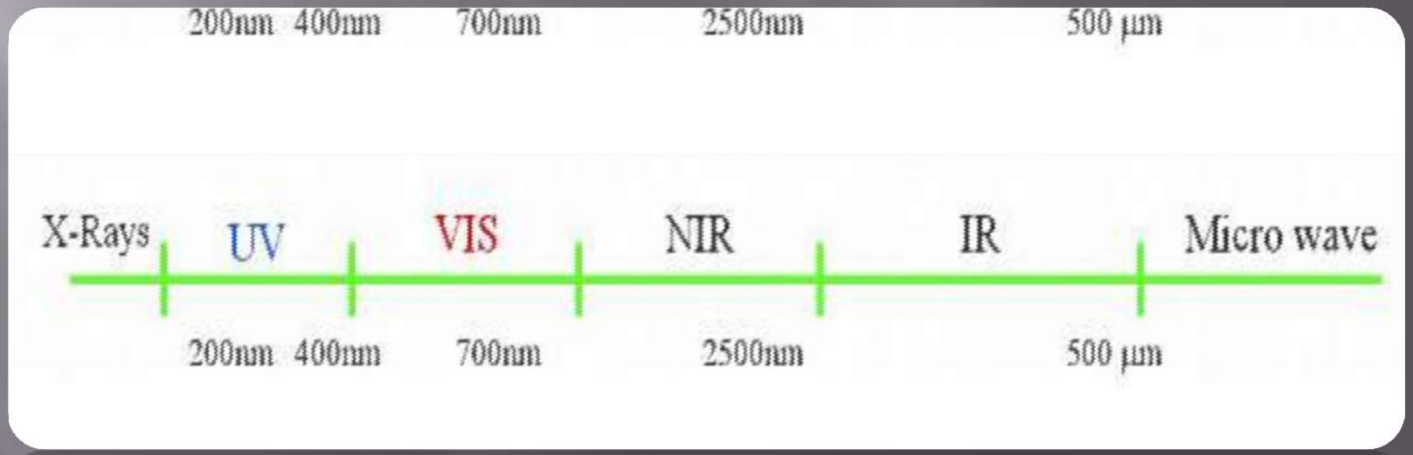
-We will get better sensitivity by directing red light through the solution because CuSO4 absorbs strongest at the red end of the visible spectrum. But to do this, we have to isolate the red wavelengths

Internal Components



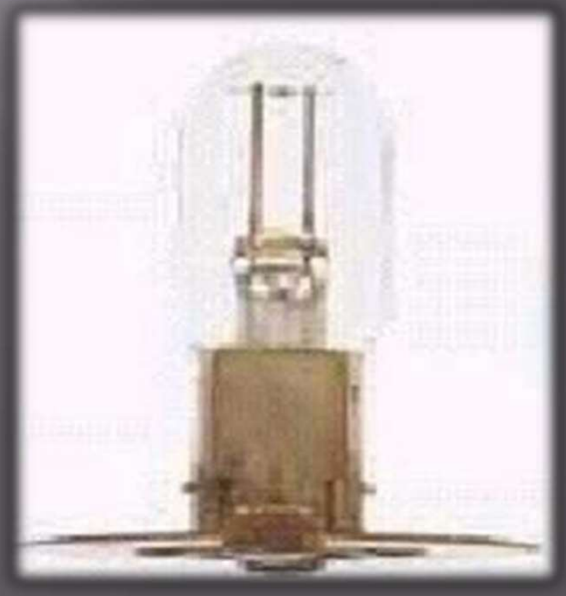
1)Light source

The function of the light source is to provide a sufficient of light which is suitable for marking a measurement. The light source typically yields a high output of polychromatic light over a wide range of the spectrum.



I) Tungsten Lamp

Tungsten Halogen Lamp, it is the most common light source used in spectrophotometer. This lamp consists of a tungsten filament enclosed in a glass envelope, with a wavelength range of about 330 to 900 nm, are used for the visible region. They are generally useful for measuring moderately dilute solutions in which the change in color intensity varies significantly with changes in concentration . It has long life about 1200h.

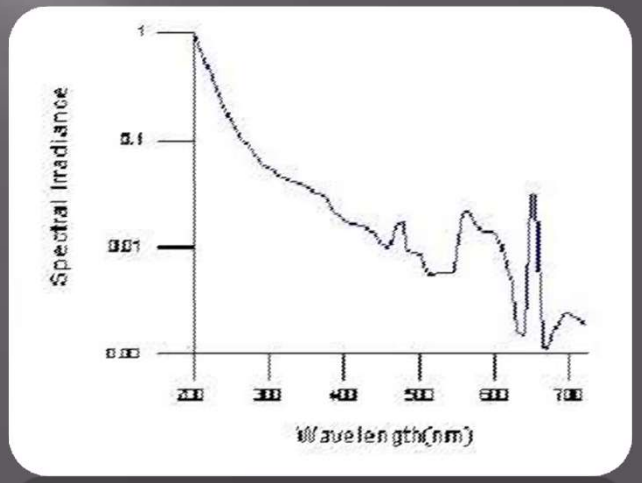


II) Hydrogen / Deuterium Lamps

For the ultraviolet region, hydrogen or deuterium lamps are frequently used.

their range is approximately 200 to 450 nm.

Deuterium lamps are generally more stable and has long life about 500h.This lamp generates continuous or discontinuous spectral.



III) Xenon flash lamps

Xenon flash lamps have several advantages as the following :

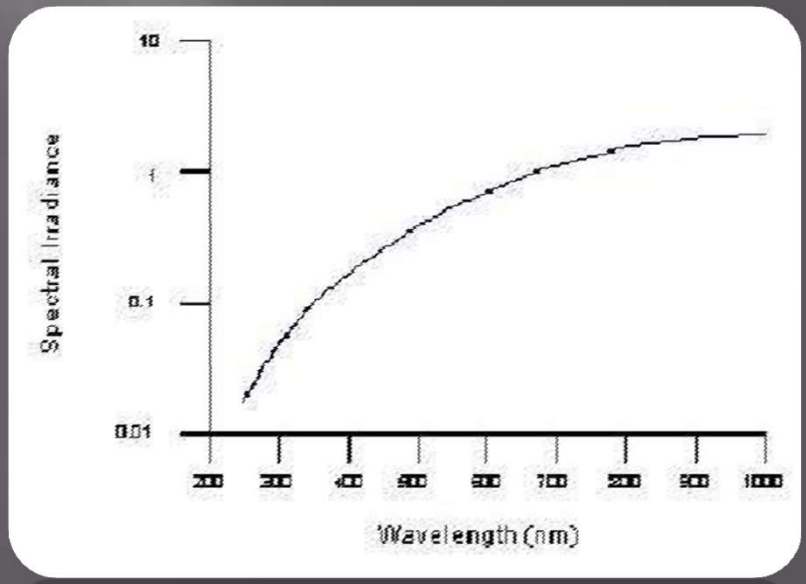
1)Their range between(190nm - 1000 nm)

2) Emit both UV and visible wavelengths

3) Long life

4) Do not heat up the instrument

5) Reduce warm up time



2) Dispersion devices

***Monochromator**

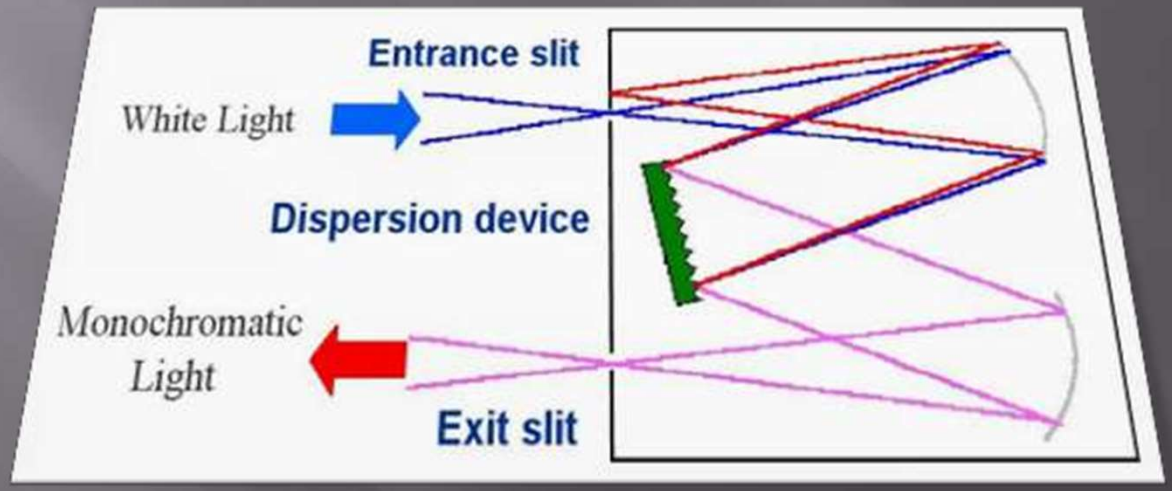
Accepts polychromatic input light from a lamp and outputs monochromatic light.

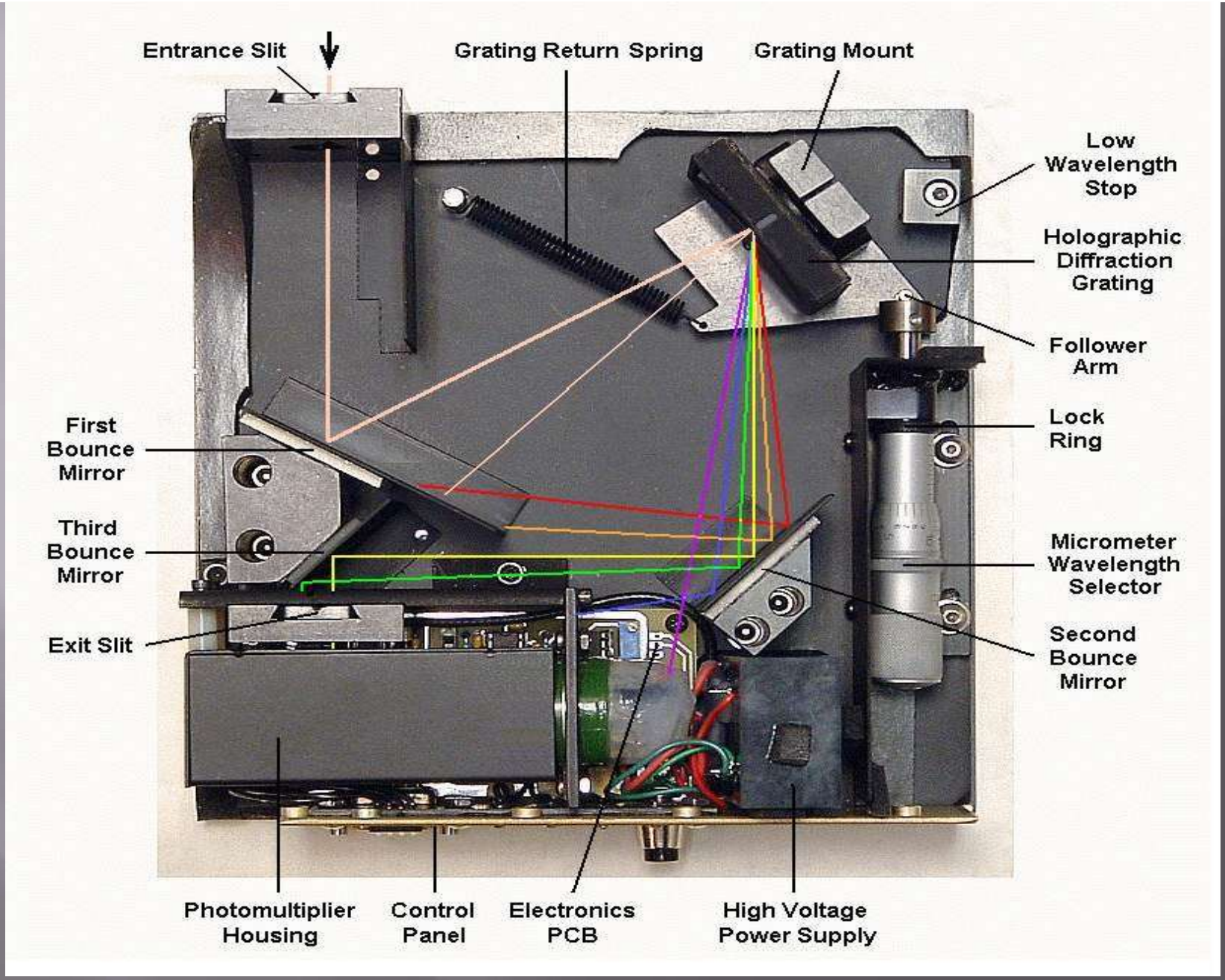
Monochromator consists of three parts:

I) Entrance slit

II) Exit slit

III) Dispersion device





Monochromator

Dispersion devices :

Dispersion devices causes a different wavelength of light to be dispersion at different angles monochromators used for function.

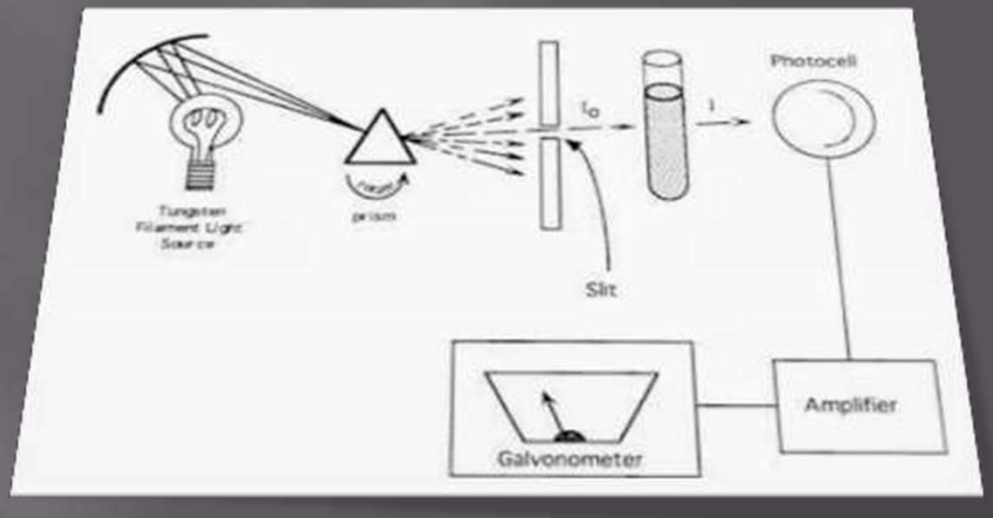
*Types of dispersion devices :

1)Prism

Prism is used to isolate different wavelength .If a parallel beam of radiation falls on a prism , the radiation of two different wavelength will be bent through different angles.

Prism may be made of glass or quartz. The glass prisms are suitable for radiation essentially in the visible range whereas the quartz prism can cover the ultraviolet spectrum also.

It is found that the dispersion given by glass is about three times that of quartz.



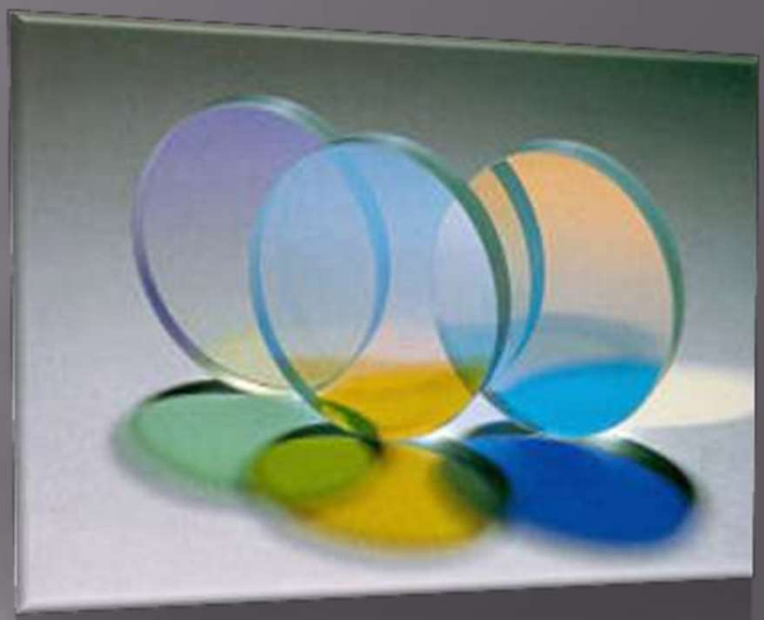
2)Filter

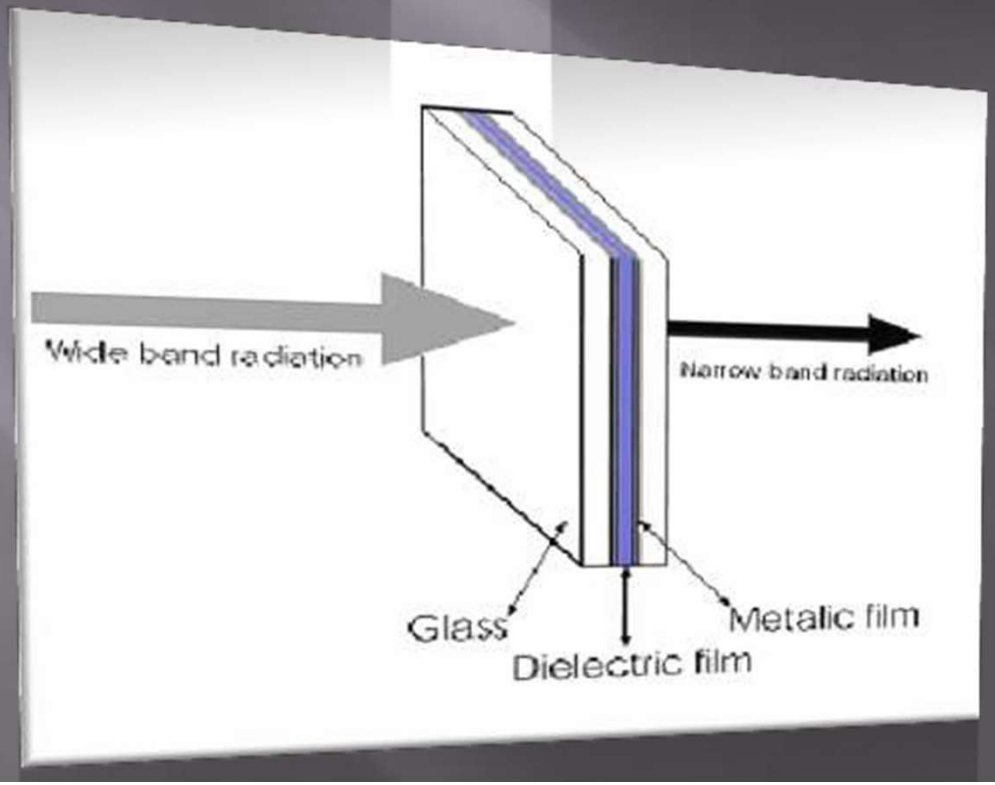
Filters separate different parts of the electromagnetic spectrum by absorbing or reflecting certain wavelengths and transmitting other wavelengths.

***Absorption filters** are glass substrates containing absorbing species that absorb certain wavelength. A typical example is a cut on color filter, which blocks short wavelength light such as an excitation source, and transmits longer wavelength light such as fluorescence that reaches a detector.

***Interference filters** are made of multiple dielectric thin films on a substrate. They use interference to selectively transmit or reflect a certain range of wavelengths.

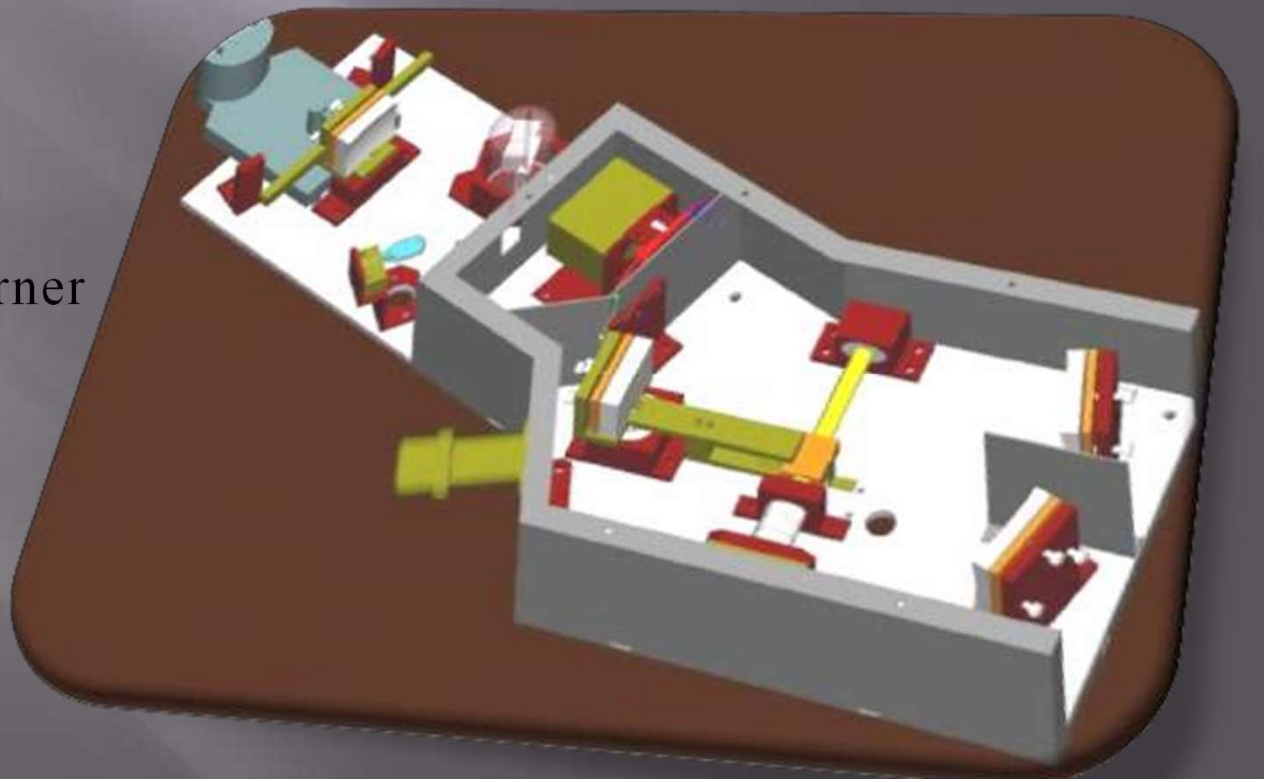
A typical example is a Bandpass interference filter that transmits a narrow range of wavelengths, and can isolate a single emission line from a discharge lamp.




Wide band radiation
Narrow band radiation
Glass
Dielectric film
Metalic film

3)Focusing devices

Combinations of lenses, slits, and mirrors. Variable slits also permit adjustments in the total radiant energy reaching the detector. The Ebert and Czerny-Turner monochromators and their variations are combinations of prisms or gratings and focusing devices .



Ebert and Czerny-Turner Monochromator.

*Optical Materials

1)Mirrors

Type of rays	Mirror material
X-rays - Ultraviolet(UV)	Aluminum
Visible	Aluminum
Near infrared	Gold
Infrared (IR)	Copper or Gold

2)Lenses

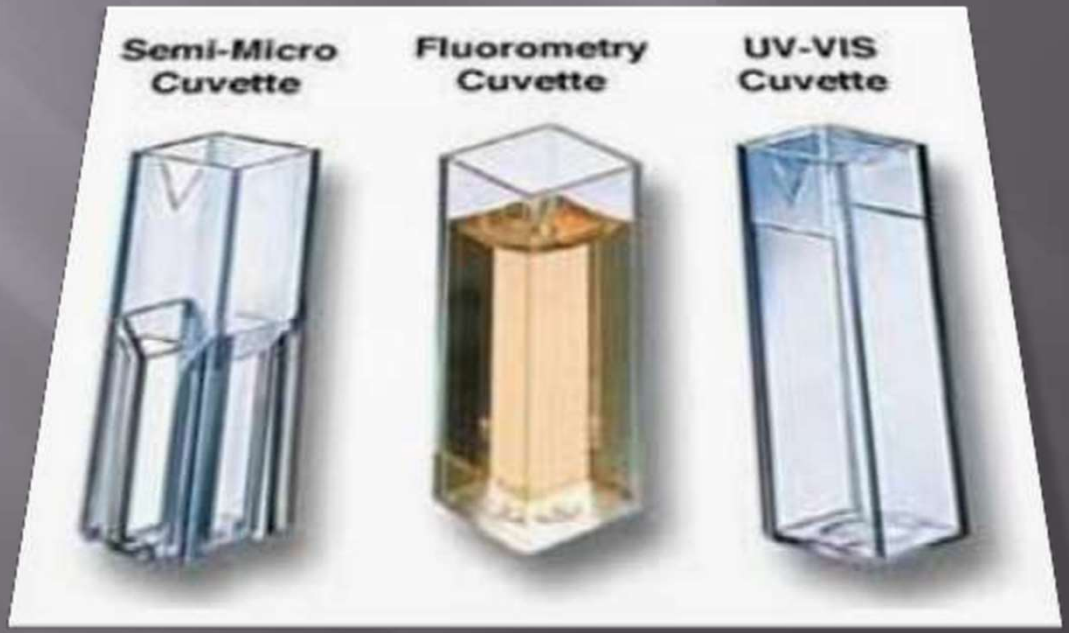
Rays	Material
X-rays Ultraviolet	Fused silica , Sapphire
Visible	Glass
Infrared	Glass

4)Absorption cells(Cuvettes)

A cuvette is a kind of cell (usually a small square tube) sealed at one end, made of Plastic, glass or optical grade quartz and designed to hold samples for spectroscopic experiments. Cuvette should be as clear as possible, without impurities that might affect a spectroscopic reading. Like a test-tube, a cuvette may be open to the atmosphere on top or have a glass or Teflon cap to seal it shut.

Cuvettes are chosen for transparency in the spectral wavelengths of interest.

For measurements in the visible region, cuvettes of optical glass are sufficient; however, optical glass absorbs light below 350 nm , and more expensive quartz or fused silica must be used for these wavelengths. The sample cuvettes are placed in a darkened analysis chamber; some chambers have rotating carousels that can hold several cuvettes.



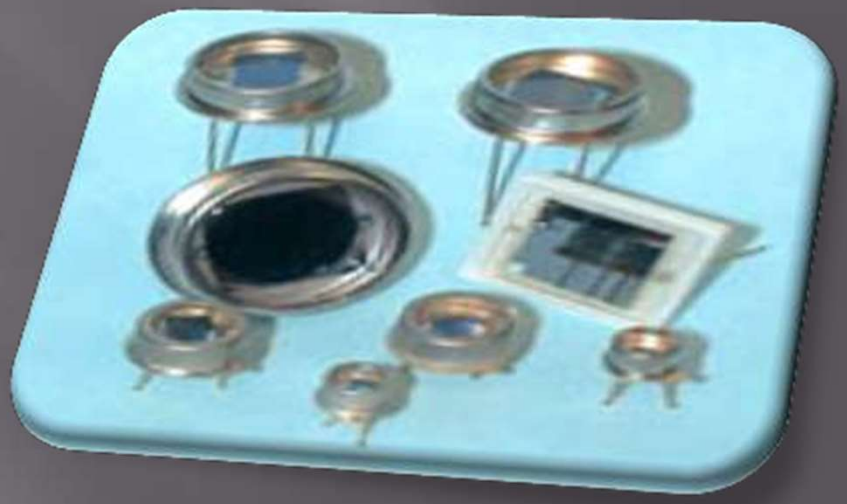
5)Detectors

Any photosensitive device can be used as a detector of radiant energy .The photocell and phototube are the simplest photodetectors, producing current proportional to the intensity of the light striking Them .

*Types of detectors

1) Silicon PIN Photodiodes Photovoltaic V-Series

Blue enhanced for spectral range from 350nm to 1100nm; designed for low-noise, D.C. to medium bandwidth applications. Active areas range from .31mm² to 100mm². Applications include: low light level measurements, particle counting, chemical and analytical measurement and detection.

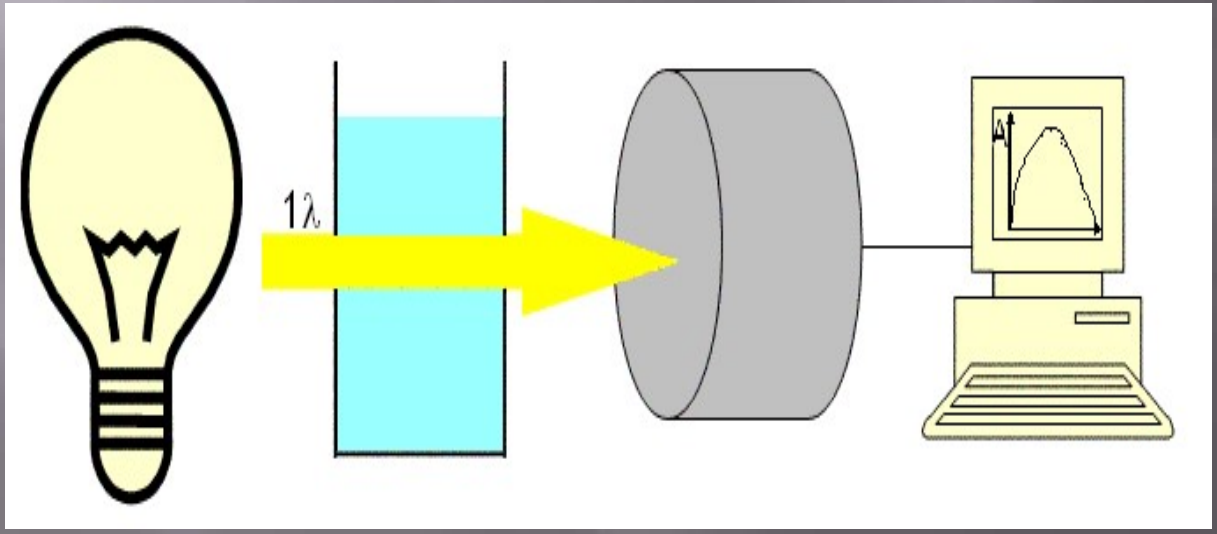


2)Gallium Nitride (GaN) UV Detectors

This family of Gallium Nitride (GaN) UV Detectors are Schottky processed fully passivated U.V. photodiodes. Spectral range from 200 nm to 365 nm and is ideal for UVA or UVB sensing applications and is packaged with a quartz window.

6)Display devices

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



Beer-Lambert Law:

Transmittance (T) : the amount of light that passes through a solution

It can be expressed as the ratio of intensity of the transmitted light I_t to the initial intensity of the light beam I_o

$$T = I_t / I_o$$

Absorbance (A) : the reciprocal of transmittance of the sample varies logarithmically with the product of three factors

Ɛ=the molar absorptivity of the solution

b=the cell or cuvette width

c=the molar concentration

$$A = \log(1/T) = Ɛ\ b\ c$$

- Absorption is proportional to concentration

$$A = Ɛ\ c$$

Concentration of unknown solution C_u

$$C_u = C_s\ A_u / A_s$$

C_u: unknown concentration

A_u: unknown absorbance

C_s: standard concentration

A_s: standard absorbance

Theoretical work of spectrophotometer

First we put the sample into a Cuvette then the light source generates light at a specific wave length or wave lengths , the light passes through the dispersion devices that separate the light into its components wavelengths .

Slits then isolate the wave lengths needed for measurement with a *Bandpass filter to improve its purity . Next , the light passes through the sample ,and a portion of radiant energy absorbed . The remaining light is transmitted to the Photometer ,which converts light energy to electrical energy can be registered on a meter or digital readout.

The amount of light absorbed depends on the nature of the concentration of the sample .

*Bandpass filter is a device that passes frequencies within a certain range and rejects frequencies outside that range.