SPECTROPHOTOMETRY

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Introduction

- Spectrophotometry is a branch of science that deals with the study of interaction of electromagnetic radiation with matter.

- During such interactions the energy is either absorbed or emitted by the matter in descrete amount called quanta.

Electromagnetic spectrum

Electromagnetic radiation –

- It may be considered as simple harmonic wave propagated from a source and travelling in straight lines except when reflected or refracted.
- This radiation will be associated with the properties of the waves.

Wavelength

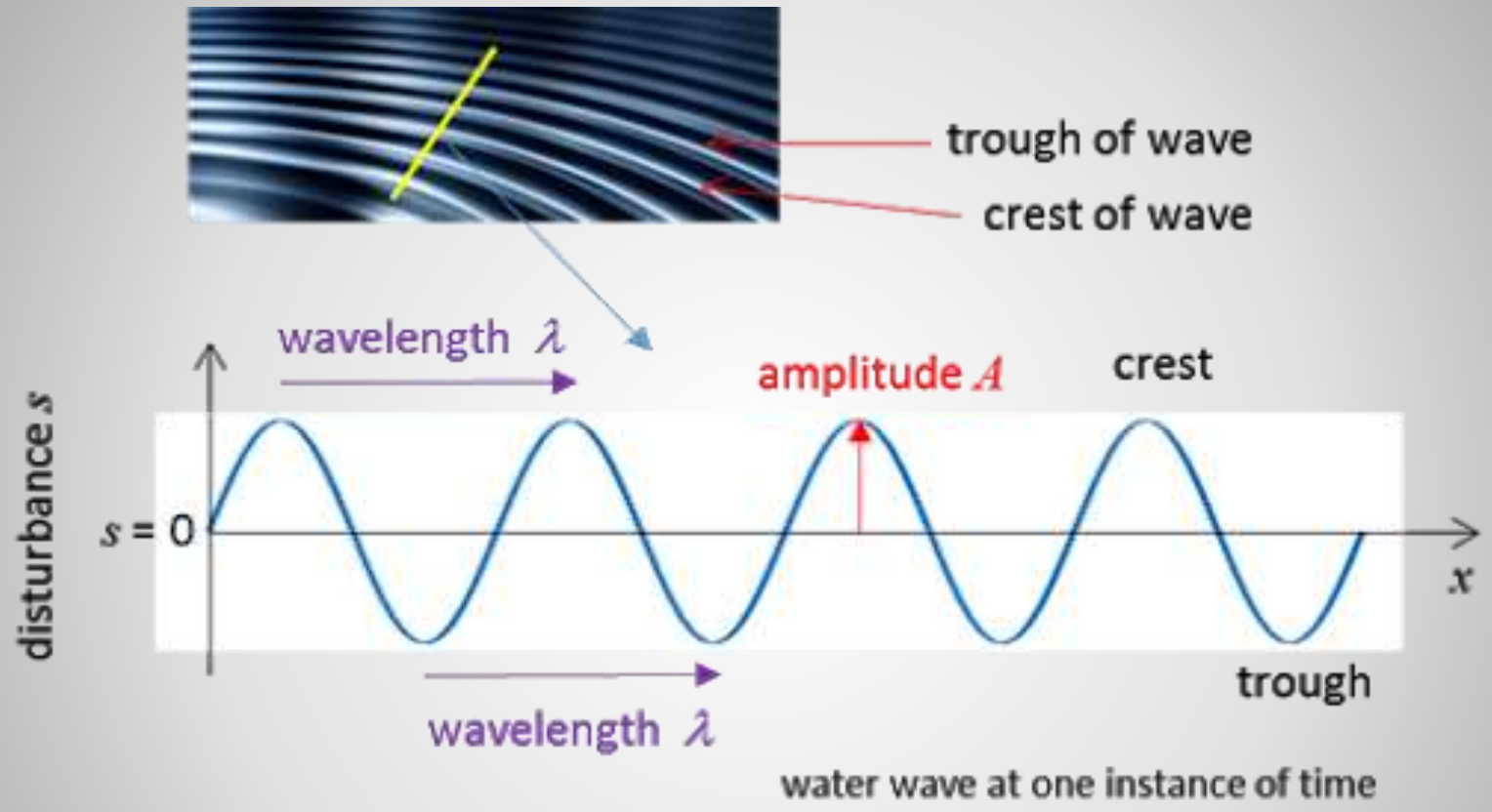
- It is the distance between two successive maxima on an electromagnetic wave.

Frequency

The number of complete wavelength units passing through a given point in unit time is called the frequency of radiation (v)

Wave number

It is the number of waves per centimeter in vaccum.



The concept of travelling wave

Relation between frequency, wave number and wavelength

- The product of wavelength and frequency is equal to the velocity of the wave in the medium. $\lambda \times \nu = velocity$

- As the velocity of light is represented by c, which is maximum in vacuum

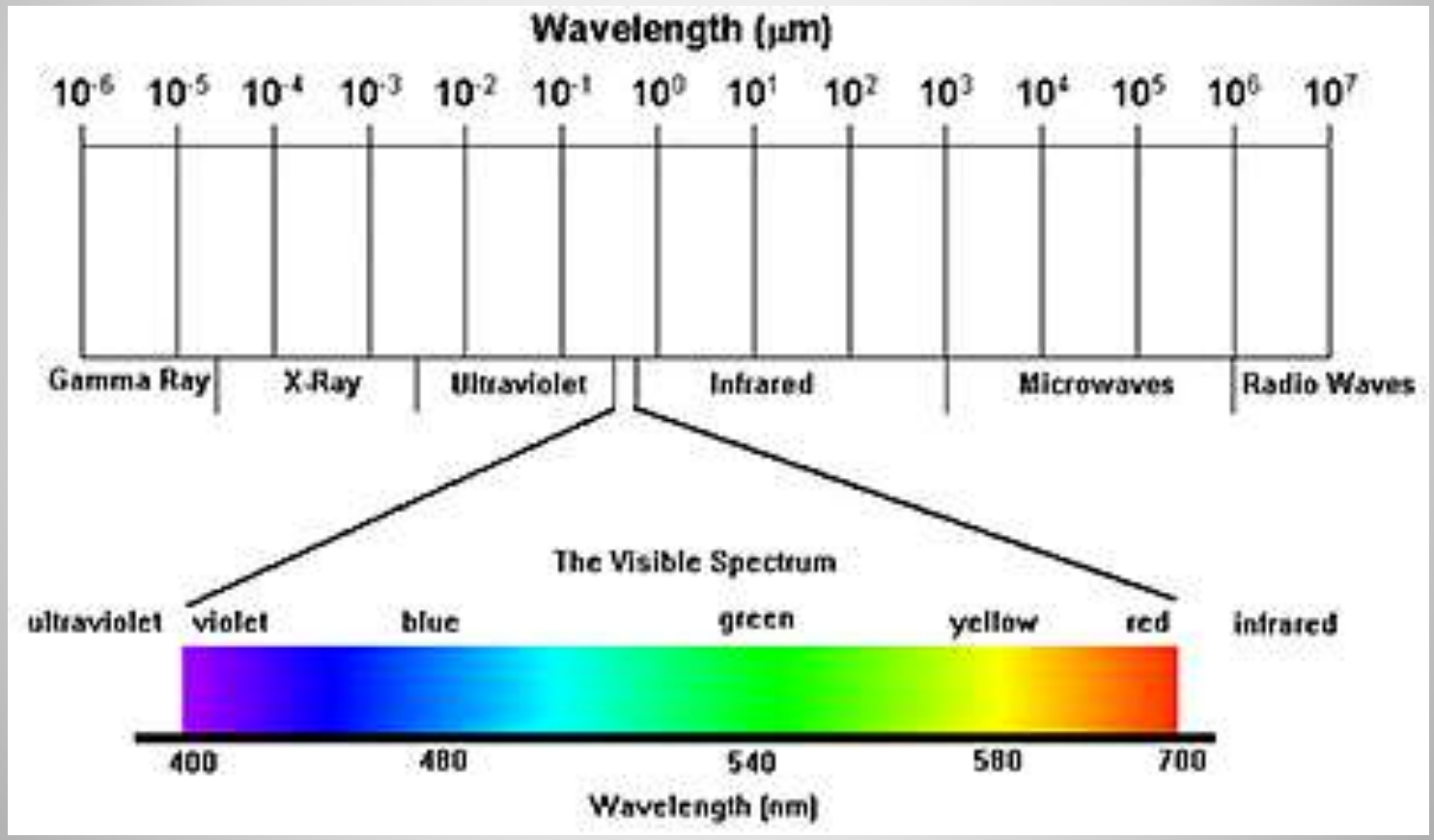
$$\lambda\nu = c = 3 \times 10^{10}\ cm\ sec^{-1}$$

Thus $1/\lambda = \nu / c = \bar{\nu}$

Interactions of Radiation with matter

- The entire range over which electromagnetic radiation exists is known as electromagnetic spectrum.
- Spectrophotometry is mainly concerned with the ultraviolet (200-400nm) and visible (400-800nm) regions.
- Main instruments – photometers, colorimeters and spectrophotometers

Regions of the electromagnetic spectrum



Terminology used in Absorption Measurement

- Radiant Energy : It is defined as energy transmitted as electromagnetic radiation. It has the properties of both particle and wave motion.
- Electromagnetic radiation possesses a certain amount of energy. The energy of a unit radiation, called photon, is related to frequency by –

$$E = h\nu = \frac{hc}{\lambda}$$

- Where E is the energy of the photon in ergs
- h is Planck's constant (6.62×10^{-27} erg sec)

- Radiant Power, P : Formerly known as Intensity (I). Energy per unit time is called as power. Radiant power is the rate at which energy is transported in a beam of radiant energy.

- Transmittance, T: It is simply the fraction of the incident power transmitted by a sample.

- Absorbance, A : It was formerly known as an optical density (O.D.) or extinction (E). The absorbance is the logarithm to base 10 of the reciprocal of the transmittance.

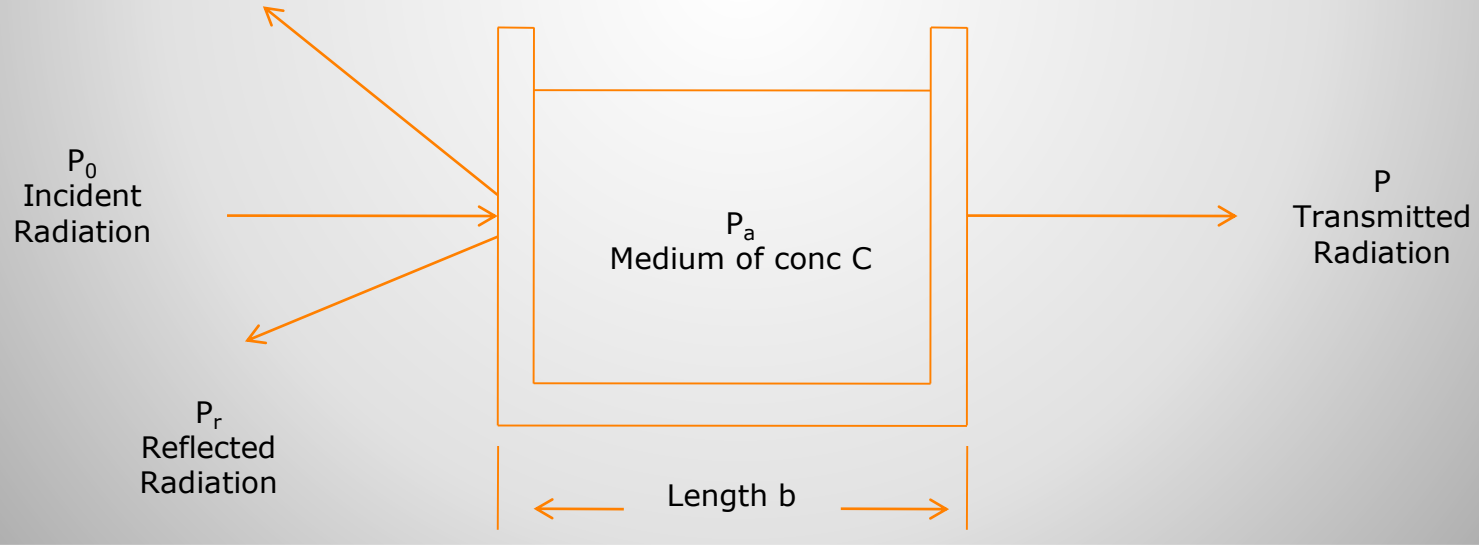
- Absorptivity, a : Formerly known as the extinction coefficient or specific extinction.
- Absorptivity is defined as the ratio of the absorbance to the product of the length of optical path b and the concentration of the sample C.
- Absorptivity is the measure of the ability of sample to absorb light.

- Molar absorptivity, € : Formerly known as molar extinction coefficient or molar absorption coefficient. Molar absorptivity is the product of absorptivity and molecular weight of the material.

- Path Length, b : It was formerly denoted by *l* or *d*. It is the internal thichkness (diameter) of the cell in which the test sample is taken.

Fundamental Laws of photometry

- When light is incident upon a homogeneous medium, a part of the radiant power of the incident light is reflected, a part is absorbed and the remainder is transmitted.



Two fundamental laws of photometry

- Lambert's Law : It states the relationship between the radiant power of absorbed light with the thickness of the medium.

Lambert's Law

- When a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease of radiant power with the thickness of the medium is directly proportional to the radiant power of the incident light.

$$\frac{-dP}{db} \propto P \qquad \frac{-dP}{db} = k_1 P$$

- dP is change in radiant power, db is small thickness of sample, k_1 is proportionality constant and minus sign indicate radiant power decrease

After rearranging and integrating above formula we get

$$A = K_1 * b$$

A = Absorbance = $\log_{10}(Po/P)$

K_1 = Absorption coefficient = $k_1/2.303$

- Beer's Law : It states the relationship between the radiant power of absorbed light with the concentration of the medium

- When a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease of radiant power with the concentration of the medium is directly proportional to the radiant power of the incident light.

- Combined formed is called Lambert-Beer's law.

Lambert-Beer's law

- The law deals with the relationship between the radiant power of the incident light and transmitted light as a function of both the thickness of the medium and the concentration of the absorbing species.

- **Lambert's law**

  Absorbance = Constant x Thickness of the medium

- **Beer's Law**

  Absorbance = Constant x Concentration of the medium

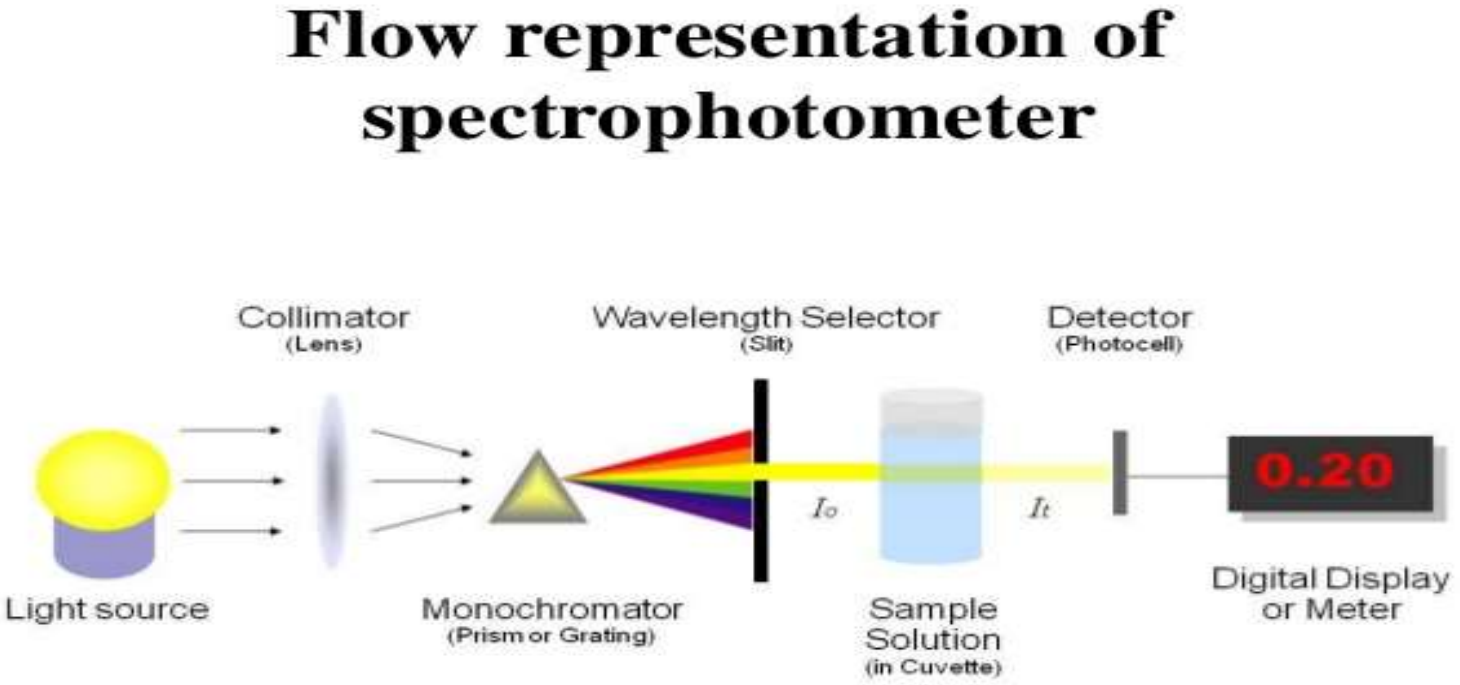
- **Lambert-Beer's Law**

The combined law may be given by the relation –

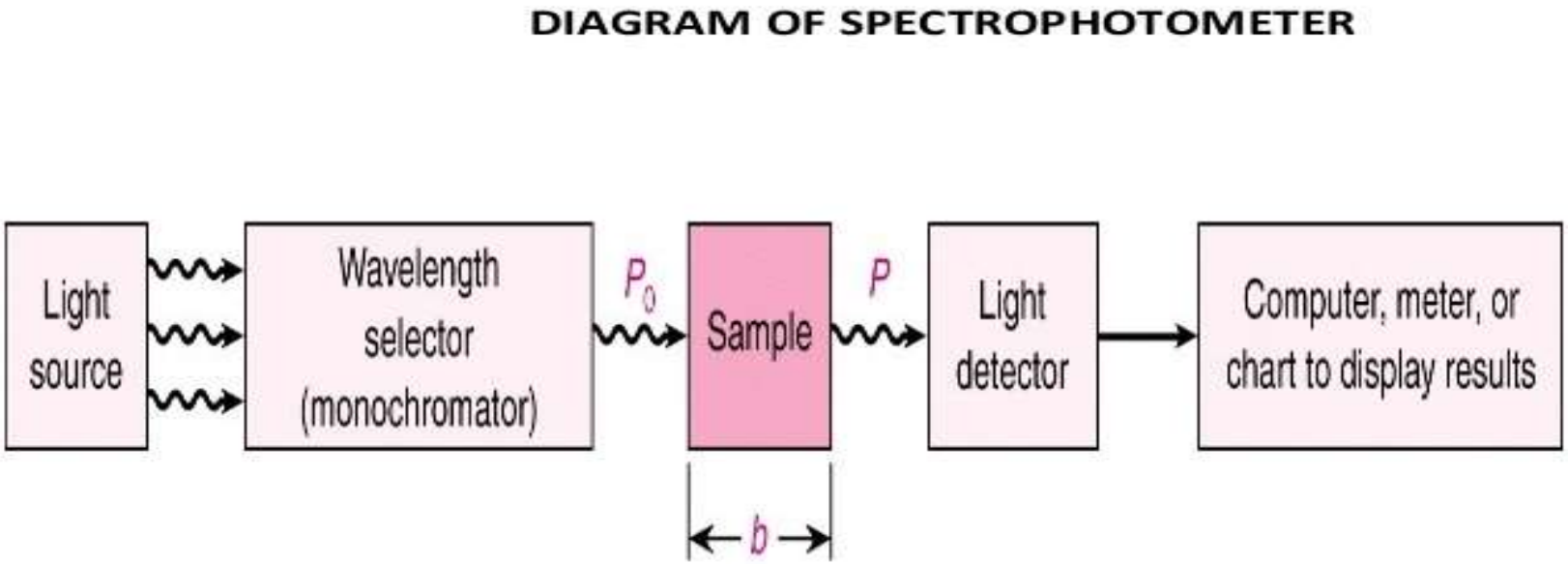
Absorbance = Constant x [Thickness of the medium] x [Concentration of the medium]

It states that, for the given system and the thickness of the medium, the absorption of the medium is directly proportional to the concentration of an absorbing species.

Instrumentation



Instrumentation

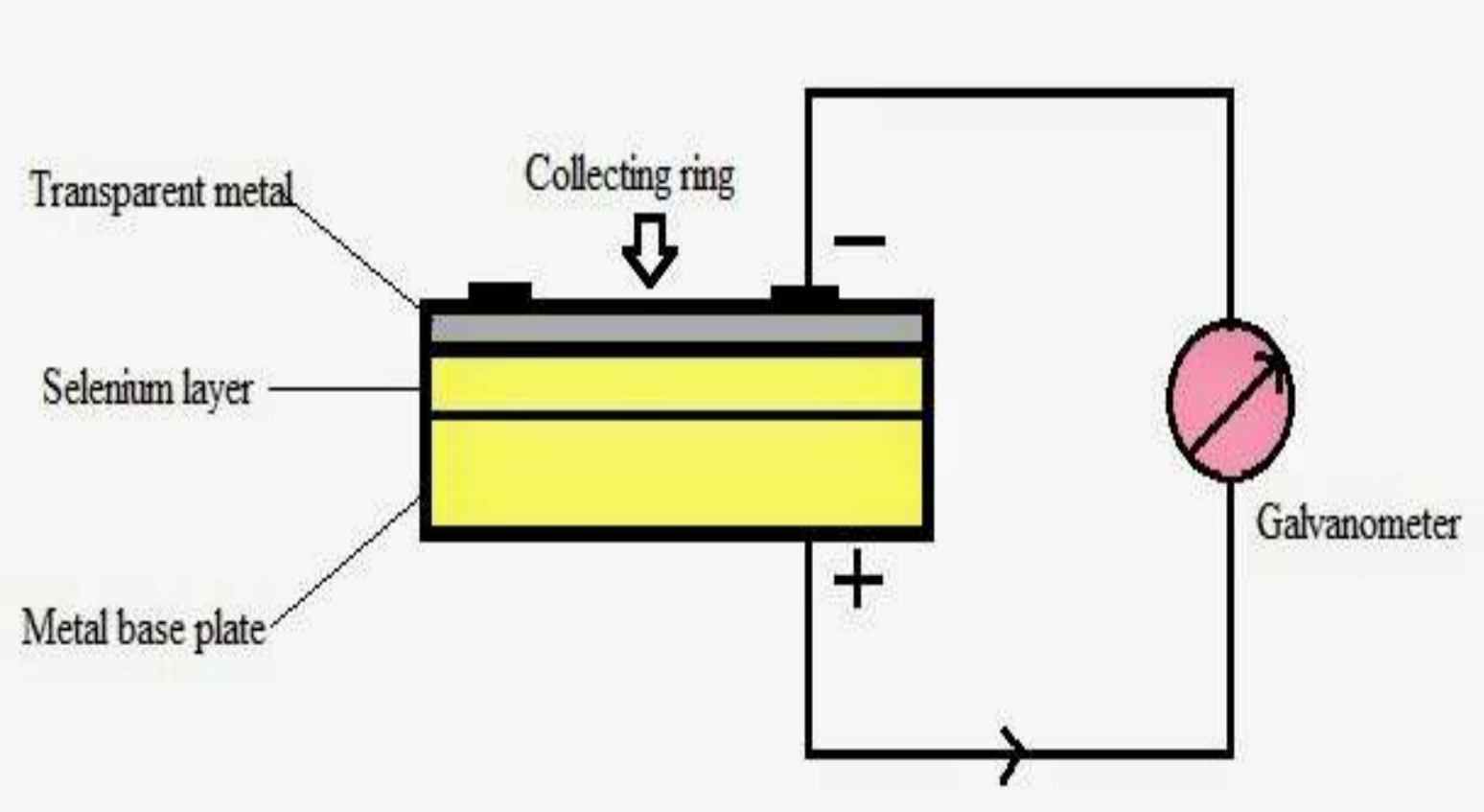


Instrumentation - Components

- Radiation source
- Filters and Monochromator – Prism, Diffraction grating
- Cuvette (sample container)
- Detection of Radiation – Detector – Photovoltaic cell or Photronic cell.

a. Photovoltaic cell

- It is also known as barrier-layer cell or photronic cell
- In this cell radiant energy falling on it generates a current at the interface of a metal and a semi-conductor.
- It operates without battery.
- It consists of a metal base plate made up of iron or copper, which acts as one electrode.
- A thin layer of semiconducting material is deposited on the surface of the metal plate.
- Then the surface of semiconducting material is covered by a very thin layer of silver or gold which acts as a second collector electrode.


Transparent metal
Collecting ring
Selenium layer
Metal base plate
Galvanometer

Characteristics of photovoltaic cell

- It is completely different in design and principle of the photomissive cell and works without the use of battery
- These cells generate their own e.m.f.
- **The magnitude of photocurrent is directly proportional to the radiant power of incident radiation striking on it.**
- The cells are sensitive over the whole visible region. The typical cell has maximum sensitivity at about 550nm
- The current output of the cell depends upon the wavelength of the incident radiation.

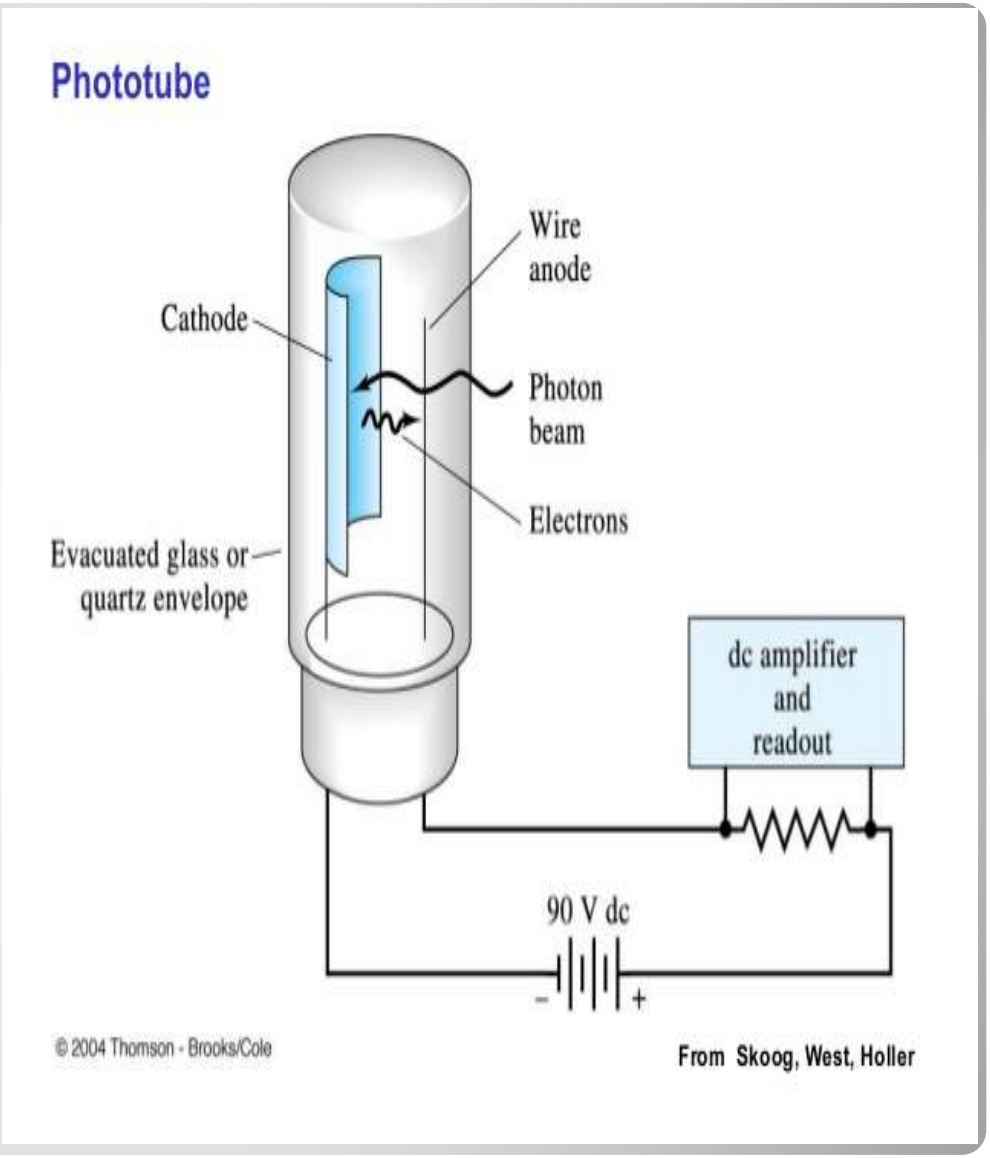
Disadvantages of photovoltaic cell

- The current produced by the cell cannot be readily amplified because of the low internal resistance

- The cells show fatigue effect – its current output decreases slowly during continued illumination. The fatigue effect can be minimised by careful selection of the optimum level of illumination.

b. Phtotubes

- These cells are also known as Photoemissive tubes
- In this tube, radiant energy falling on photosensitive solid surface causes emission of electrons.



- When light (photons) falls on cathode, electrons are emitted by the cathode due to photoelectric effect.
- The liberated electrons are attracted to the anode, causing an electric current to flow through an external circuit.
- The current is amplified and measured by readout device.
- The current measures the radiant power of light radiation striking the photosensitive surface.

C. Photomultiplier tubes

- It contains a photosensitive surface as well as many other surfaces that emit a cascade of electrons from the photosensitive area.
- It is more sensitive than a phototube for the visible and UV regions.
- It is mostly used in spectrophotometer

Instrumentation

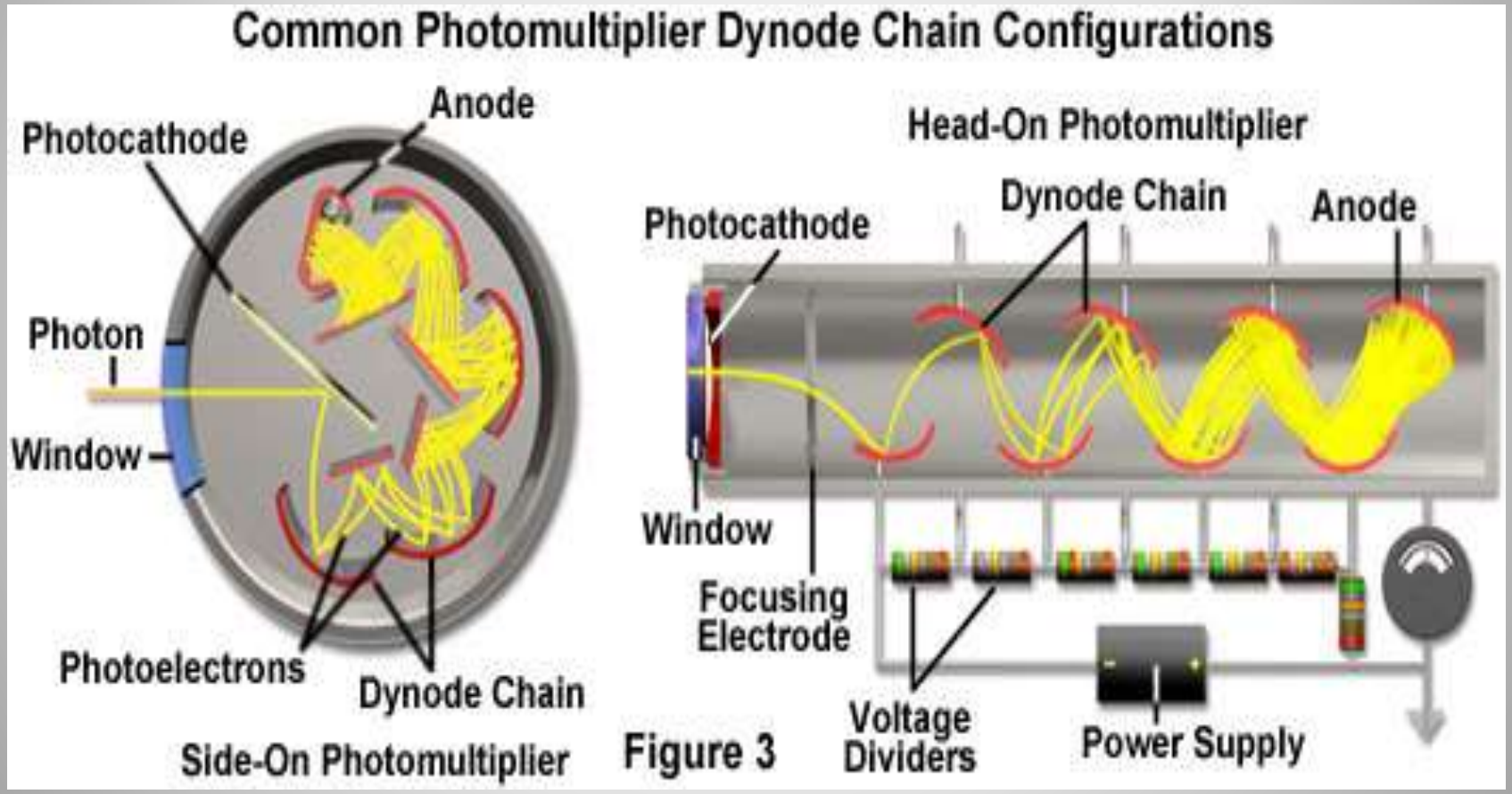


Figure 3

Construction

- A photomultiplier tube consists of a cathode, an anode and many additional electrodes that are called **dynodes**.
- All these are enclosed in an evacuated glass tube.
- A photoemissive cathode is in the form of a half cylinder of metal.
- The inner surface of cathode is coated with a light sensitive material such as oxide of cesium or potassium or silver.
- Most photomultiplier tubes have nine dynodes.
- A dynode is an electrode with a coating of cesium which emits several electrons (2-5) for each striking on its surface.

Working

- When the light radiation strikes the cathode surface, it ejects electrons due to photoelectric effect.
- These ***primary electrons*** get accelerated in the electrostatic field between the cathode and the first dynode and fall on dynode.
- When electrons strike dynode 1, each electron causes emission of several electrons. These ***secondary electrons*** in turn are accelerated towards dynode 2
- Likewise the process is repeated nine times, where each secondary electron releases several electrons (2-5).
- The resulting current is then amplified and measured.

Colorimeter

- A colorimeter is a device used in colorimetry.
- In scientific fields the word generally refers to the device that measures the absorbance of particular wavelengths of light by a specific solution.
- This device is commonly used **to determine the concentration of a known solute in a given solution** by the application of the Beer-Lambert law.
- Beer Lambert Law states that the **concentration of a solute is proportional to the absorbance.**
- It is invented by Louis Jules Duboscq in 1870.
- It involves quantitative estimation of colour.
- The colour of light is the function of its wavelength.

Single beam photoelectric colorimeter

The basic components are –

- A source of light such as tungsten filament lamp with concave reflector and collimating lens
- An adjustable diaphragms or slits
- A coloured glass filter for monochromatic light
- A cuvette such as glass tube for holding the solution/solvent
- A detector such as photovoltaic cell
- A recorder such as galvanometer

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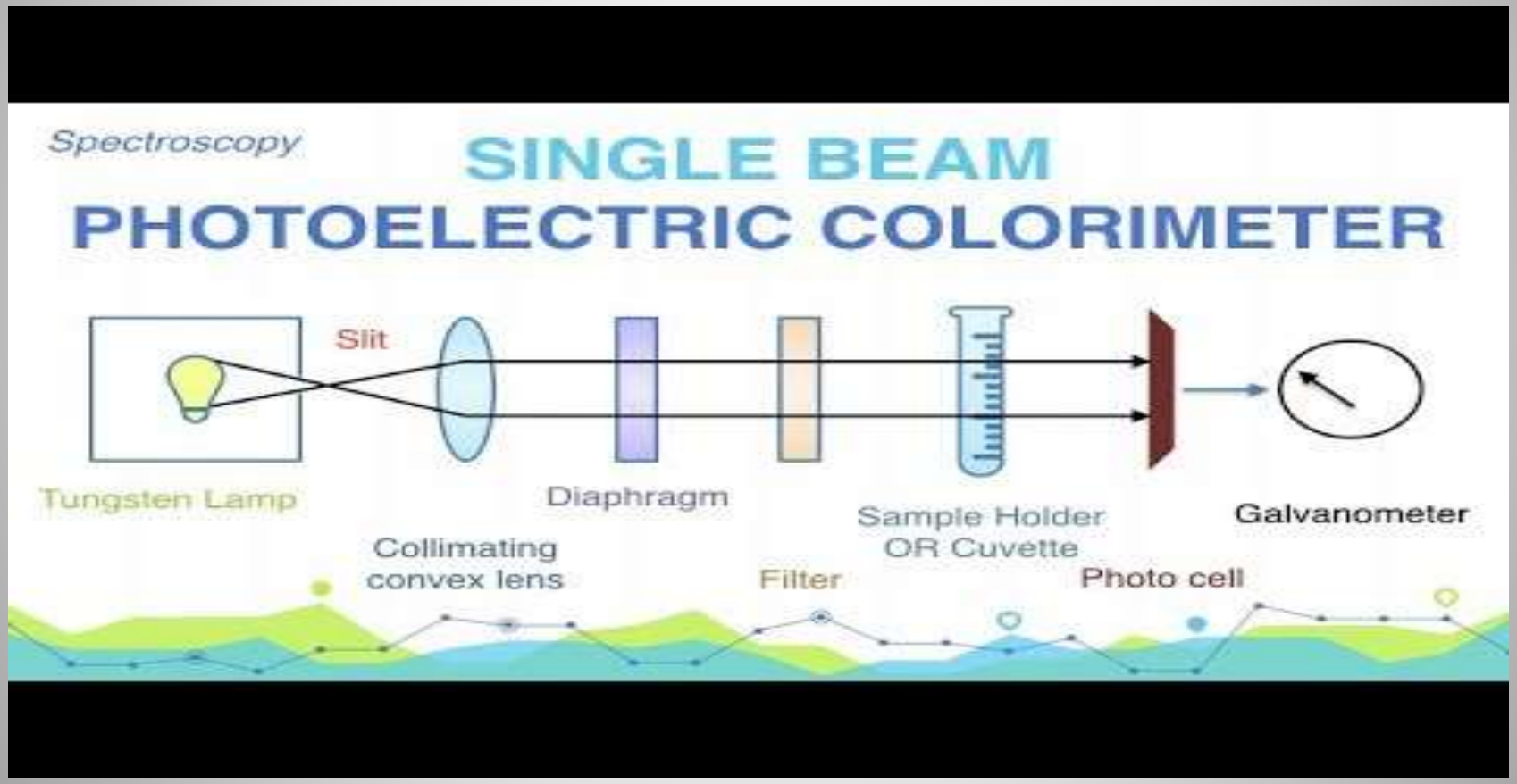
Instrumentation



Functioning

- Polychromatic light from a incandescent tungsten filament lamp reflects by concave mirror in the direction of the filter and also the light passes through the collimating lens.
- It makes the beam of light parallel
- The light passes further through slit (1) which controls the in-going light.
- The filter converts the polychromatic light to narrow band width wavelength light.
- It passes further through absorbing material where the material absorbs the part of it depending upon the **colour density of the solution** and transmits the remaining intensity through slit (2) towards the detector.
- The detector **converts the light energy into electrical energy** and feeds it to the recorder.
- The signal **magnitude of the recorder is the measure of the absorbance or transmittance** of the solution.

Spectrophotometer

- If you pass white light through a colored substance, some of the light gets absorbed.
- A solution containing hydrated copper(II) ions, for example, looks pale blue because the solution absorbs light from the red end of the spectrum.
- The remaining wavelengths in the light combine in the eye and brain to give the appearance of cyan (pale blue).
- Some colorless substances also absorb light - but in the ultra-violet region.
- Since we can't see UV light, we don't notice this absorption.

- Different substances absorb different wavelengths of light, and this can be used to help to identify the substance - the presence of particular metal ions, for example, or of particular functional groups in organic compounds.
- The amount of absorption is also dependent on the concentration of the substance if it is in solution.
- Measurement of the amount of absorption can be used to find concentrations of very dilute solutions.
- An absorption spectrometer measures the way that the light absorbed by a compound varies across the UV and visible spectrum.

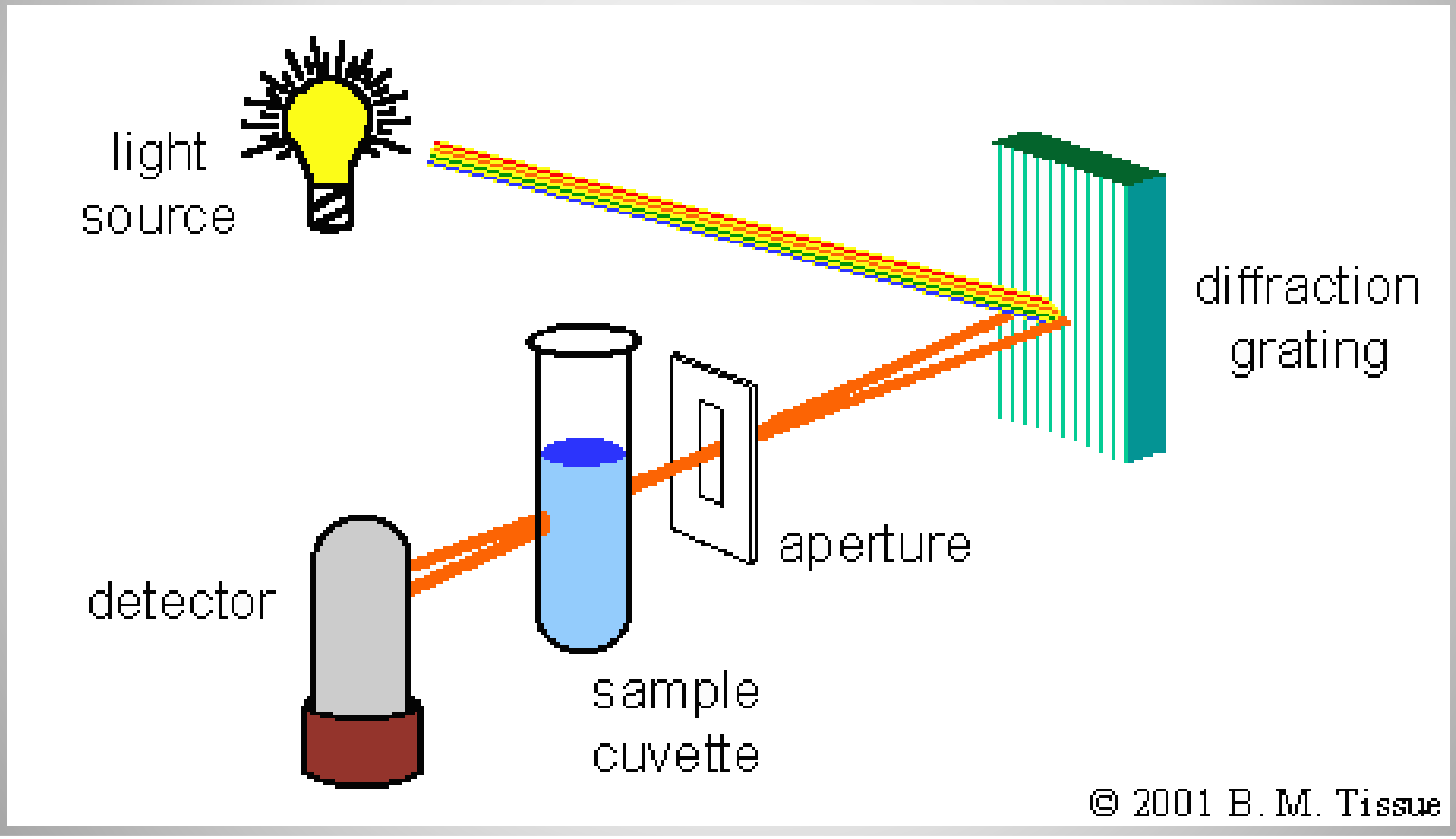
Single beam spectrophotometer

- Single-Beam spectrophotometers are often sufficient for making quantitative absorption measurements in the UV-Vis spectral region. The concentration of an analyte in solution can be determined by measuring the absorbance at a single wavelength and applying the Beer-Lambert Law.

Instrumentation

- Single-beam spectrophotometers can utilize a fixed wavelength light source or a continuous source.
- The simplest instruments use a single-wavelength light source, such as a light-emitting diode (LED), a sample container, and a photodiode detector.
- Instruments with a continuous source have a dispersing element and aperture or slit to select a single wavelength before the light passes through the sample cell.
- In either type of single-beam instrument, the instrument is calibrated with a ***reference cell*** containing only solvent to determine the P_o value necessary for an absorbance measurement.

Instrumentation



Double beam spectrophotometer

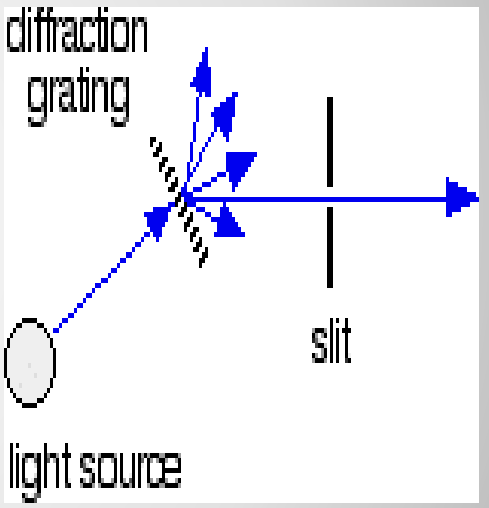
It has components as –

The light source

- You need a light source which gives the entire visible spectrum plus the near ultra-violet so that you are covering the range from about 200 nm to about 800 nm.
- You can't get this range of wavelengths from a single lamp, and so a combination of two is used - a deuterium lamp for the UV part of the spectrum, and a tungsten / halogen lamp for the visible part.
- The combined output of these two bulbs is focused on to a diffraction grating.

The diffraction grating and the slit

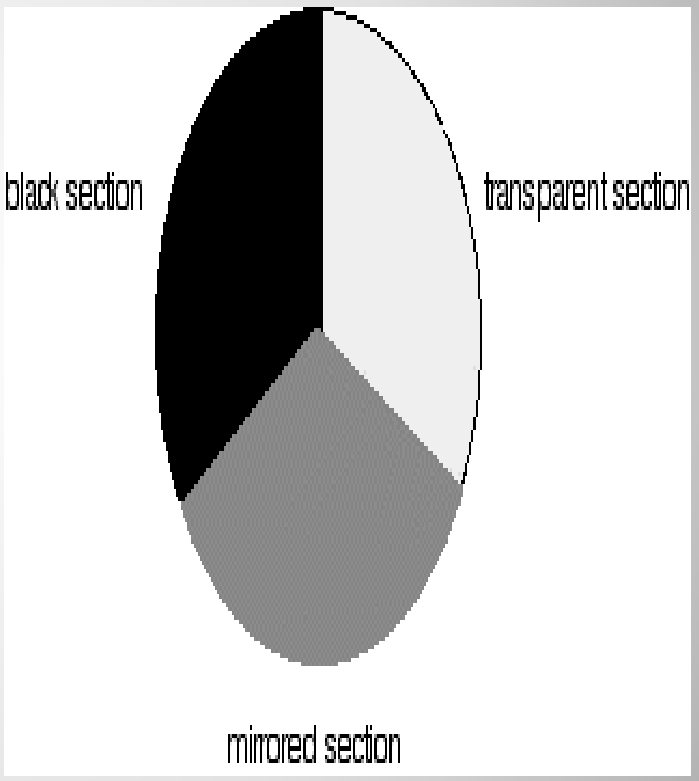
- You are probably familiar with the way that a prism splits light into its component colors.
- A diffraction grating does the same job, but more efficiently.



- The blue arrows show the way the various wavelengths of the light are sent off in different directions.
- The slit only allows light of a very narrow range of wavelengths through into the rest of the spectrometer.
- By gradually rotating the diffraction grating, you can allow light from the whole spectrum (a tiny part of the range at a time) through into the rest of the instrument.

The rotating disks

- Each disk is made up of a number of different segments.
- Those in the machine we are describing have three different sections - other designs may have a different number.



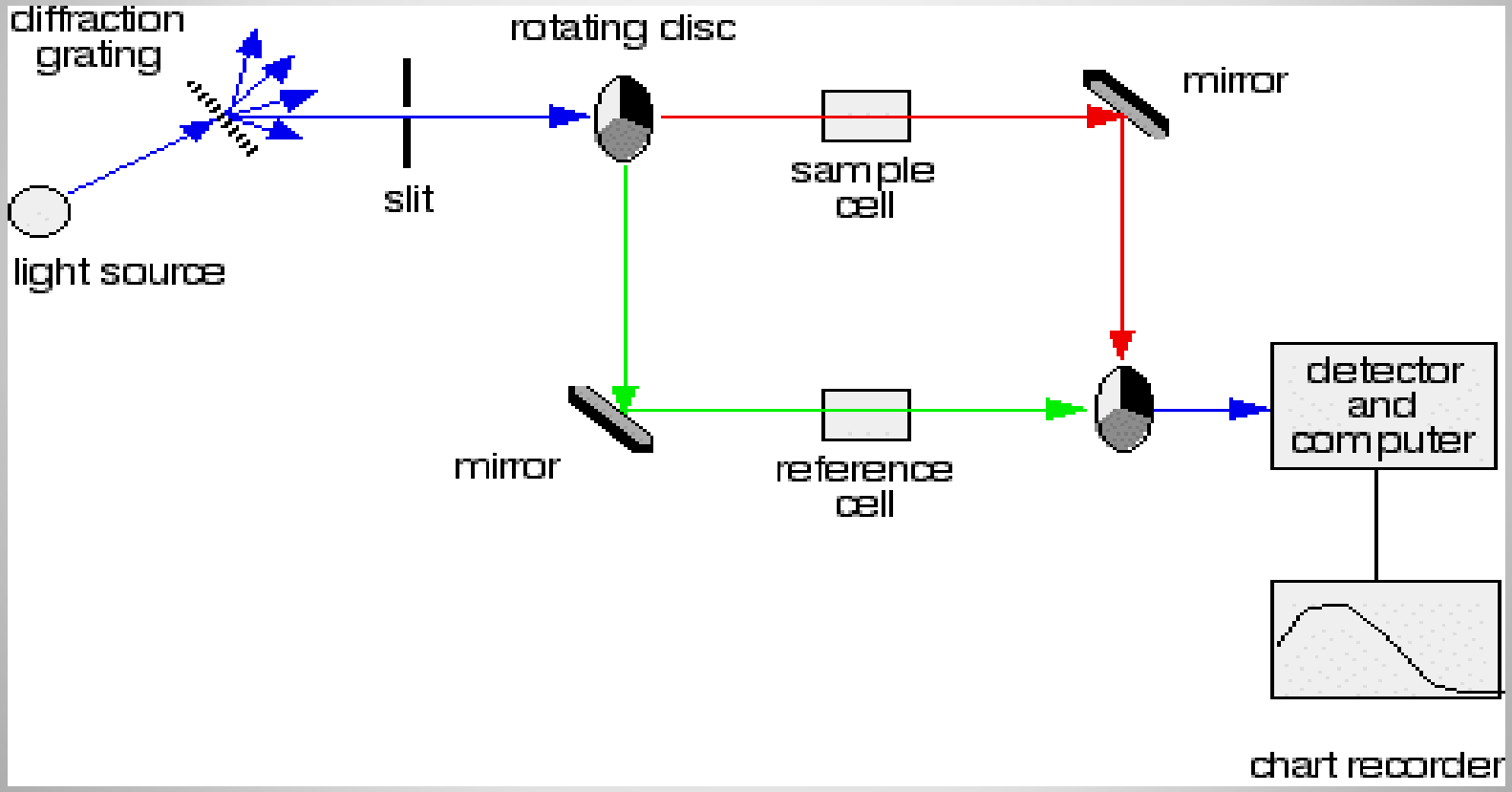
The sample and reference cells

- These are small rectangular glass or quartz containers.
- They are often designed so that the light beam travels a distance of 1 cm through the contents.
- The sample cell contains a solution of the substance you are testing - usually very dilute.
- The solvent is chosen so that it doesn't absorb any significant amount of light in the wavelength range we are interested in (200 - 800 nm).
- The reference cell just contains the pure solvent.

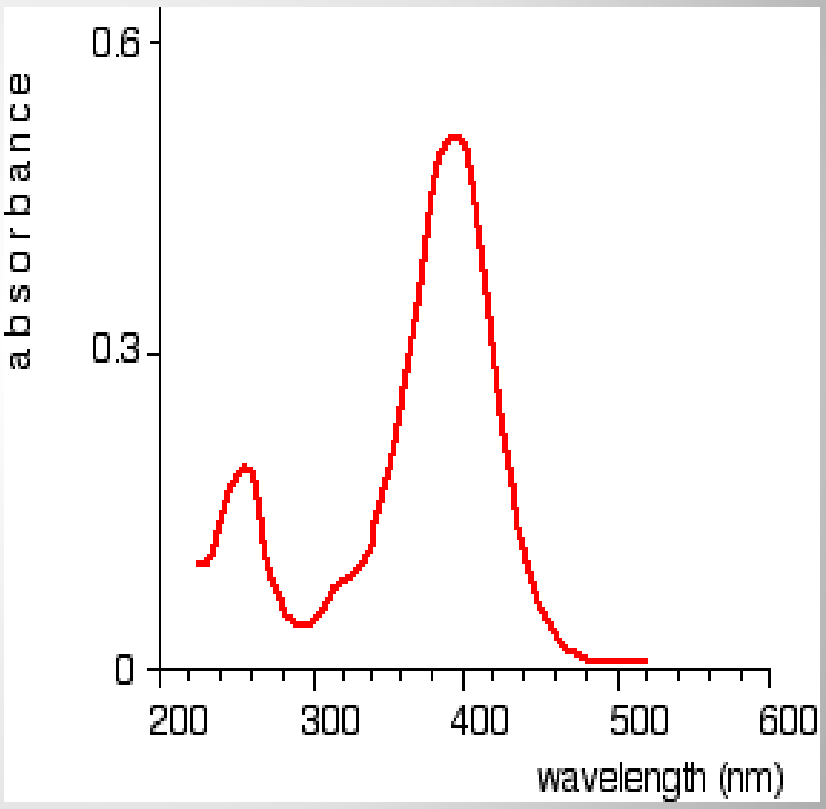
The detector and computer

- The detector converts the incoming light into a current.
- The higher the current, the greater the intensity of the light.
- For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured.

Instrumentation



- The absorption spectrum is a plot of absorbance against wavelength.



Colorimeter Vs Spectrophotometer

Spectrophotometric titrations

- **Definition:** The process of determining the quantity of a sample by adding measured increments of a titrant until the end-point, at which essentially all of the sample has reacted, is reached.
- The titration is followed by measuring the absorbance of radiation in the range ultraviolet to near-infrared (0.1--2.5 \\mum) by the sample.
- The titration in which absorbance of a solution is used to determine the end point are called spectrophotometric titrations.

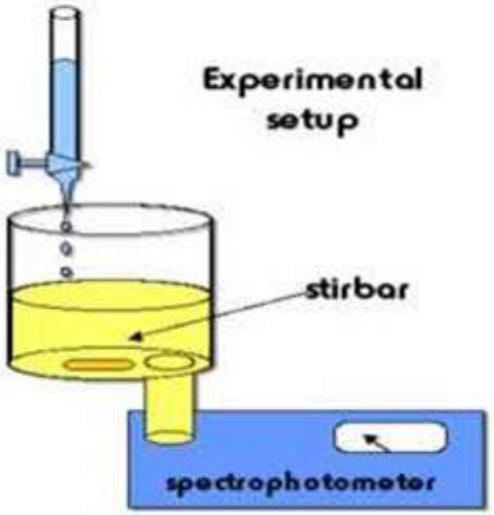
Instrumentation

Spectrophotometric titrations

The absorbance of a solution can be monitored during a titration.

In this example, stirring will cause the solution to circulate in the cuvette and the response can be measured.

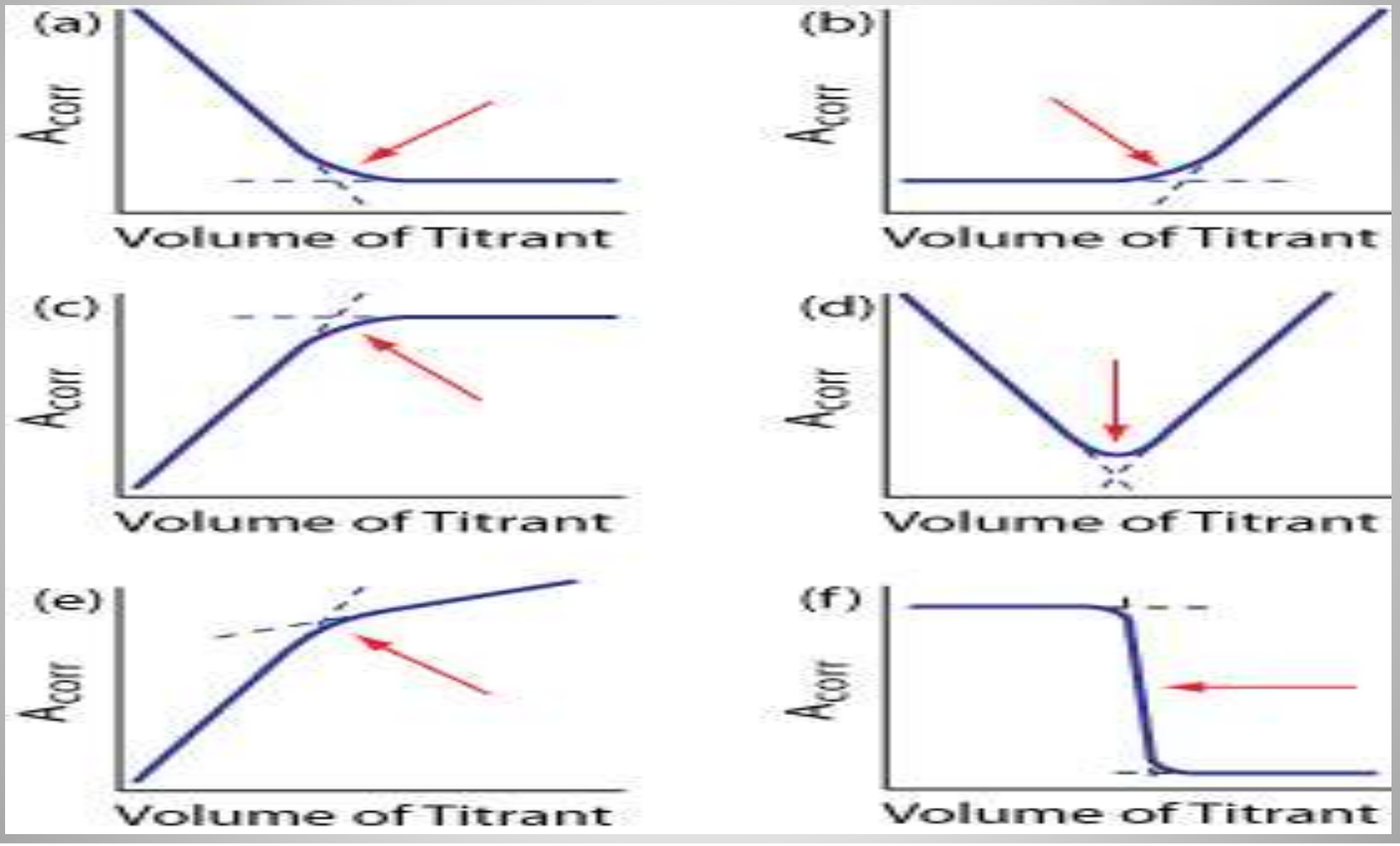
You could also use a pump or simply transfer a sample at known intervals.



Technique

- The absorbance measurements are made at a fixed wavelength.
- First an optimum wavelength is selected and zero adjustment is made.
- The solution to be titrated (tirand) is taken in the cell
- Then the cell is kept in the beam of light in the instrument.
- A known volume of titrant is added to the stirred solution in the cell.
- Thus the absorbance of the titrand solution is measured after each addition of the titrant and the end point of the titration is detected.
- The absorbance is plotted against the volume of the titrant added.

Titration curve



Advantages of photometric titrations

- Useful to solutions with lower or higher ionic strength or non-aqueous solvents
- Used for highly coloured solutions which cannot be determined the visual indicators.
- The slight changes in colour are readily detcted by the spectrophotometer and hence end point determination is sharp and accurate.
- Other absorbing species do not interfere with the actual titration, because only the changes in absorbance are taken into account and not the absolute values of absorbance.

- These titrations can be applied to a large number of non-absorbing constituents, since only one absorber is necessary among the reactant, the titrant or the reaction products.
- These titrations provide more accurate results tan a routine analysis because the data fro many measurements are brought together in determining the end point.
- It is particularly suitable to titration reactions where a relatively large degree of reaction incompletion exists at the equivalence point.

Applications

- https://www.slideshare.net/rey216/spect

- https://slideplayer.com/slide/5256756/