SPECTROPHOTOMETRY

Spectrophotometry is the quantitative measurement of the absorbance of transmission property of material as a function of wavelength.

It measures the intensity as a function of wavelength. It does this by diffracting the light beam into spectrum of wavelength, detecting the intensity with charged coupled device and displaying the result as a graph on the detector and then the display device.

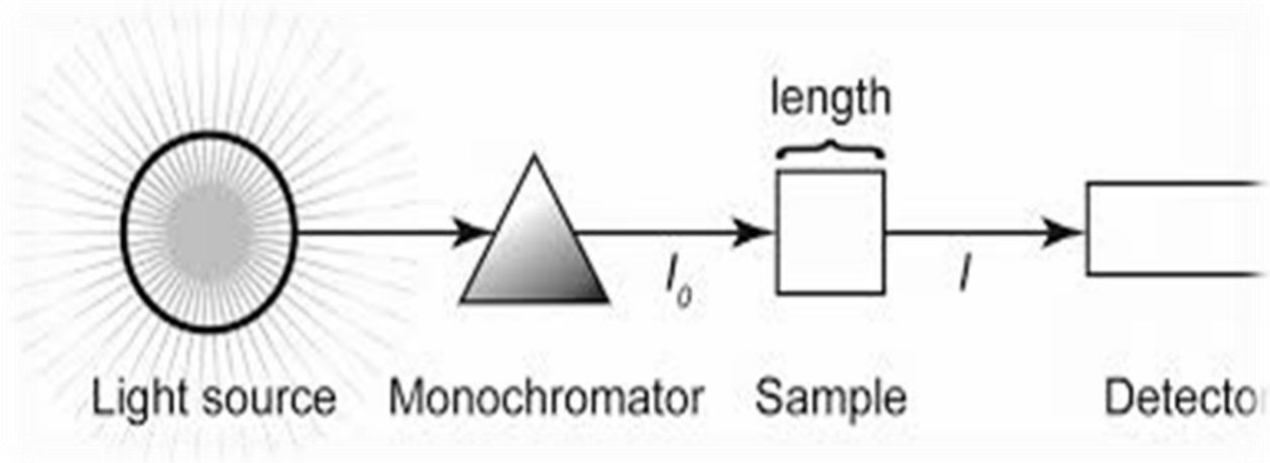
COMPONENTS OF SPECTROPHOTOMETER

Following are the main component of spectrophotometer-

- Light source- for absorption measurement in the ultraviolet region, a high pressure hydrogen or deuterium lamp is used for the source of light. These lamp produce radiation in the 200-340nm in range.
- Monochromator- this is an optical device that transmittes a mechanical narrow band of light or other radiation choosen from wider range of wavelength available of the input.

- Sample chamber- The processed monochromatic light is then directed into a sample chamber which can accommodate a wide variety of sample holder. The sample is placed in cuvette. Sample chamber of spectrophotometer comes in two varieties-

  Those holding only one cuvette at a time called single beam and those holding two cuvette one for reference and other for sample called double beam.
- Detector- At last the detector are placed, it is an instrument that a give a direct readout of absorbance in digital or analog form. This is an photosensitive device that detect how much light is transmitted through the sample, usually a photomultiplier tube called PMT. New detectors are called photodiode composed of silicon crystal.



UV-visible spectroscopy

Ultra violet and visible absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface.

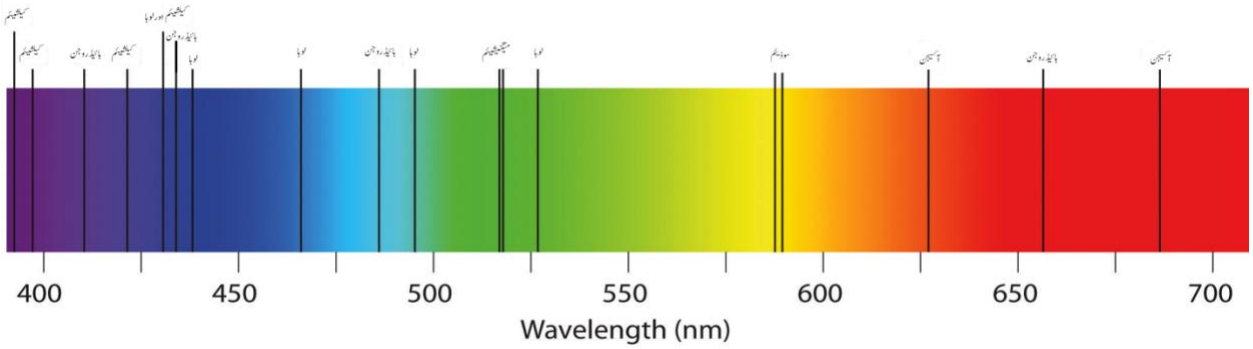
The principle of UV and visible based on the measurement of spectrum of a sample containing atom or a molecule.

Spectrum is a graph of intensity of absorbed or emitted radiation by sample versus frequency or a wavelength.

Ultraviolet absorption spectrum arises from transition of electron within a molecule from lower level to higher level. A molecule absorb ultraviolet radiation of frequency, electron in that molecule undergo transition from lower level yo higher level.

Absorption spectrum

When a white light passes through a prism, light split into different color constituent because white light is made up of many colors, this process is known as dispersion, like we see in rainbow.



When we see the line diagram, we found pattern of different colors of band i.e. violet blue, green, yellow, orange and red, actually these bands are called spectrum

These bands are continuous because all color are merged in between and the colors are change continuously, we cannot see when blue color changes into green color that's why it is called continuous spectrum.

When white light passes through gas or transparent material, then a dark line form, these bands are called absorption spectrum, these bands are because of transparent material absorb the specific wavelength of light

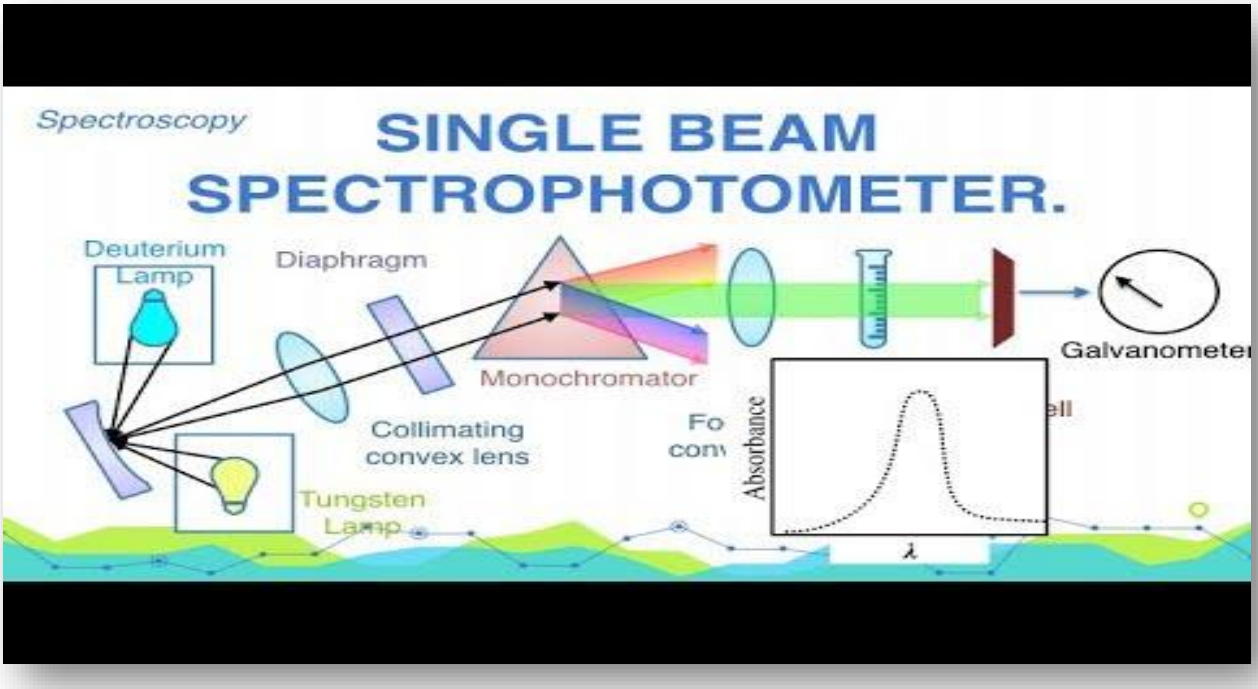
TYPE OF UV-VISIBLE SPECTROPHOTOMETER

There are two types of UV-visible spectrophotometer-

1. Single beam spectrophotometer
2. Double beam spectrophotometer

Single beam spectrophotometer-Component of single beam spectrophotometer includes the source of light or infra ray, uv-light can be obtain by heating the filament with hydrogen.

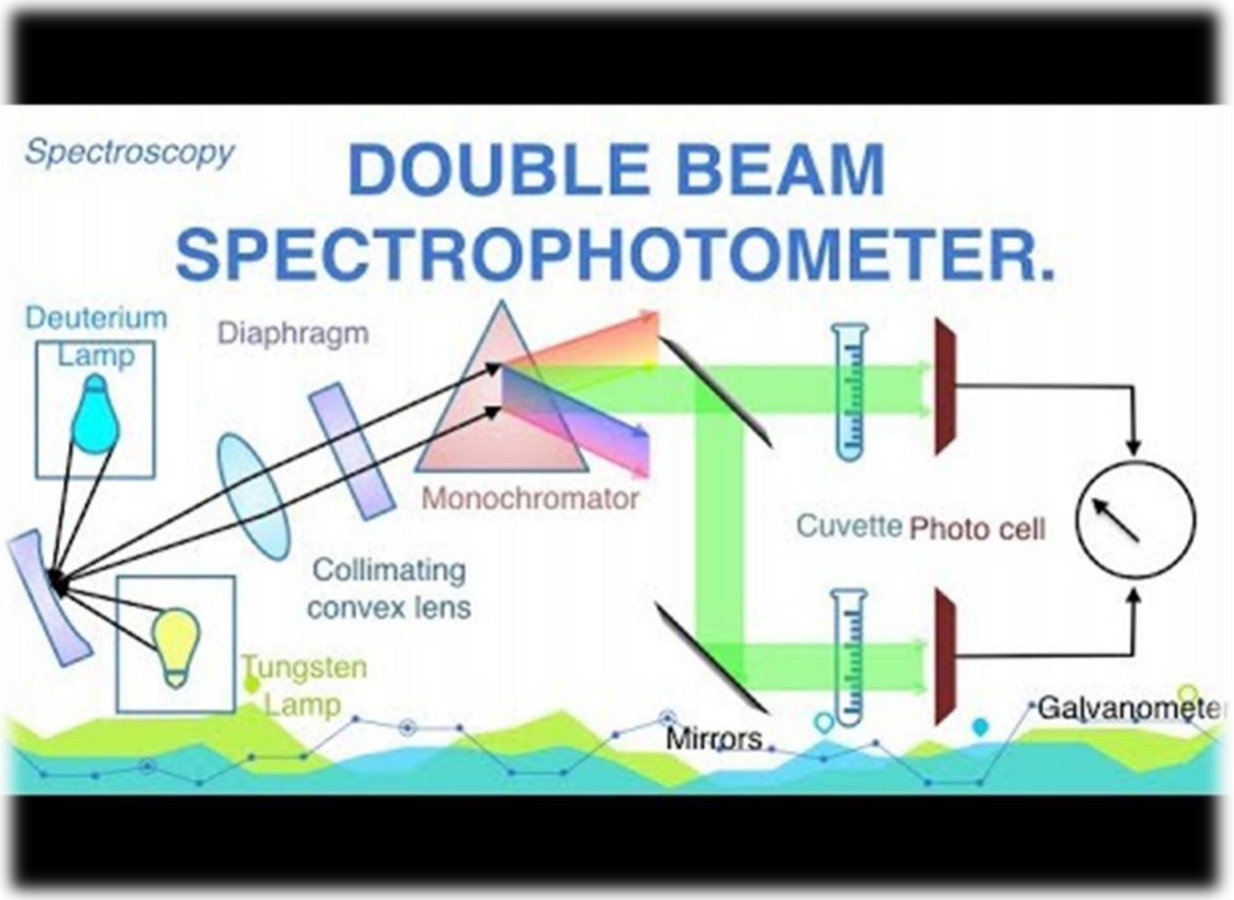
Second is collimating convex lens and then the monochromator, it may b prism or granting next come cuvette and finally the detectors.



WORKING SYSTEM OF SINGLE BEAM SPECTROPHOTOMETER-

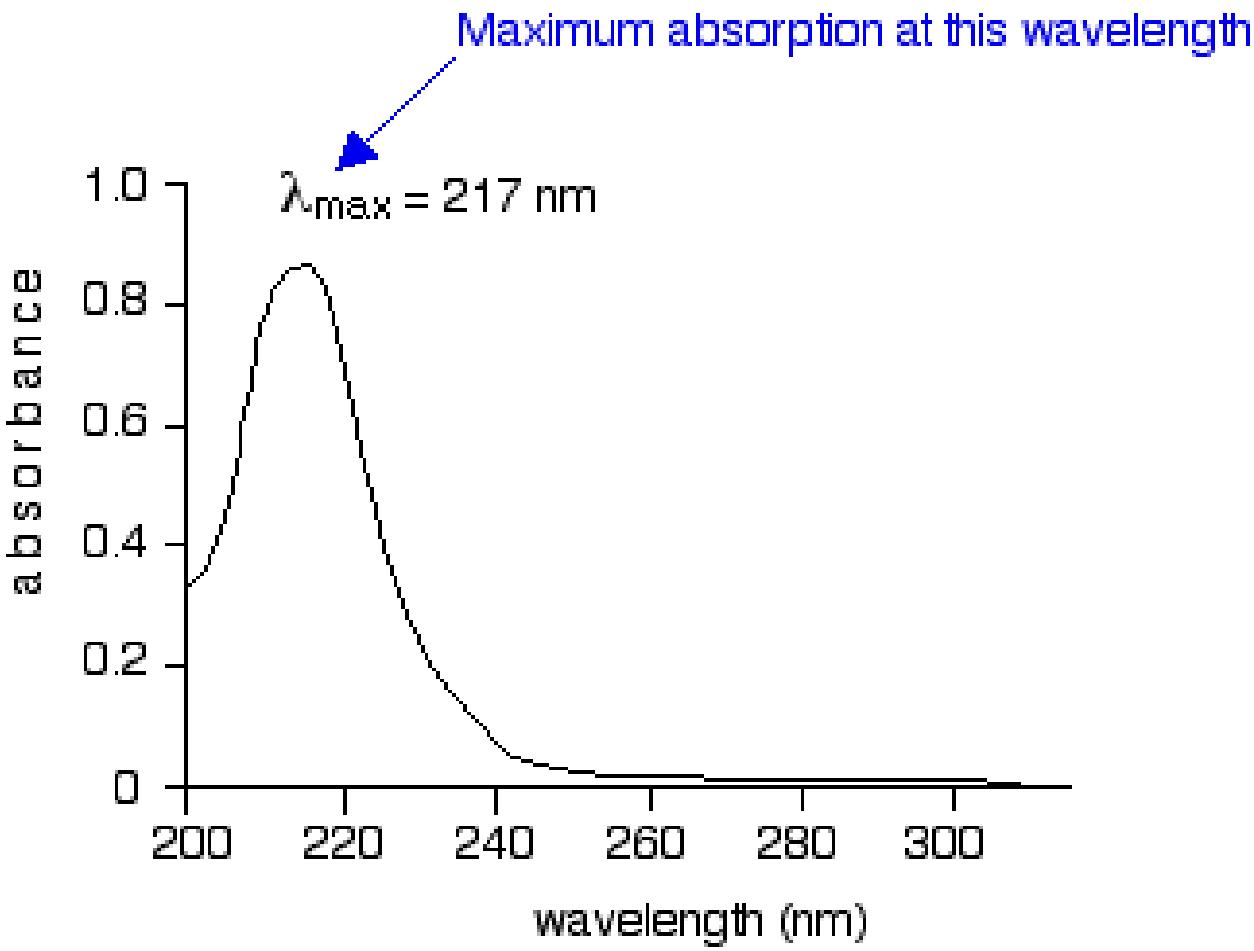
First of all the source of radiation is comes out, the light from the source is collected by collimating convex lens and allowed to fall on the monochromator, for each radiation incident on the monochromator we are getting the radiation of one wavelength only, this light is passed on the sample kept in the cuvette and some part of the light is absorbed and remaining is transmitted which pas through focusing lens and fall on photocell, photocell converted this radiation into current, the value of wavelength can be change by rotating the monochromator.

DOUBLE BEAM SPECTROPHOTOMETER



First the source of radiation, it may be UV-light, visible light or infra ray, UV-ight can be obtained by heating the filament with hydrogen. Second is collimator lens and then the monochromator, after monochromator there are two mirror are placed, after mirror there are two cuvette ,one for sample and other for blank solution finally the photocell connected to computer from which we get signal.

WORKING- first of all the source of light radiation is come out, the light from the source is collected by collimating lens and allowed to fall on the monochromator, for each radiation incident on the monochromator we are getting the radiation of one wavelength only. The monochromator light is then separated into two beam of light with the help of mirror, one passed through one cuvette containing sample solution and other through cuvette containing blank solution and both are finally incident on photocell which is converted it into current.



Graph of absorbance vs. wavelength is plotted the wavelength where the absorbance is maximum is known as λ max which is characteristic property of the compound and give the qualitative analysis of sample, while knowing the value of absorbance we can find out the concentration of solution using Beer –Lambert law thus qualitative analysis is possible.

ADVANTAGE OF DOUBLE BEAM SPECTROPHOTOMETER

- ◊ As the two beam of radiation are passed simultaneously from sample solution and blank therefore any fluctuation in voltage can be compensated.
- ◊ The advantage of the double beam spectrophotometer design is high stability because reference and sample are measured virtually at the same moment in time.
- ◊ Accuracy is more than single beam spectrophotometer.

BEER- LAMBERTS LAW

BEER LAW-The intensity of monochromatic light passing through an absorbing medium decreases exponentially as the concentration of absorbing medium increases.

$$I_E = I_0 e^{-kc}$$

LAMBERT LAW-The intensity of light or a ray of monochromatic light that passes through an absorbing medium decreases exponentially as the length of absorbing medium increases.

$$I_0 = I_0 e^{-kl}$$

If we combine both laws

$$I = I_0 e^{-k.cl}$$

$$I/I_0 = e^{-k.cl}$$

$$\text{Log } I_0/I = k/2.303 \times c.l$$

$$A = \varepsilon . C . l$$

A= Absorbance of medium optical density

ε = Extinction coefficient of component

TRANSMITTANCE-Transmittance is the ratio of light transmitted to the amount of light that initially fall on the surface.

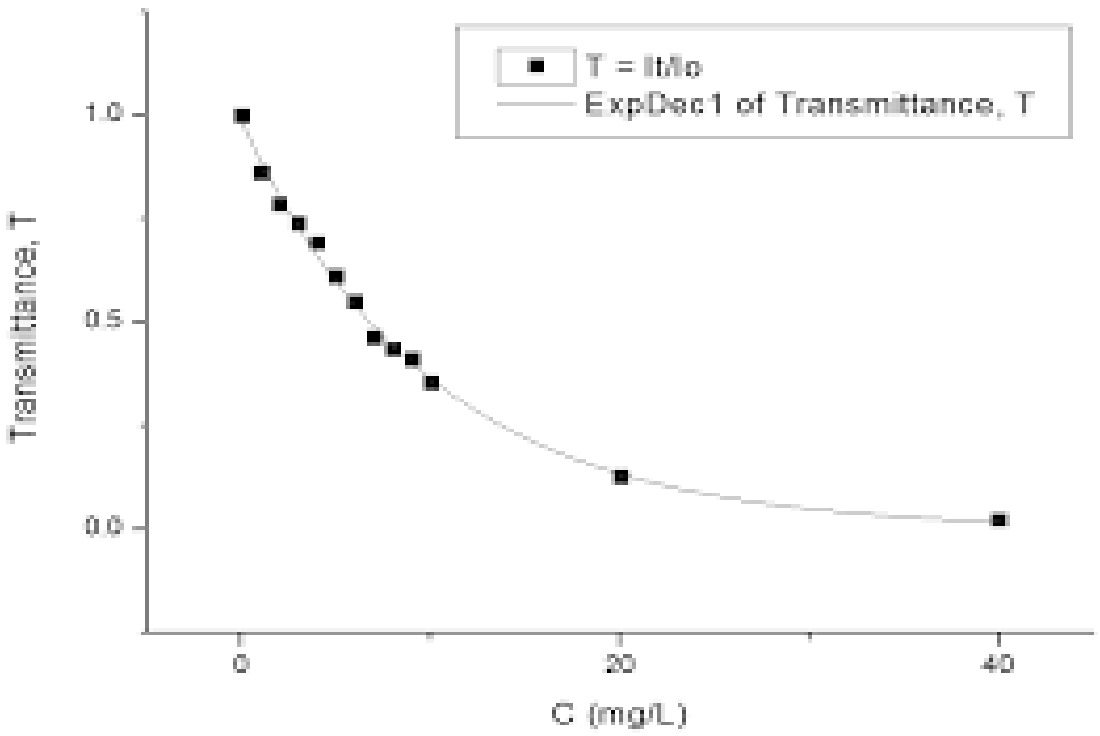
$$T=I/Io$$

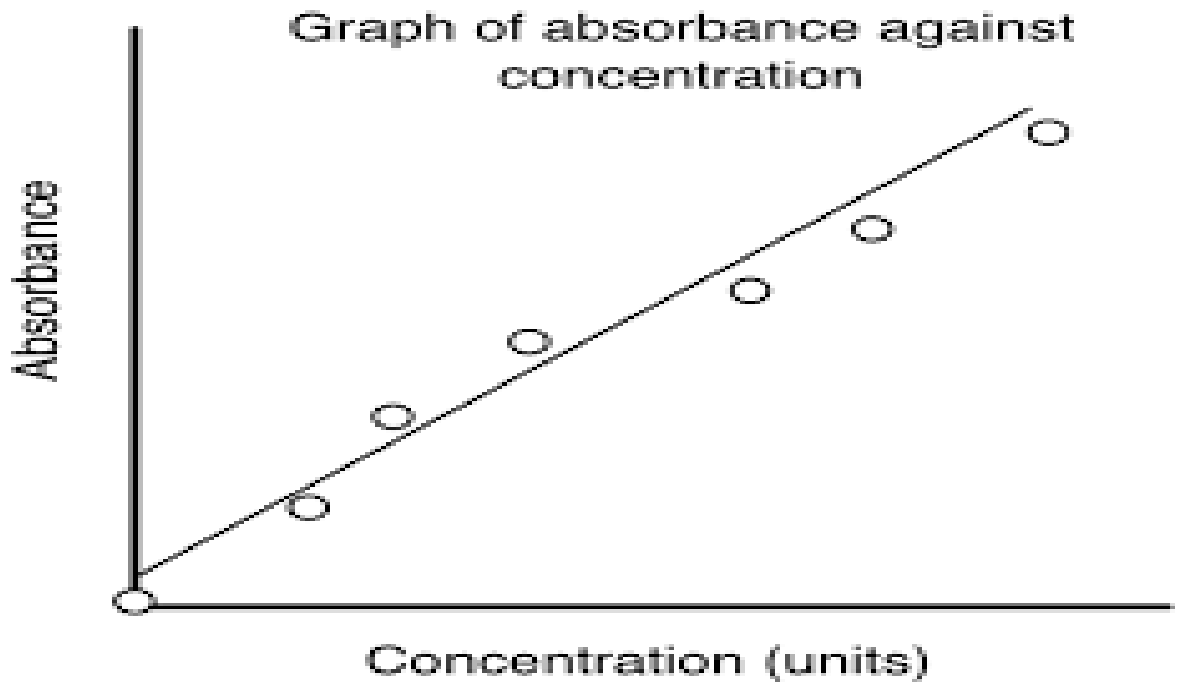
Where, I=amount of transmitted light

Io=amount of incident light

ABSORBANCE-Absorbance is defined as the negative logarithm of the transmittance

$$-\log T=-\log 10\ I/Io.$$





If we see the graph, transmittance is not give us much data because if we put the transmittance in the graph, the concentration is less than transmittance and transmittance should be maximum, absorbance in that case will be very less so we will get a graph like slope not linear.

The problem is with this graph is that although we are getting the data directly from the detector as transmittance VS concentration; it will be very difficult for us to extrapolate the data.

Let's see in graph 2 we know the sample and we utilize same test sample the concentration of which we know and then plotted graph, we are testing unknown data ,if some of the testing are above 40mg/l this become very difficult for us to finally calculate what exactly the concentration of that unknown sample that's why we can plotted the graph absorbance Vs concentration then it will very easy to find the concentration of any unknown sample because we are getting the linear curve.

LIMITATION OF BEER'S-LAMBERT LAW

- The linearity of Beer- Lambert law is limited by chemical and instrumental factor-
- The electronic radiation should be monochromatic. T
- The light beam should not be scattered
- The solution should be diluted

APPLICATION OF UV- VISIBLE SPECTROPHOTOMETER

Spectrophotometer can be used for-

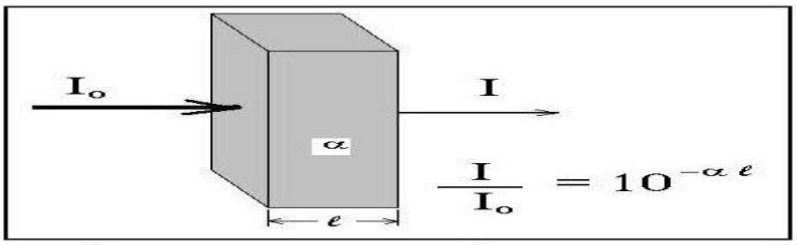
- **QUANTITATIVE ANALYSIS**- the metallic salt solution which are coloured foe example, Cu, Fe, Ni etc and some organic coloured compound can be easily analyzed quantitatively by using Beer's Lambert law. Thus from optical density we can calculate the concentration of solution.
- **IDENTIFICATION OF FUNCTIONAL GROUP(QUALITATIVE ANALYSIS) –** The spectrophotometer are the instrument working on the principle of λ-max. This λ-max is constant for each functional group present. If we know λ- max for the particular sample we can decide the type of functional group present in it as λ-max is characteristic property.

- **DISTINGUISH BETWEEN GEOMETRIC ISOMER**- Each geometrical isomer have its own characteristic λ-max therefore from Λ-max we can identify geometrical isomer.
  e.g., cinnamic acid exists in Cis and Trans form.
- **CHEMICAL ANALYSIS**-With the help of spectrophotometer we can find out the rate constant of any chemical reaction.
- We can also use spectrophotometer for the study of kinetics.
- Detection of impurities.
- It can also be use for detection of specific molecules in human cell and tissue of haemoglobin protein.

COLORIMETER

Colorimeter is an instrument used for the measurement of colored substance in solution. This instrument is operative in visible range og the electromagnetic spectrum.

PRINCIPLE-When a monochromatic light passes through a colored solution, some specific wavelength of light are absorbed which is related to color intensity. The amount of light absorbed or transmitted by a color solution is an accordance with two law i.e., Beer and lamberts law.



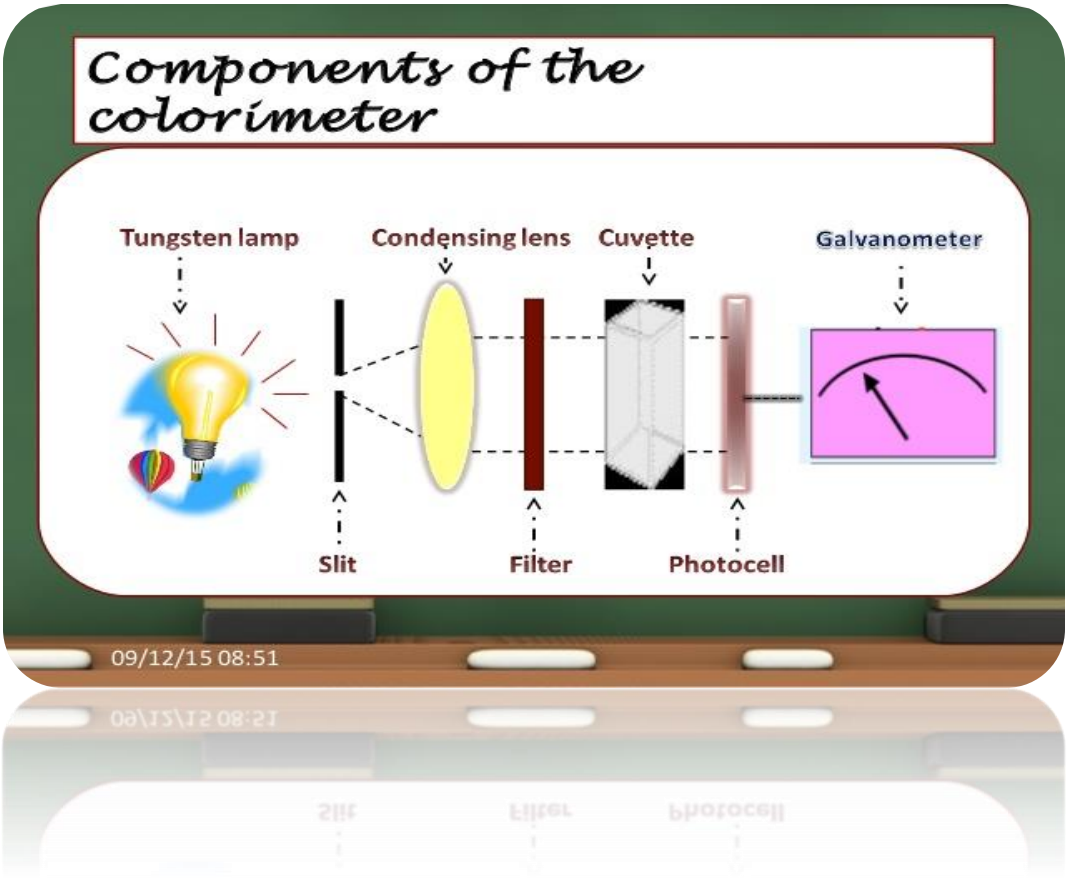
The Beer Lambert Law

COMPONETS OF COLORIMETER

There are some essential parts in a colorimeter –

- Light source-the most common source used in colorimeter is a tungsten filament .
- Monochromator—To select the particular wavelength filter monochromator are used to split the light from the light source.
- Sample holder- test tube or cuvette are use to hold the color solution they are made up of glass at the visible wavelength.

- Photo detector system- when light fall on the detector system, an electric current is generated this reflects the galvanometer reading.
- Measuring device- the current from the detector is fed to the measuring device, the galvanometer show the meter reading that is directly proportional to the intensity of light.



WORKING SYSTEM OF COLORIMETER-When using a colorimeter it requires being calibrated first which is done by using standard solution of the known concentration of the solute that has to be determined in the test solution. For this, the standard solution are filled in the cuvette holder in the colorimeter.

There is a ray of light with a certain wavelength that is specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of different filter and lenses. These lenses are used for navigation of the colored light in the colorimeter and the filter splits beam of light into different wavelength and allows the required wavelength to pass through it and reaches the cuvette containing the standard solution. It analyzes the reflected light and compared with predetermined standard solution.

When the monochromatic light reaches the cuvette some of the light is reflected and some part of; light is absorbed by the solution and the remaining parts if transmitted through the solution which falls on the photo detector system. The photo detector system measures the intensity of transmitted light and converts it into electrical signal that are sent to the galvanometer.

The galvanometer measures the electrical signals and displays it in the digital form. that digital representation is in the absorbance or optical density of the solution analyzed.

If the absorption of the solution is higher then there will be more light absorbed by the solution and if the absorption of the solution is low then more light will be transmitted through the solution which affect the galvanometer reading and corresponds to the concentration of the solute in the solution.

APPLICATION OF COLORIMETER

- The colorimeter is commonly used f9or the determination of coloured compound
- It can also be used for the determination of the course of the reaction.
- By colorimeter, a compound can be identified
- They are used to measure and monitor the color in various food and beverages.
- Detection of impurities
- Detection of specific molecules in human cells and tissue such as hemoglobin protein.
- Molecular weight determination.
- tautomeric equilibrium