Microbiology

US05EMIC26

Bioinstrumentation and Biotechniques

Unit- I

Spectroscopy

(a) Beer's and Lamberts Law.

(b) Principle, working and applications of :

- UV-Visible Spectrophotometer

- Atomic Absorption spectroscopy

- Infra Red Spectroscopy

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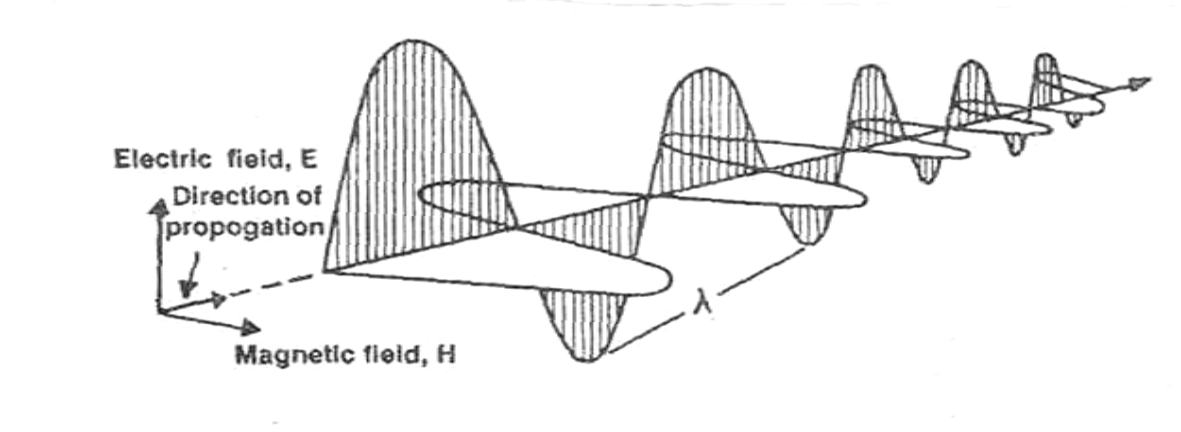

A beam of light from a bulb consists of many randomly oriented plane polarized components being propagated in the same direction. The distance along the direction of propagation for one complete cycle is known as wavelength [λ]. Wavelength may be measured in centimeters (cm), micrometers (μm), nanometers (nm) or ang-strom units (A^{O}).

Wavelength and frequency share an inverse relationship; this means that as the wavelength increases, the energy of the radiation decreases, while the energy increases with the increase in frequency.

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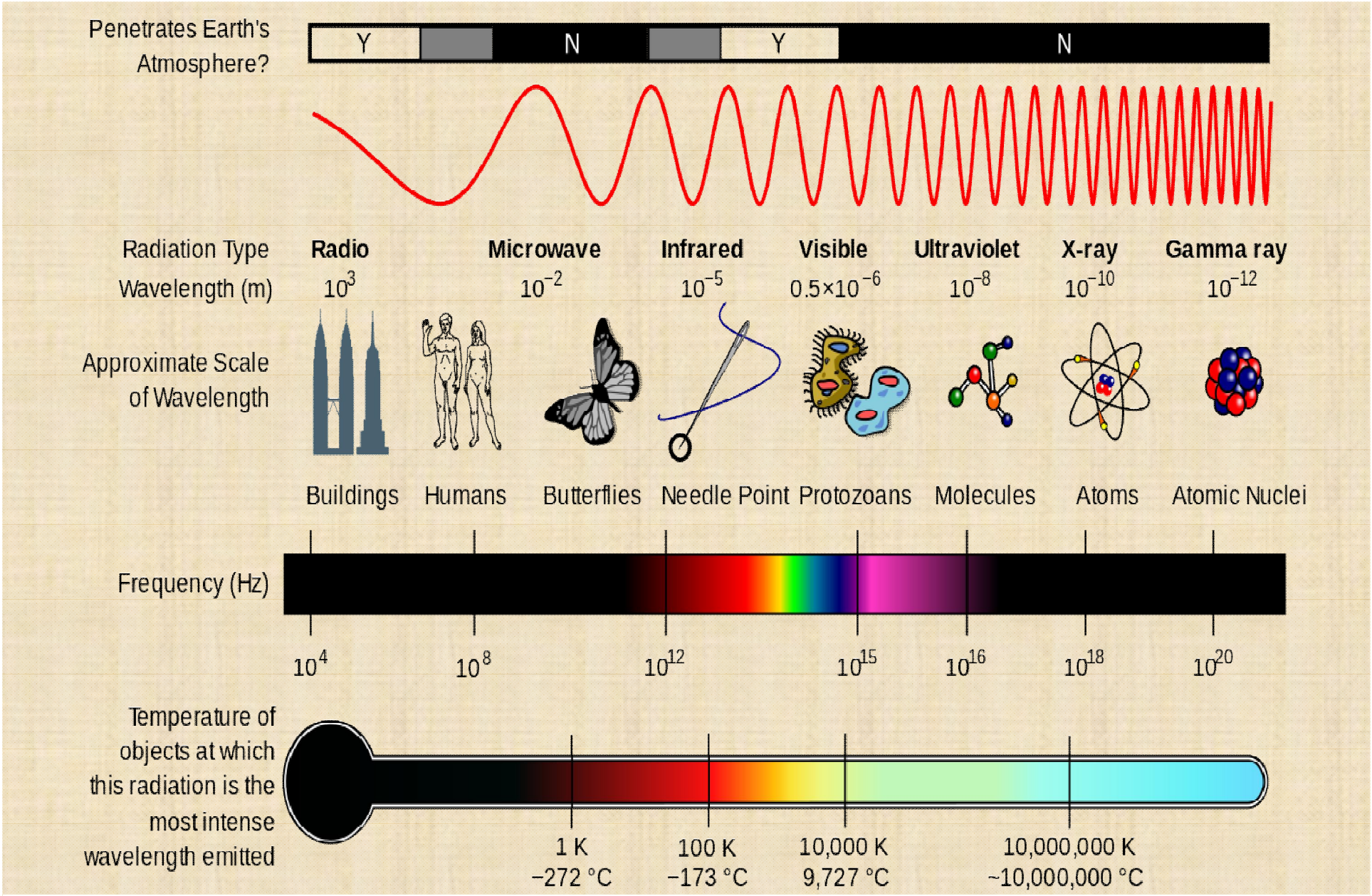

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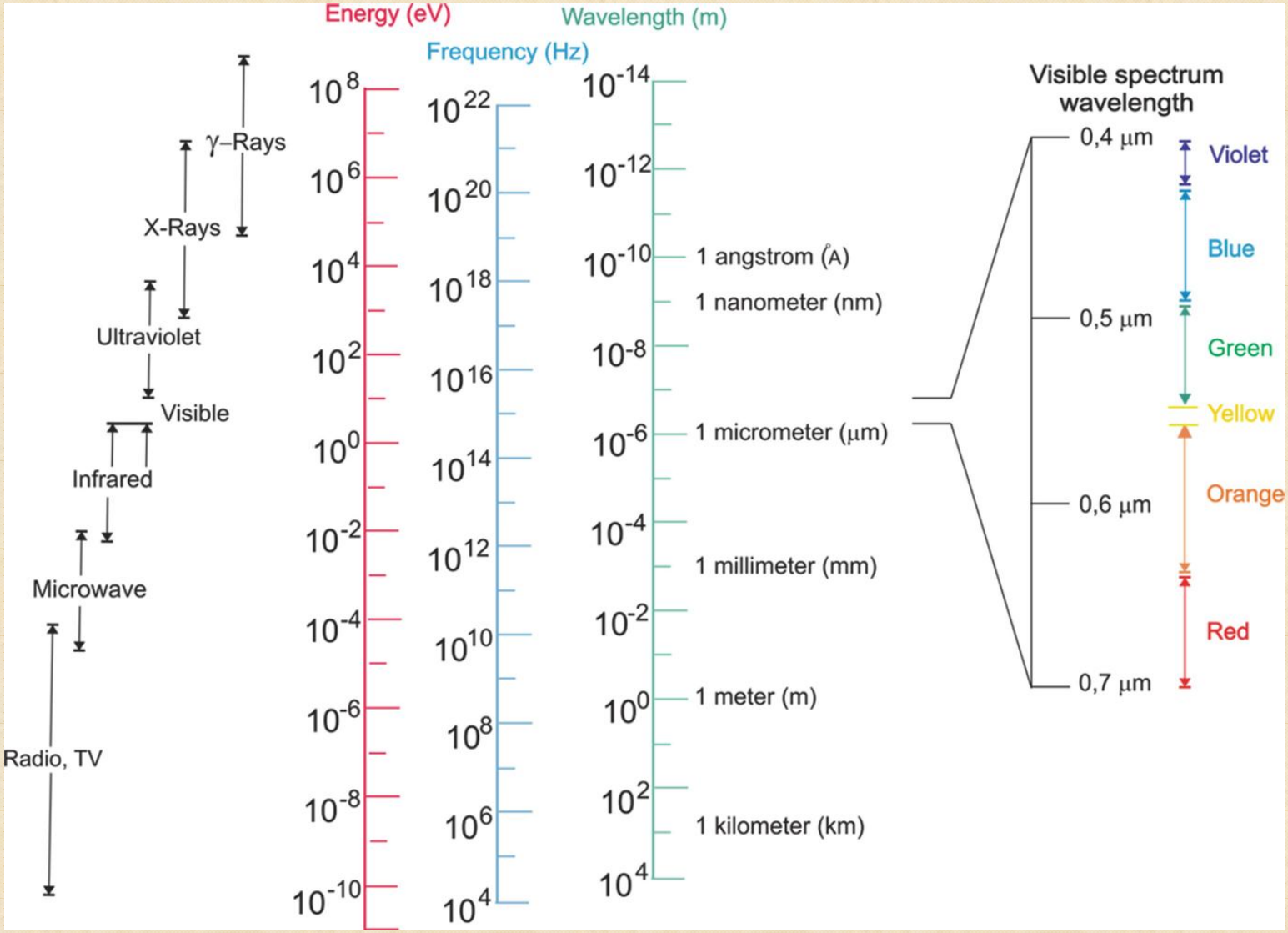

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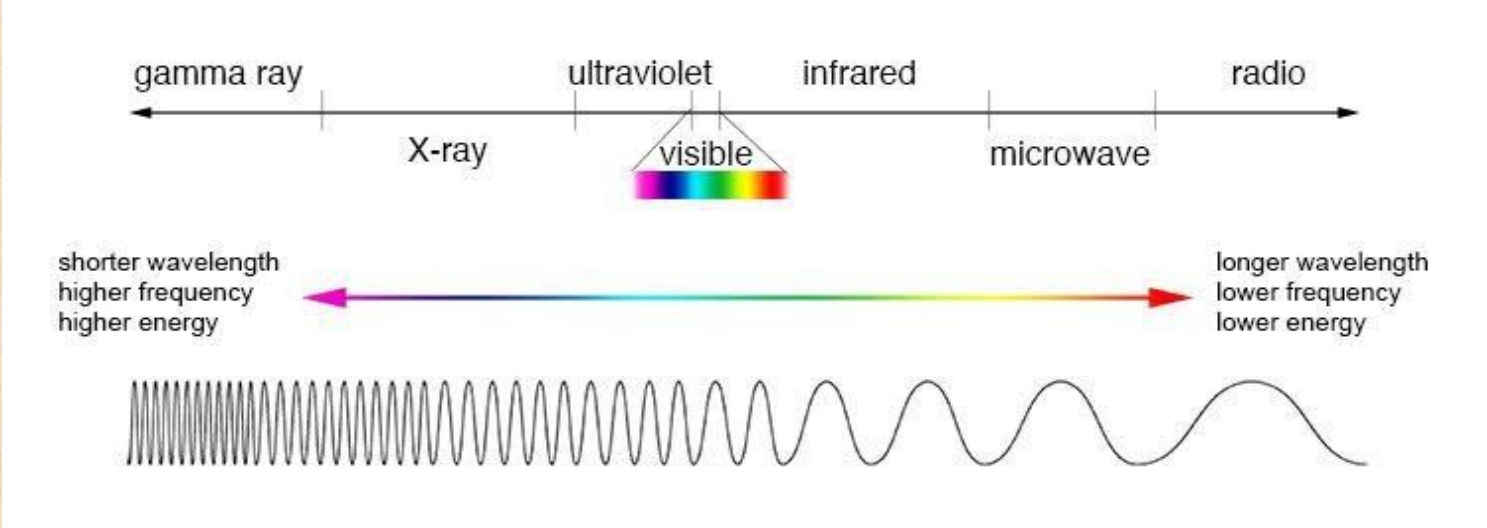

Color	Wavelength
violet	380–450 nm
blue	450–495 nm
green	495–570 nm
yellow	570–590 nm
orange	590–620 nm
red	620–750 nm

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A Colorimeter is generally any tool that characterizes color samples to provide an objective measure of color characteristics. In chemistry, the colorimeter is an apparatus that allows the absorbance of a solution at a particular frequency (color) of visual light to be determined.
it is proportional to the absorbance.

A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light.

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Colorimeter	Spectrophotometer
Detection of solute only in color solution	Detection of solute both in color & colorless solution
Wavelength of visible length	Wavelength of in or out of visible length
Filter for wavelength	Prism or diffraction grating
Glass or plastic cuvette	Quartz or silica glass cuvette
Cheaper and less Accurate	Costly and more Accurate

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The Beer –Lambert Law

When a monochromatic light of initial intensity Io passes through a solution in a transparent vessel, some of the light is absorbed so that the intensity of the transmitted light I is less than Io.

There is some loss of light intensity from scattering by particles in the solution and reflection at the interfaces, but mainly from absorption by the solution.

The relationship between I and Io depends on the path length of the absorbing medium, l, and the concentration of the absorbing solution. These factors are related in the laws of Lambert and Beer.

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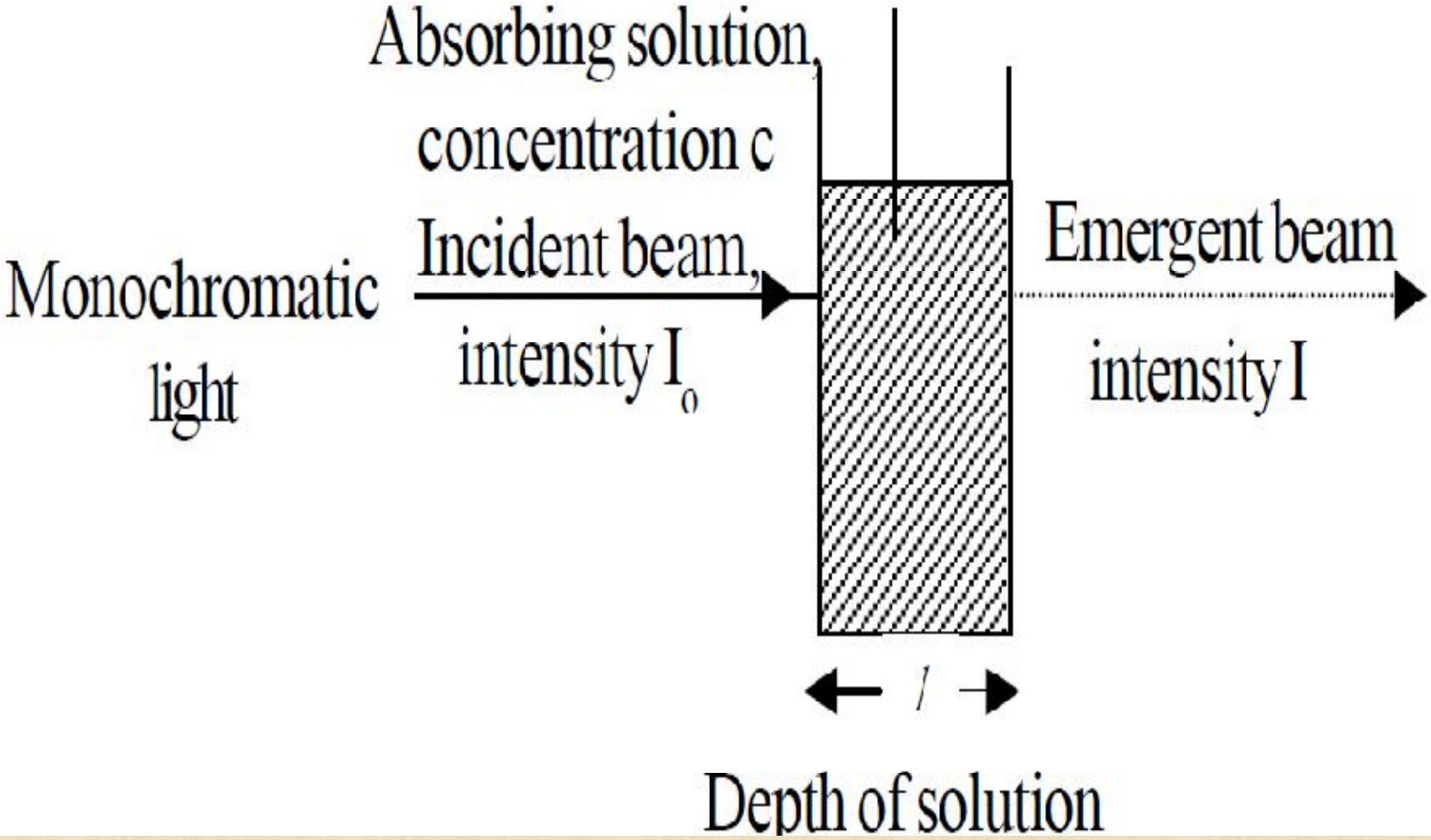

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Lambert's law:

When a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

Beer's law :

When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

A beam of radiation from an electric bulb consists of several wavelengths and is known as polychromatic. A beam in which all the rays have the same wavelength is known as monochromatic.

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Principle, working, instrumentation and applications of UV-Visible Spectrophotometer

Principle:

The principle of uv-visible spectroscopy is depends on lows of absorption of beer and Lambert.

Lambert law: "It states that the amount of light absorbed is proportional to the thickness of the absorbing material and is independent of the intensity of incident light."

Beer's law: "This law states that the amount of light absorbed by a material is proportional to the number of absorbing solution."

A 100% value of T represents totally transparent substance with no radiation being absorbed, where as zero value of T represents totally opaque substance Represents complete absorption.

Absorbance used to be called "optical Density" ranges from 0(100%T) or infinite(0 % T)

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Instrumentation:

Photometers, colorimeter and spectrophotometers have the following basic components.

(A) A stable and cheap radiant energy source,
(B) Filter or monochromator to break the polychromatic radiation into component wavelengths or "bands" of wavelengths,
(C) Transparent vessels (cuvettes) to hold the sample,
(D) A photosensitive detector and an associated readout system (meter or recorder).

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[A] Radiant Energy Sources :-

Materials which can be excited to high energy states by a high voltage electric discharge or by electrical heating serve as excellent radiant energy sources. As the electrons of these materials return to their ground state, they emit radiation of characteristic energies corresponding to the energy difference between the excited and the ground energy levels.

(a) Sources of Ultraviolet Radiation
(b) Sources of Visible Radiation

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(a) Sources of Ultraviolet Radiation :-

Most commonly used sources of ultra-violet radiation are the hydrogen lamp and the deuterium lamp. Deuterium lamp is expensive and used when high intensity is required. Xenon discharge lamp may also be used for ultraviolet radiation, but the radiation produced is not as stable as the hydrogen lamp.

(b) Sources of Visible Radiation :-

Tungsten filament lamp is the most commonly used source for visible radiation. It is inexpensive and emits continuous radiation in the visible region.

Carbon arc, which provides more intense visible radiation is used in a small number of commercially available instruments.

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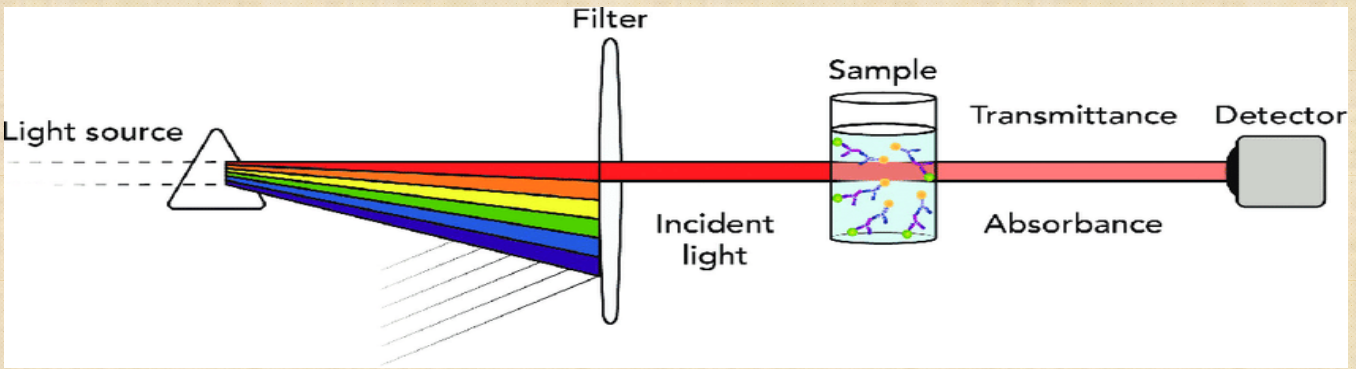
(B) Filter or monochromator to break the polychromatic radiation into component wavelengths or "bands" of wavelengths.

A source is generally emitting a continuous spectra therefore a device is required to select a narrow band from the wavelength of continuous spectra. For this selection filter Or monochromator or both are used.

(1)Filters: A light filter is a device that allow light of the required wavelength to pass but absorbs light of other wavelength completely or partially. It means particular filter is used for the Specific analysis.



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(2)Monochromator :

It accepts polychromatic input light from a lamp and outputs monochromatic light.

The monochromator of the instrument is composed of an

(a) Entrance slit (to narrow the beam to a usable size)

(b) A dispersion device

(usually a diffraction grating or prism that separates polychromatic white light into bands of monochromatic light of a single wavelength)

(c) An exit slit (to select the desired monochromatic wavelength).

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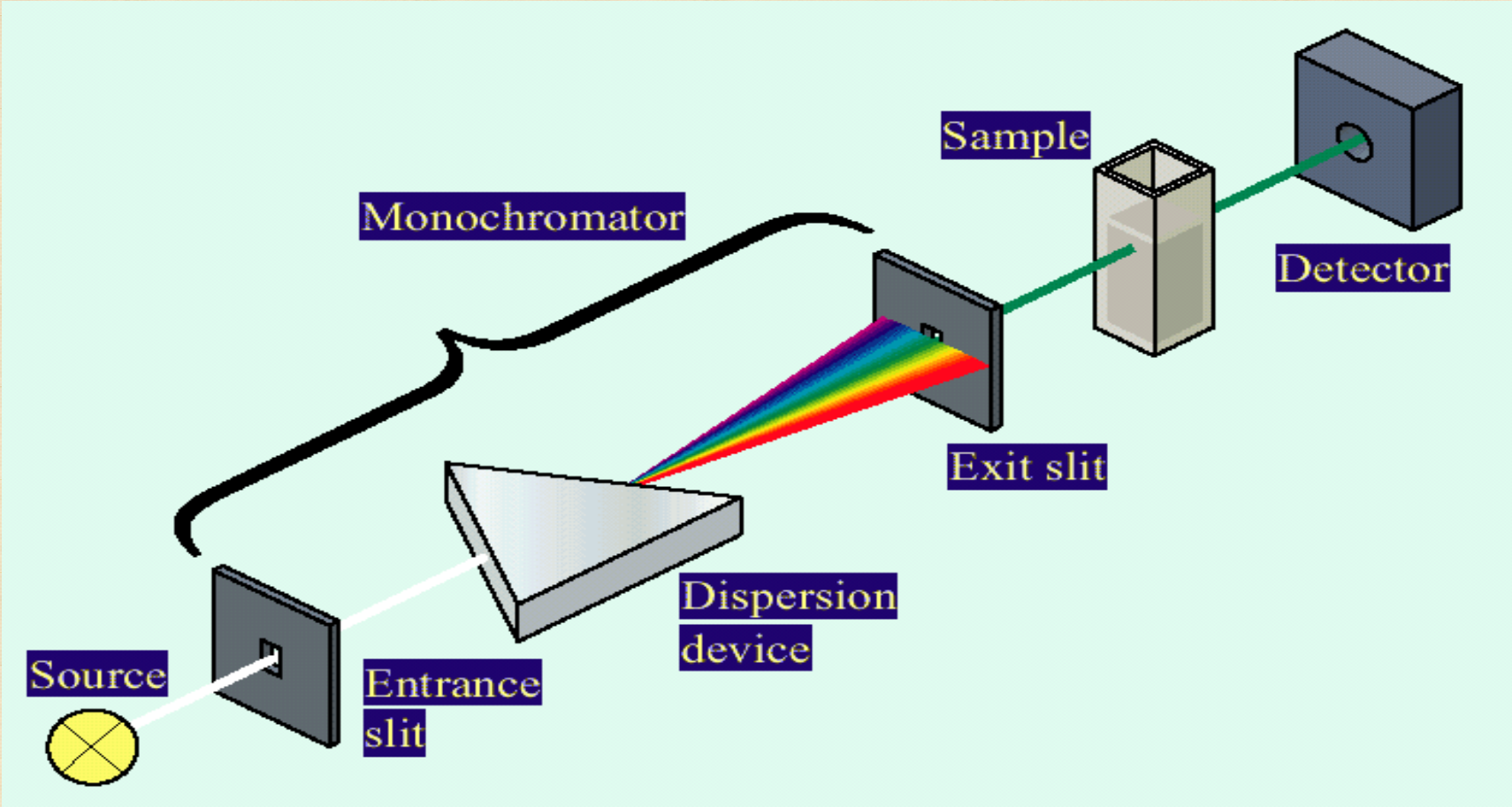

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A dispersion device usually a diffraction grating or prism that separates polychromatic white light into bands of monochromatic light of a single wavelength)

Prism :

Prism is used to isolate different wavelength .If a parallel beam of radiation falls on a prism , the radiation of 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.

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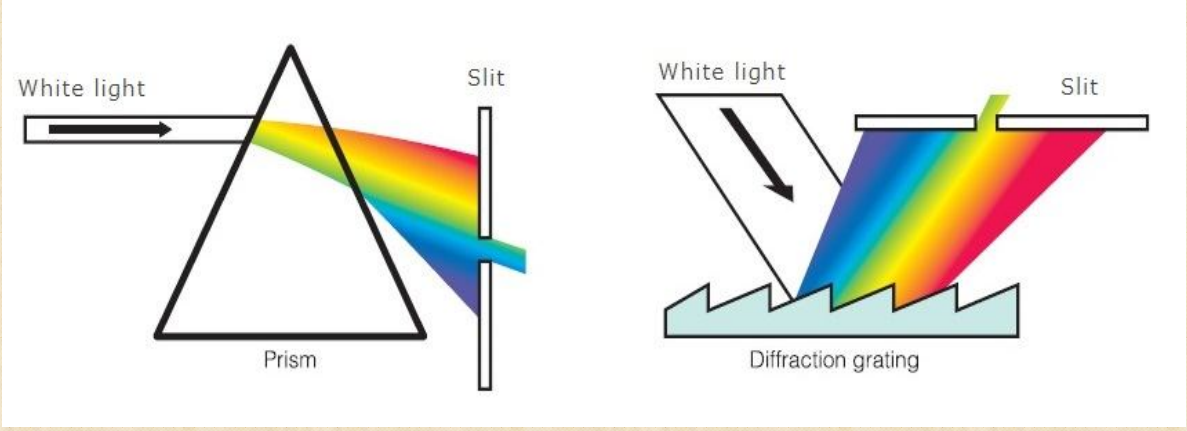
Diffraction gratings:

Diffraction grating is an optical component with a regular pattern, which splits (diffracts) light into several beams travelling in different directions. The directions of these beams depend on the spacing of the grating and the wavelength of the light so that the grating acts as a dispersive element. The diffraction grating disperses the light into a linear spectrum of its component wavelengths, which is then directed, in whole or in part along the light path of the instrument



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(C) Transparent vessels (cuvettes) to hold the sample(cells)

- Samples to be studied in the ultraviolet or visible region are usually gases or solutions and are put in cells known as cuvettes. Cuvettes for the visible region are made up of either color corrected fused glass and for the ultraviolet region, quartz or fused silica cells are used.
- Standard path length of these cuvettes is usually 1 cm (internal distance). However, cuvettes of path-length of 1 mm to 10 cm are available for special purposes.
- cells may be rectangular or cylindrical in shape or cylindrical with flat ends.

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(D) A photosensitive detector and an associated readout system (recorder).

- 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

- The following three types of detectors are used in uv-visible spectrophotometer

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

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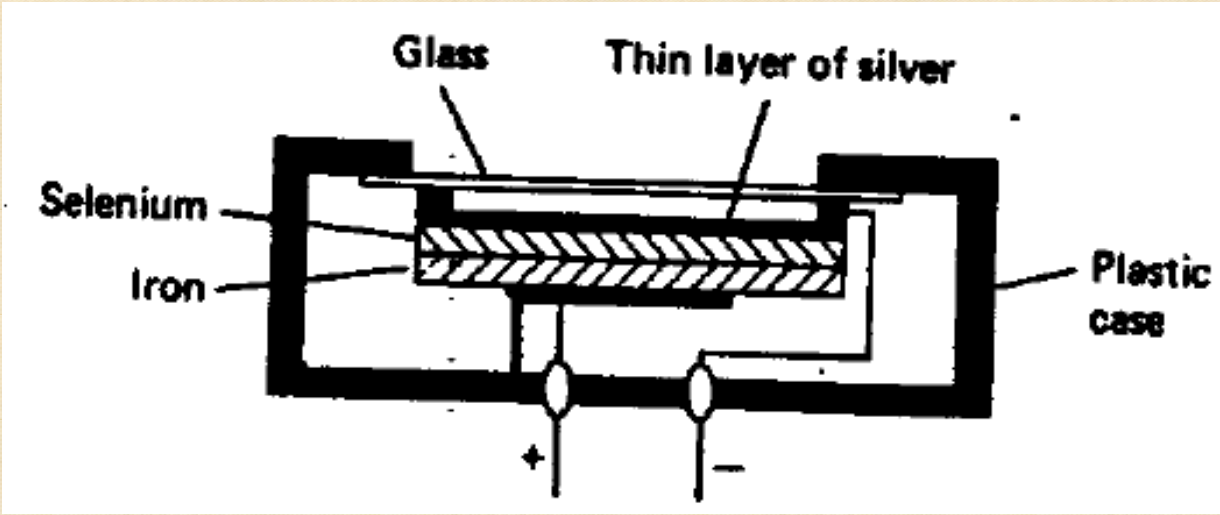
Requirements of an ideal detector:-

- 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 to wide spectrum of radiation received.

1. Barrier layer cell / Photovoltaic cell



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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.

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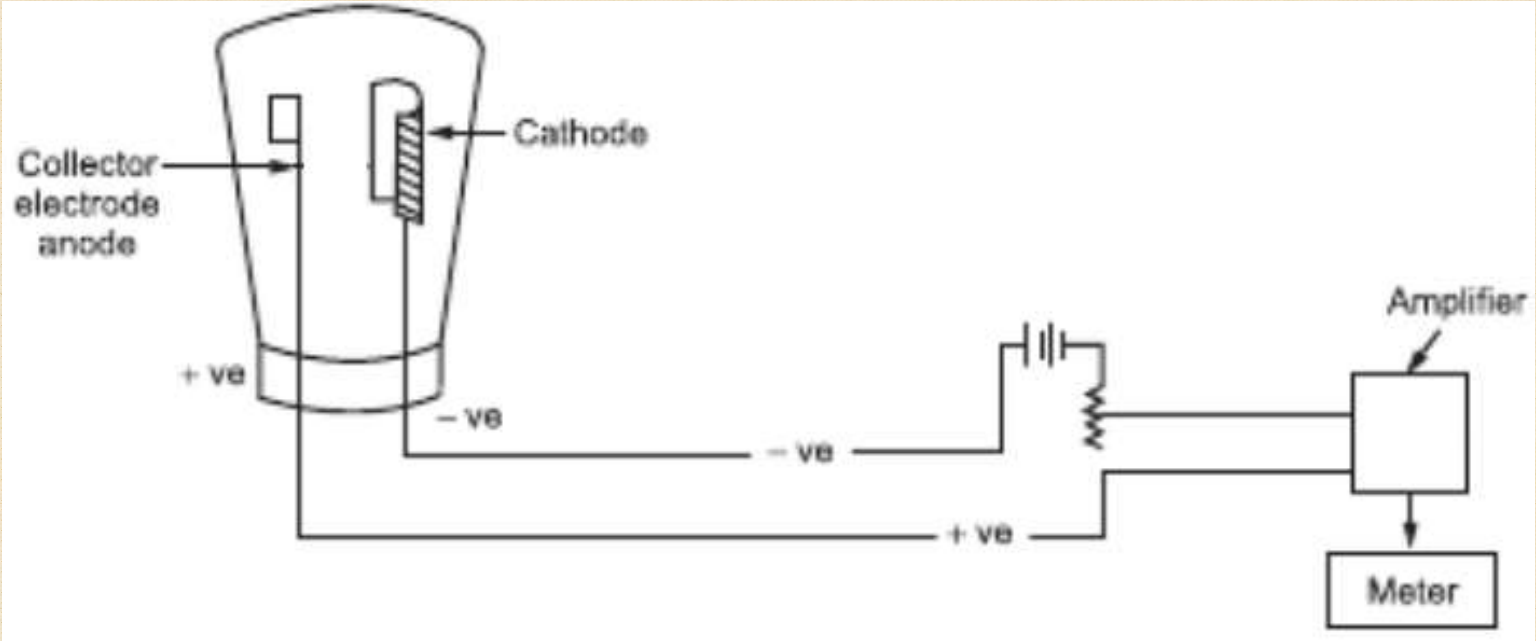
2. Phototubes/ Photo emissive tube



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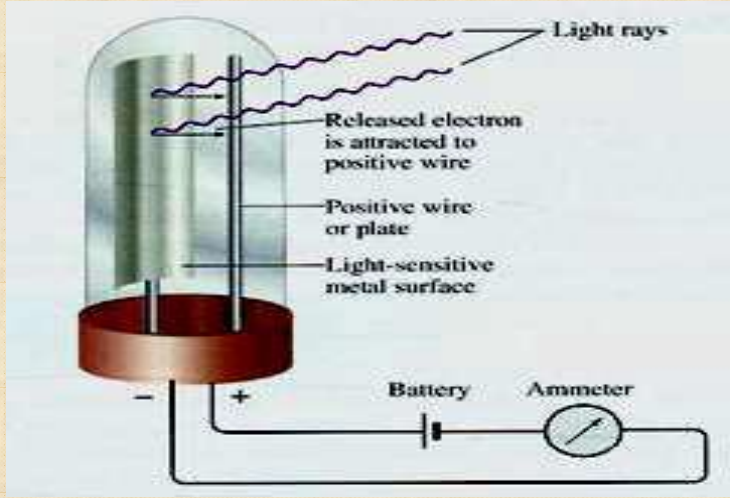

- **Consists of a evacuated glass tube with a photocathode and a collector anode.**
- **The surface of photocathode is coated with a layer of elements like cesium, silver oxide or mixture of them.**
- **When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.**
- **More sensitive compared to barrier layer cell and therefore widely used.**

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3. Photomultiplier tube

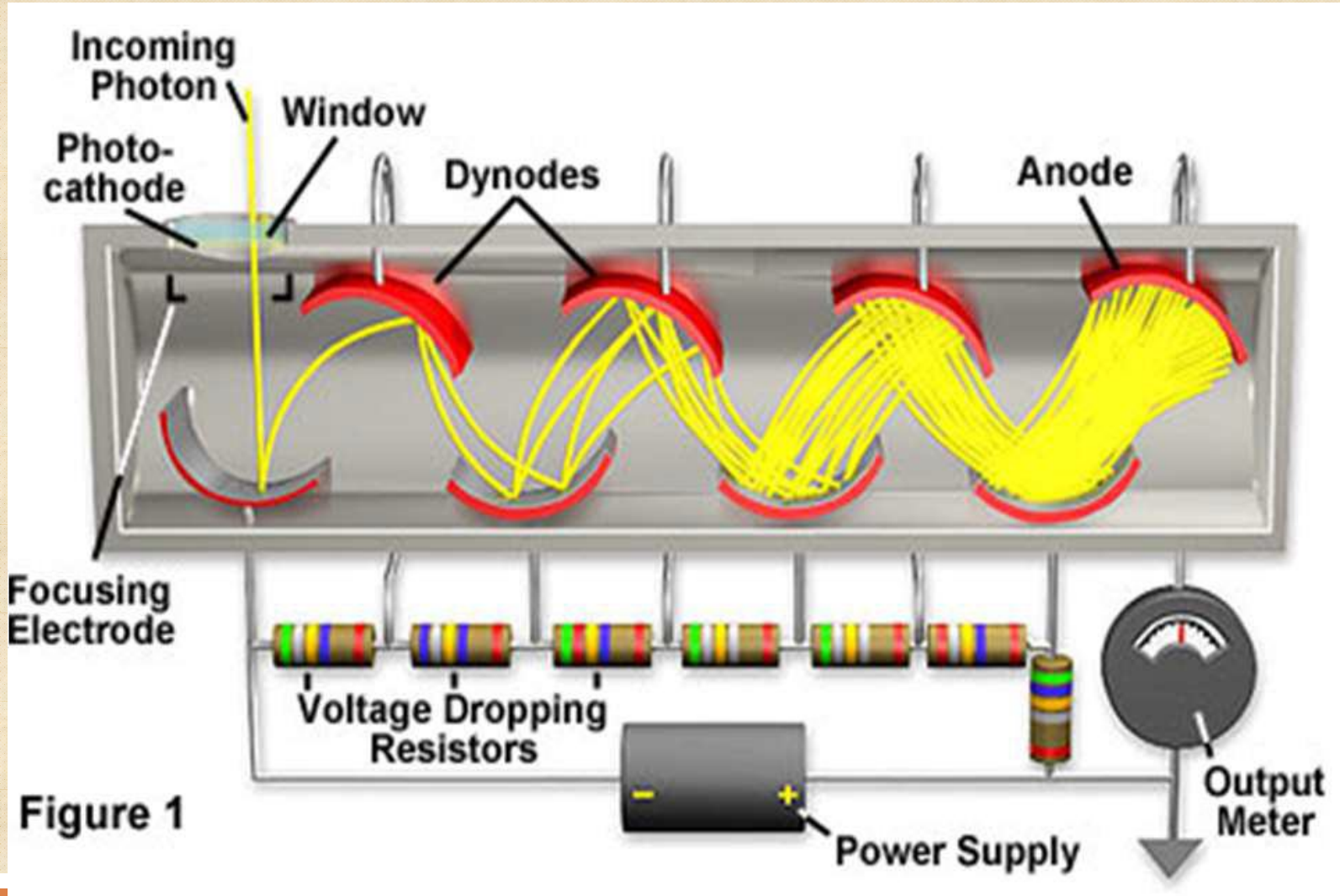

Figure 1

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- **The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.**
- **In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample.**
- **Some eight to ten dynodes are fixed each with increasing potential of 75-100V higher than preceding one.**
- **Near the last dynode is fixed an anode or electron collector electrode.**
- **Photo-multiplier is extremely sensitive to light and is best suited where weaker or low radiation is received**

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Applications of UV-Visible Spectrophotometer

[1] Many substances which do not possess significant extinction co-efficient in the visible region will react quantitatively with some other reagent to give a colored product. This property is used as a basis for assaying such substances. The color (chromospheres) is produced under standard conditions from known quantities of the substances and the extinctions of these samples are measured.

[2] Qualitative Analysis :- Visible and ultraviolet spectra may be used to identify classes of compounds in both the pure state and in biological preparations. E.g. proteins, nucleic acids,cytochromes, chlorophylls. This technique may also be used to indicate chemical structures and intermediates occurring in a system.

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[3] Quantitative Spectrophotometric Analysis :- A number of important classes of biological compounds may be measured semi-quantitatively using ultra-violet and visible spectrophotometers. E.g. proteins at 280 nm and nucleic acids at 260 nm. Corrections may be made for the presence of impurities by also measuring the extinction at wavelengths where the impurities absorb more than the compound under investigation and applying the appropriate algebraic formula.

[4] Enzyme Assays and Kinetic Analysis :- Ultraviolet and visible spectrophotometry provide the most commonly used assays of enzymes and their substrates.

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[5] Difference Spectra :- A different spectrum is the difference between two absorption spectra. It may be obtained indirectly by subtracting one absolute absorption spectrum from another.

[6] Nucleic Acid Structural Studies :- The extinction at 260 nm of double stranded DNA in solution increases (hyperchromicity) when it is heated through its transition temperature due to denaturation of its helical structure. The reverse occurs on renaturation of the DNA by cooling. Thus the effects of pH, temperature and ionic strength on the secondary structure of DNA may be studied.

[7] UV and visible spectrophotometry is also used for the study of protein structures.

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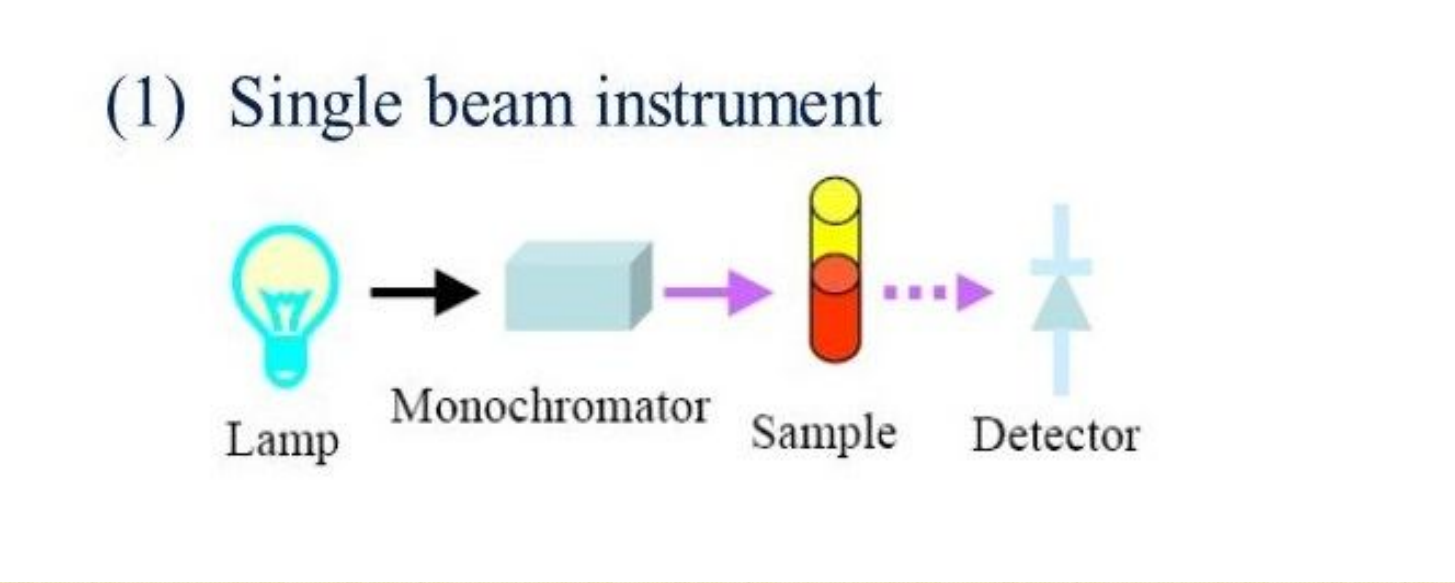
- **Spectrophotometers used here detects the percentage transmittance of light radiation, when light of certain intensity & frequency range is passed through the sample.**
- **Spectrophotometer can be a single beam or double beam optical system.**

1. Single Beam spectrophotometer:



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- 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 color of the solution.
- The sample solution to be analyzed is placed in curettes.
- After passing through the solution, the light strikes the surface of detector (barrier-layer cell or phototube) and produces electrical current.
- The output of current is measured by the deflection of needle of light-spot galvanometer or micro ammeter. This meter is calibrated in terms of transmittance as well as optical density.
- The readings of solution of both standard and unknown are recorded in optical density units after adjusting instrument to a reagent blank.

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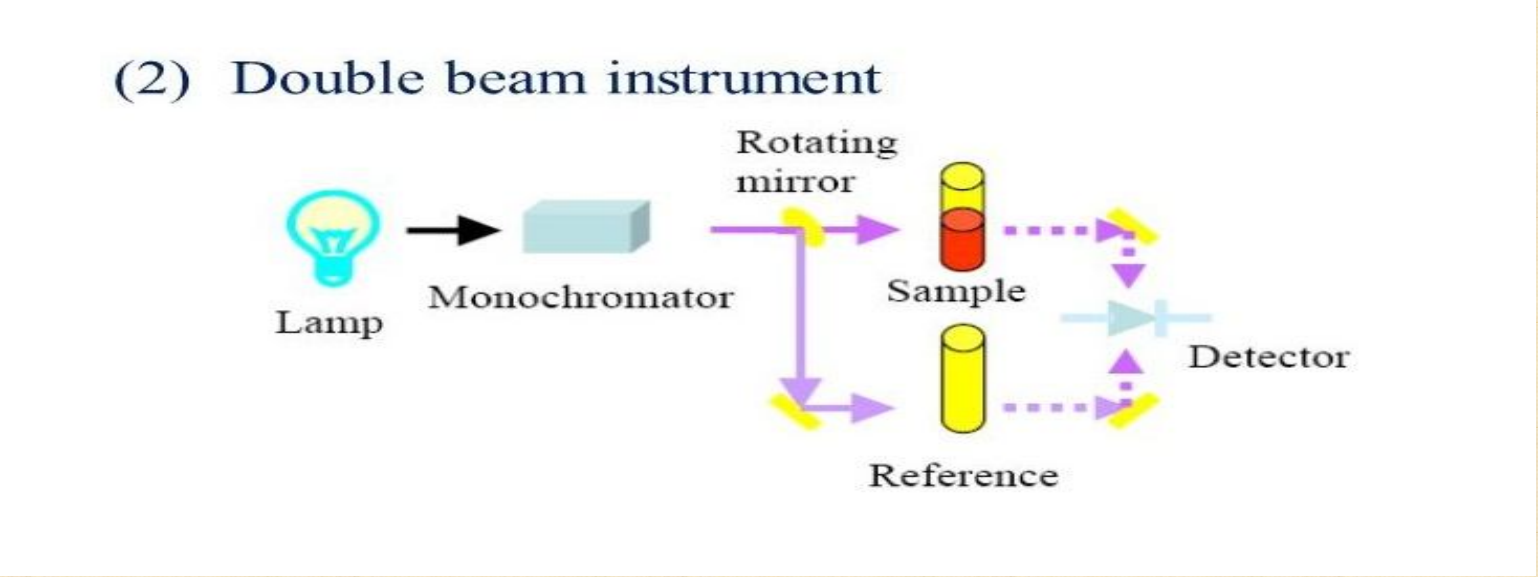
2. Double Beam spectrophotometer:



- **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.**

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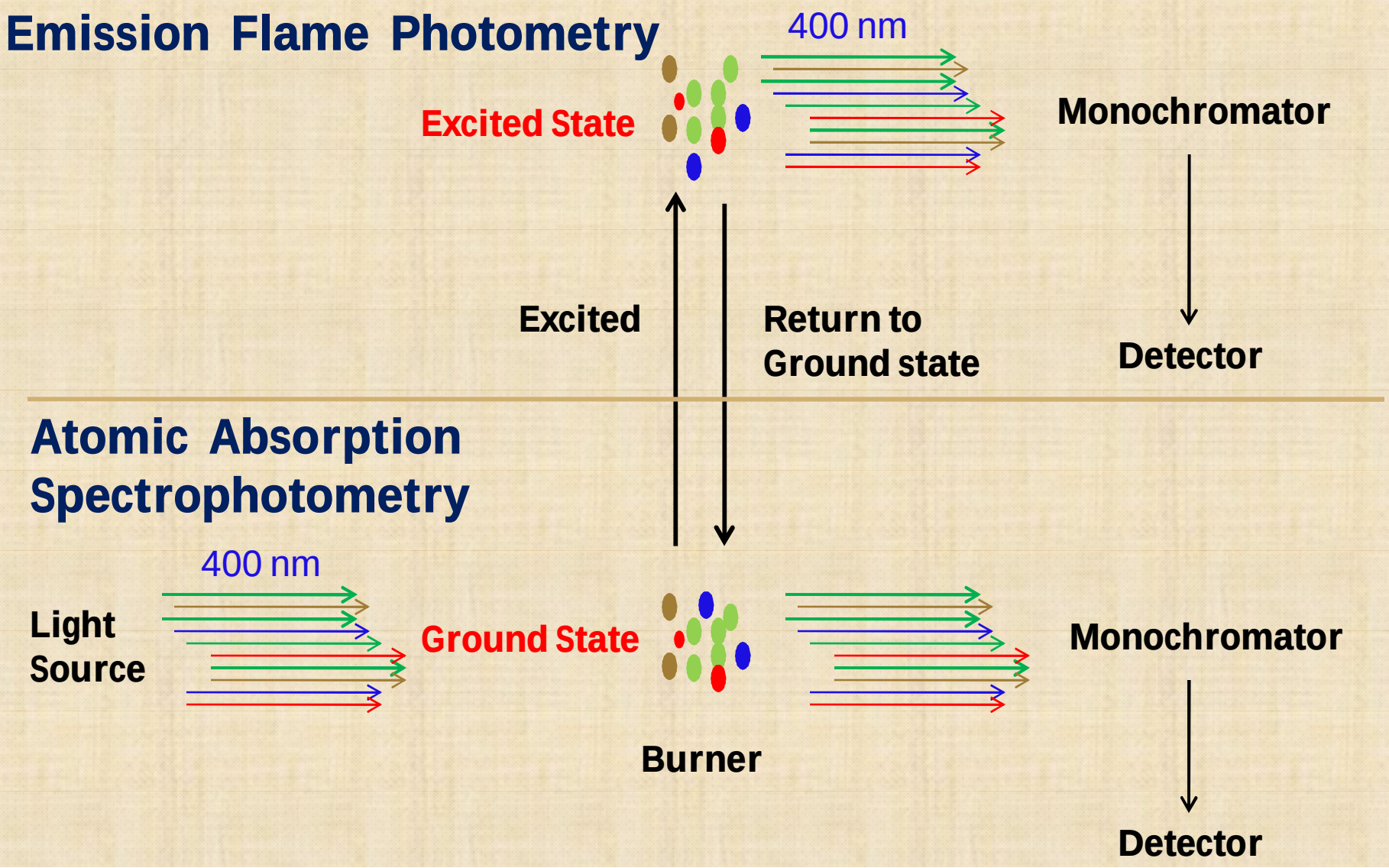
FLAME SPECTROPHOTOMETRY :-



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FLAME SPECTROPHOTOMETRY :-

- **The flame photometric analysis method is more or less similar to that of spectrophotometry with the exception that the place of the sample cell is taken by a flame. consequently, it is the absorption or emission of specific wave-lengths by excited atoms that is studied by this technique.**
- **The optical system and even the photo-detectors used in spectrophotometry and flame spectro-photometry are identical.**
- **The general method of flame photometry can be applied in two complementary ways:**
  [1] Emission Flame Photometry
  [2] Atomic Absorption Spectrophotometry

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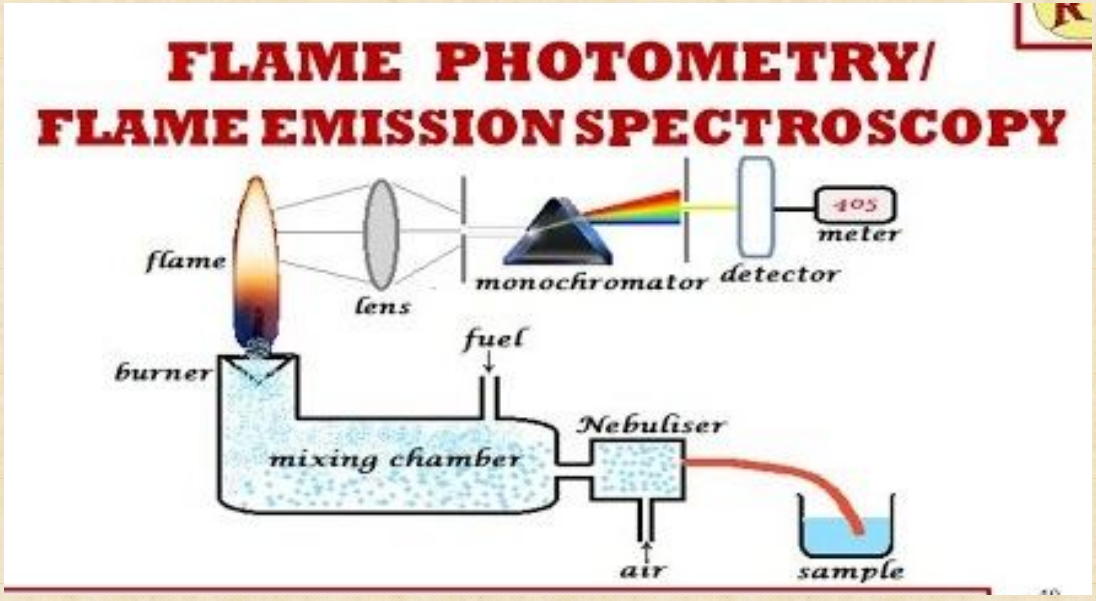

Volatilization of molecules in a flame produces free atoms and then excites them to higher energy levels. The characteristic emission spectrum of the element is produced when the excited atoms return to their ground state. This is the principle of emission flame photometry.

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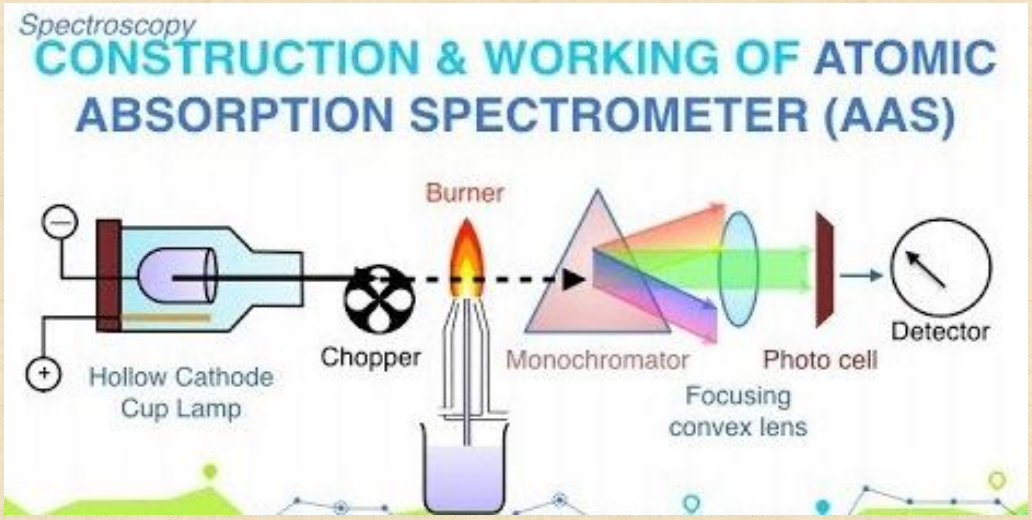

Principle of Atomic absorption spectrophotometry, to measures the absorption of a beam of mono-chromatic light by atoms in a flame.

Moreover, the energy absorbed or emitted is proportional to the number of atoms present in the optical path. Thus, apart from giving the identity of the element(s) present in a sample, flame photometry also provides information about the quantity of the element(s) present.

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Principle, working and applications of Atomic Absorption spectroscopy

Introduction

- Alan Walsh in mid 1950s introduced Atomic absorption spectroscopy.
- Most powerful technique for quantitative determination of trace element in metal liquids.
- Provides a total metal content of the sample and is almost independent of the molecular form of the metal in liquid.
- 60-70% elements including the most common to rare earth metals can be determined in concentration ranging from trace to macro quantities.
- Determination can be made in the presence of many other elements and non only restricted to aqueous solution but also not aqueous solution.
- It is a method of elemental analysis.
- Ideal tool for nonchemist-engineer-biologist.

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Principle:

- The absorption of energy by ground state atoms in the gaseous state forms the basis of atomic absorption spectroscopy.
- When a solution containing metallic species is introduced in to a flame ,vapors of metallic species is obtained. Some metal atoms may be raised their energy level at which it emit characteristics radiation.
- Majority of metal atoms will remain in the ground state or non emitting state. The ground state atoms can absorb light radiation of their own specific resonance λ (same λ that would be emitted if the atoms are excited).
- Thus when a light of this λ is allowed to pass through a flame having atoms of metallic species part of that light will be absorbed and the absorption will be proportional to density of the atoms in the flame.

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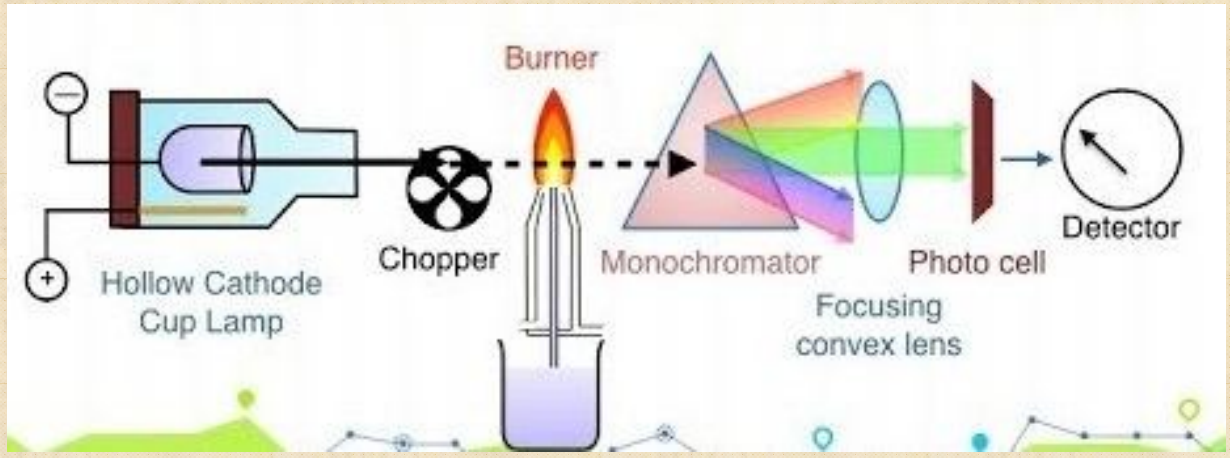
Instrumentation:



1. **Radiation Source**
2. **Chopper**
3. **Burner(Atomizer)**
4. **Monochromator**
5. **Photocell**
6. **Detector**

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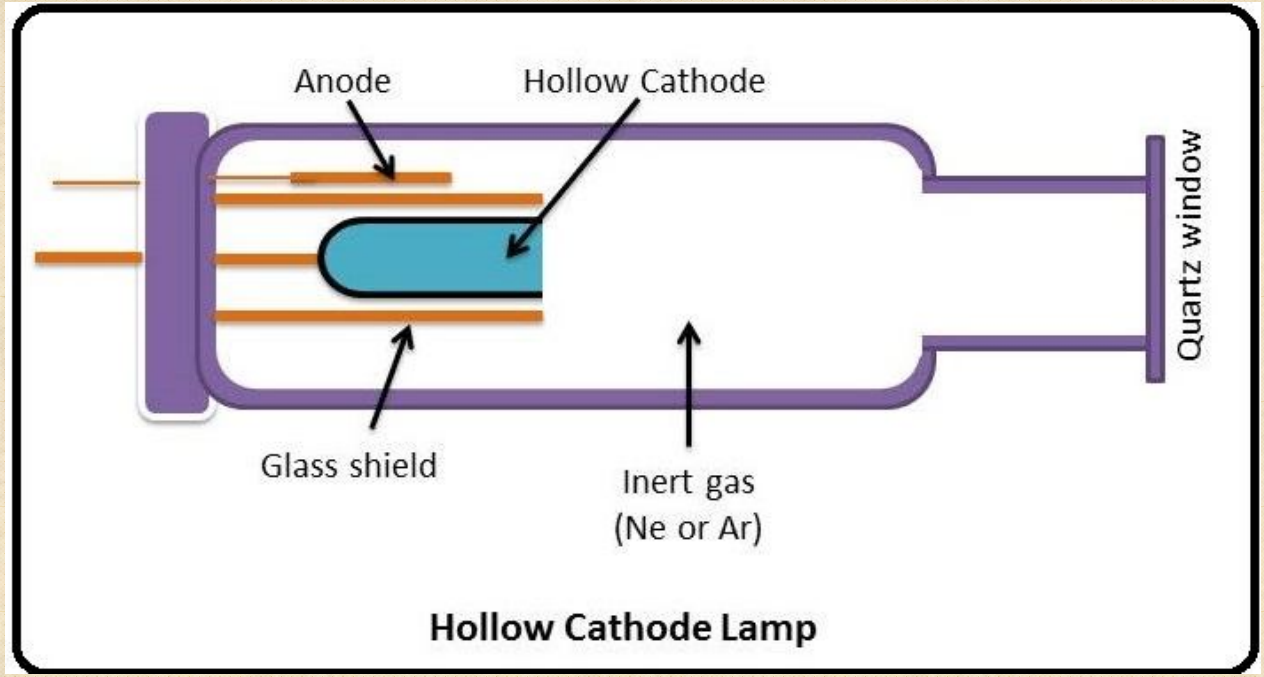
1. Radiation Source

Two basic types of light source are used for atomic absorption.
(a) hollow-cathode lamp (b) Electrodeless discharge lamp

hollow-cathode lamp



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Hollow cathode lamp

- Hollow cathode lamp is more commonly used.
- This is a lamp in which the cathode is coated with the analyte metal of interest.
- Within the lamp, inert filler gas (neon or argon) is ionized by an electric current and these ions are then attracted by the cathode.
- The inert gas ions bombard the cathode and in so doing excite the metal ions coated on it.
- It is this excitation of the metal that produces the emission of radiation with wavelengths characteristic of the analyte.
- Hollow cathode lamps are available for most metallic elements.

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2. Chopper

- It is rotating wheel placed between lamp and flame ,which breaks steady light from the lamp in to, pulsating light which gives pulsating current in the photocell.
- pulsating current is also amplified and recorded.
- Absorption of light is measured without interference from the light emitted by flame.

3. Burner(Atomizer)

- In order to achieve absorption by atom it becomes necessary to reduce the sample to atomic state.
- For this purpose burners are used, which produces flame and convert liquid sample to gaseous state, and gaseous element to atomic vapor.

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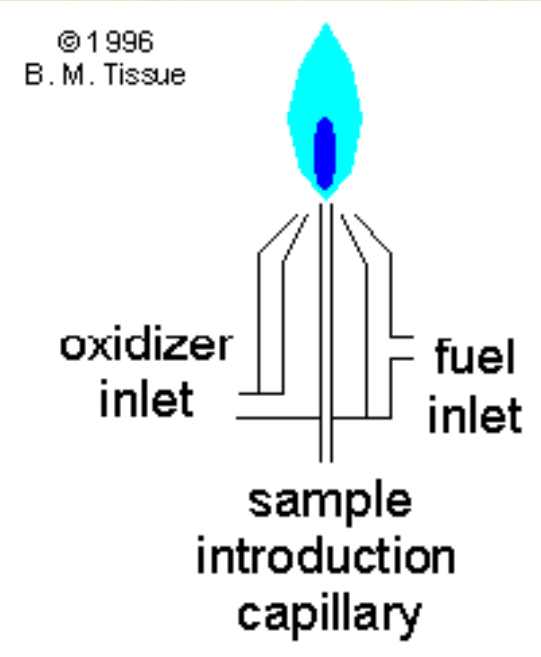
There are two types of burners generally used.

(a) Total consumption burner

(b) Premixed burner

(a)Total consumption burner:



- In total-consumption burner, the fuel (usually acetylene), oxidant (usually air) and sample all meet at the base of flame.
- As the sample containing metallic element to be estimated, is liquid.
- Flame breakup liquid sample in to droplets which are evaporated which is reduced to atoms.
- In these burner O_2 used as oxidants and H_2 or Acetylene as Fuel which gives very hot flame which is very noisy.
- Efficiency of burner is not good.

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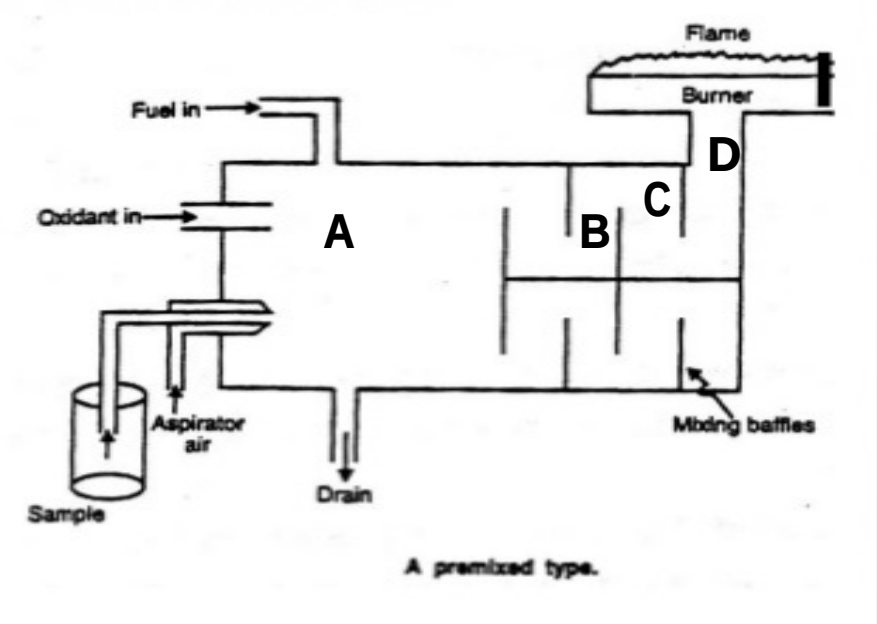
(b) Premixed burner:



A premixed type.

- Here liquid sample and premixed gases (O2+ H2) is Allowed to enter from different inlet, they mixed with each other in region A.
- From region A the unburnt hydrocarbon gaseous mixtures and liquid droplets are allowed to enter region B ,which is a region of free heating.
- In region B liquid is evaporated leaving a residue.
- In this region heating is done by the heat obtained from the region C by convection, conduction & diffusion of radicals in to it, which initiate the combustion.
- After this residue sample is burnt into region C & D to produce atoms. From D region atoms enters into the flame.

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4. Monochromator:

A monochromator produces monochromatic light by removing unwanted wavelengths from the source light beam. The function of the monochromator is to isolate a single atomic resonance line from the spectrum of lines emitted by the hollow cathode lamp.

5. Photocell:

- Photovoltaic cell is widely used in Atomic absorption spectroscopy.
- It has a thin metallic layer coated with silver or gold which act as electrode, also has metal base plate which act as another electrode
- Two layers are separated by semiconductor layer of selenium, when light radiation falls on selenium layer.
- This creates potential diff. between the two electrode and cause flow of current.

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6. Detector (Read out device):

- It is capable of displaying the absorption spectrum as well absorbance at specific wavelength.
- Nowadays the instruments have microprocessor controlled electronics that provides outputs compatible with the printers and computers.
- Thereby minimizing the possibility of operator error in transferring data.

Application:

- Applicable in the field of chemistry, Mineralogy Biochemistry Water supplies, Metallurgy and Soil analysis.
- 60-70 various metals including earth metal can be detected.
- Detect metallic element in biological material.
- In food industry to detect calcium, magnesium, sodium, potassium or toxic metal if present.
- Determination of lead in petroleum products.

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Principle, working and applications of

Infrared spectroscopy

Introduction:

- **Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light**
- **Infrared Spectroscopy is the analysis of infrared light interacting with a molecule.**
- **It is based on absorption spectroscopy.**
- **It is one of the most powerful analytical technique which offers the possibility of chemical identification and used for quantitative analysis.**
- **It provide useful information about structure of the molecule quickly without tiresome evaluation methods.**

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- **IR spectroscopy is concerned with the study of absorption of infrared radiation, which causes vibrational transition in the molecule. Hence, IR spectroscopy also known as Vibrational spectroscopy.**
- **IR spectra mainly used in structure elucidation to determine the functional groups.**
- **Instrument are common, relatively inexpensive and easy to operate.**

IR region: 0.8 μm (800nm) to 1000 μm (1mm)

1. **Near IR: 0.8-2 μm**
2. **Middle IR: 2-15 μm**
3. **Far IR: 15-1000 μm**

Most of the analytical applications are confined to the middle IR region because absorption of organic molecules are high in this region.

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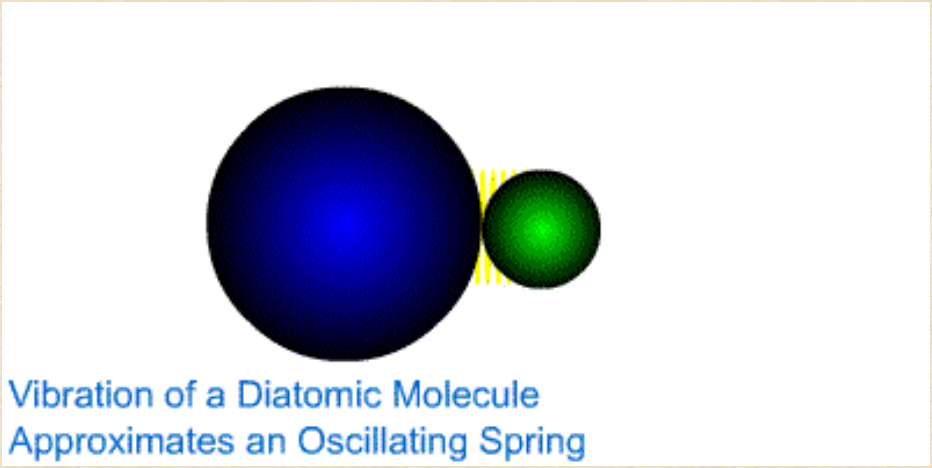
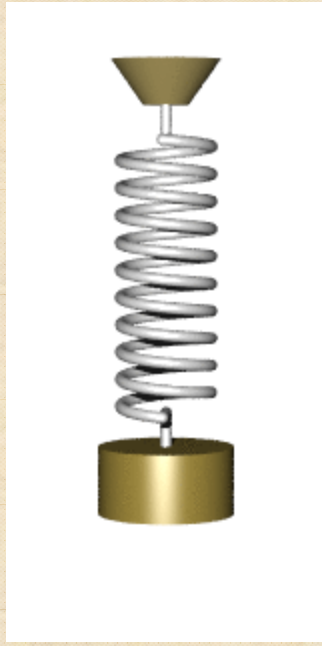
Principle of IR spectroscopy

- **Molecules are made up of atoms linked by chemical bonds. The movement of atoms and the chemical bonds like like spring and balls (vibration)**



Vibration of a Diatomic Molecule
Approximates an Oscillating Spring



- **This characteristic vibration are called Natural frequency of vibration.**

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- When energy in the form of infrared radiation is applied then it causes the vibration between the atoms of the molecules and when,
  Applied infrared frequency = Natural frequency of vibration
  Then, Absorption of IR radiation takes place and a peak is observed
- When a compound is exposed to IR radiation, it selectively absorbs the radiations resulting in vibration of the molecules of the compound, giving rise to closely packed absorption bands, called as IR absorption spectrum
- Different functional groups absorb characteristic frequencies of IR radiation. Hence gives the characteristic peak value.
- Therefore, IR spectrum of a chemical substance is a finger print of a molecule for its identification.

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- The phenomenon which is involved here is the “Bond Vibration”.
- Bond can either stretch or deform(bend),if molecule contains n atoms there will be 3n-6 fundamental vibrations in total. of these 2n-5 cause deformation and n-1 cause streching.

Molecular vibrations

There are 2 types of vibrations:

1. Stretching vibrations
2. Bending vibrations

1. Stretching vibrations:

- Vibration or oscillation along the line of bond
- Change in bond length
- Occurs at higher energy: 4000-1250 cm-1

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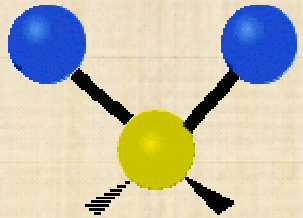
Stretching Vibration are of two types:
(a) Symmetrical stretching
(b) Asymmetrical stretching

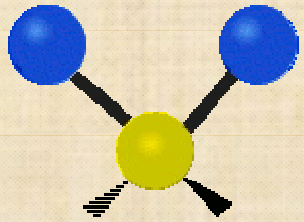
(a) Symmetrical stretching:

- **two bonds increase or decrease in length simultaneously.**



(b) Asymmetrical stretching:

- **in this, one bond length is increased and other is decreased.**



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2. Bending vibrations

- Vibration or oscillation not along the line of bond
- These are also called as deformations
- In this, bond angle is altered
- Occurs at low energy: 1400-666 cm-1
- These are of two types:

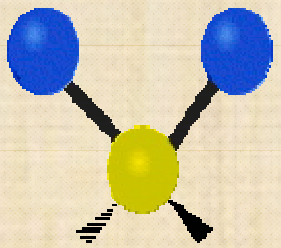
  (a)In plane bending: scissoring, rocking

  (b)Out plane bending: wagging, twisting

(a) In plane bending:

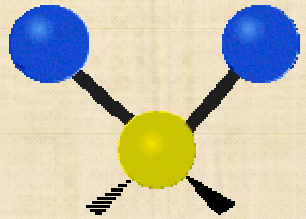
I. Scissoring:

- This is an in plane blending
- Two atoms approach each other
- Bond angles are decrease



II. Rocking:

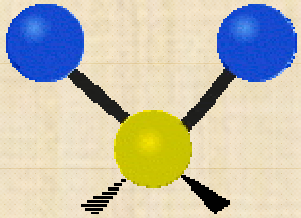
- Movement of atoms take place in the same direction.



(b) Out plane bending

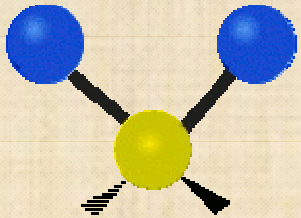
III. Wagging:

- Two atoms move to one side of the plane. They move up and down the plane.



IV. Twisting:

- One atom moves above the plane and another atom moves below the plane.



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Instrumentation of IR SPECTROSCOPY

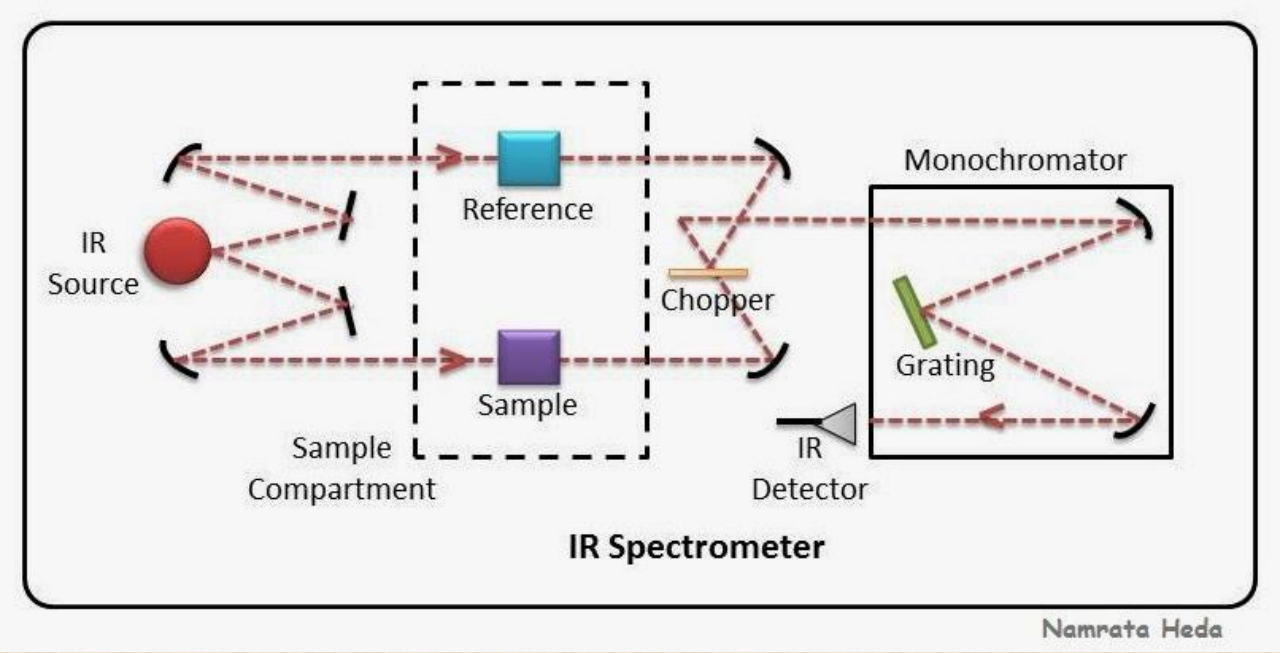
The main parts of IR spectroscopy are as follow:

(1)IR radiation source

(2) Monochromator

(3) Sample cell and sampling of substance

(4) Detector



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(1)IR radiation source:

- The IR spectrometer consists of a source of infrared light, emitting radiation throughout the whole frequency range of the instrument.
- An inert solid is electrically heated to a temperature in the range of 1500-2000K.
- This heated material will then emit IR radiation.

Following are some of the sources:

(a) The Nerst Glower
(b) The Globar source
(c) The Incandescent wire source
(d) Mercury Arc

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(a) The Nerst Glower:

- **It is a hollow rod of about 2mm in diameter and 30mm in length.**

- **It composed of rare earth oxides such as zirconia vttria and thoria. Glower is generally heated to a temperature between 1000 – 1800 ° C.**

Advantage:

- **It emits IR radiation over wide wavelength range,radiation remains steady and constant over long period of time.**

Disadvantage:

- **Frequent mechanical failure and energy concentration in the visible and near infrared region.**

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(b) The Globar source:

- It is a silicon carbide rod which is electrically heated to around 1300 and 1700 ° C.

- It is about 50 mm in length and 4 mm in diameter It strongly emits radiation in IR region.

Advantage:

- Self starting ,positive temperature coefficient and conveniently controlled with variable transformers.

Disadvantage:

- Less intense source than the Nernst Glower

(c) The Incandescent wire source:

- This is a tightly wound coil of nichrome wire, which is electrically heated to 1100K.

- It produces a lower intensity of radiation than the above mentioned Nerst or Globar sources, but it has a longer working life.

(d) Mercury Arc:

- Used in far infrared region.

- Quartz mercury lamps emits radiations at shorter wavelength when they are heated. However longer wavelength radiations can be achieved by mercury plasma through quartz.

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(2) Monochromator:

- Radiation source emits radiations of various frequencies and sample in IR spectroscopy absorbs only at certain frequencies.
- Therefore monochromator are necessary to select desire Frequency from the radiation source and rejecting the Radiation of other frequencies.

Two types of monoschromator are used:

(a)Prism monochromator:

- Used prism as dispersive element.
- Constructed of various metal halide salts, which transmit in the infrared.
- Sodium chloride is most commonly prism salt used.

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(b)Grating monochromator:

- Here prism is replaced by grating.
- Gratings are nothing but rulings made on some materials like glass, quartz or alkyl halides depending upon the instrument.
- In grating for radiation of different wavelength the angle of dispersion is different.

Grating monochromator posses certain advantages over prism monochromator:

(1) In prism monochromator salts are subjected to mechanical & thermal instability or water solubility but grating is not because it is made up of material like aluminum.

(2) Grating monochromator can be used over considerable wavelength range.

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(3) Sample cell and sampling of substance:

- Infrared spectra may be obtained for gases, liquids or solids (neat or in solution)
- Material containing sample must be transparent to the IR radiation. So, the salts like NaCl, KBr are only used.

(a) Sampling of solids: various techniques used for preparing solid samples are as follows:-

(1) Solid run in Solution:

- In this technique, solid sample may be dissolved in a non aqueous solvent.
- Provided that there is no chemical interaction with the solvent and the solvent is not absorbed in the range to be studied.
- A drop of solution is placed on the surface of alkali metal disc and the solvent is allowed to evaporate thin film of the solute.

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(2) Solid film technique:

- If the solid is amorphous in nature.
- The sample is deposited on the surface of a KBr or NaCl cell by evaporation of a solution of the solid.
- This technique is useful for only qualitative analysis.

(3) Mull technique:

- In this technique, the finely crushed sample is mixed with Nujol (mineral oil) to make a thick paste.
- A thin film is applied onto the salt plates.
- This is then mounted in a path of IR beam and the spectrum is recorded.

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(4)Pressed pellet technique:

- In this technique, a small amount of finely ground solid sample is mixed with 100 times its weight of potassium bromide and compressed.
- To form thin transparent pellet(1-2mm thick & 1cm in diameter) using a hydraulic press.
- These pellets are transparent to IR radiation and it is used for analysis.

Advantages:

- Kbr pellets stored for long period of time.
- Resolution of spectrum is superior.
- Concentration can be suitably adjusted in the pellets and so used for quantitative analysis.

Disadvantages:

- Due to high pressure polymorphic changes occurs.
- Not successful for polymers. (difficult to grind with Kbr)

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(b) Sampling of liquids:

- Liquid samples at room temperature are usually put frequently with no preparation into rectangular cells made up of NaCl, KBr or ThBr and their IR spectra can be obtained directly.
- For most liquids, the sample cell thickness is 0.01-0.05 mm

(c) Sampling of gases:

- It is similar to the liquid sample cell.
- The sample cell is made up of NaCl, KBr etc
- A sample cell with a long path length (10 cm – 1m long) is needed because the gases show relatively weak absorbance.
- Gas must not react with the reflecting surfaces or windows.
- Moisture must be avoided.

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(4) Detector:

various types of detector used in IR spectroscopy.

(a)Thermocouples:

- **A thermocouple is made by welding together at each end two wires made from different metals.**
- **If one welded joint (called the hot junction) becomes hotter than the other joint (the cold junction).**
- **In IR spectroscopy, the cold junction is carefully screened in a protective box and kept at a constant temperature.**

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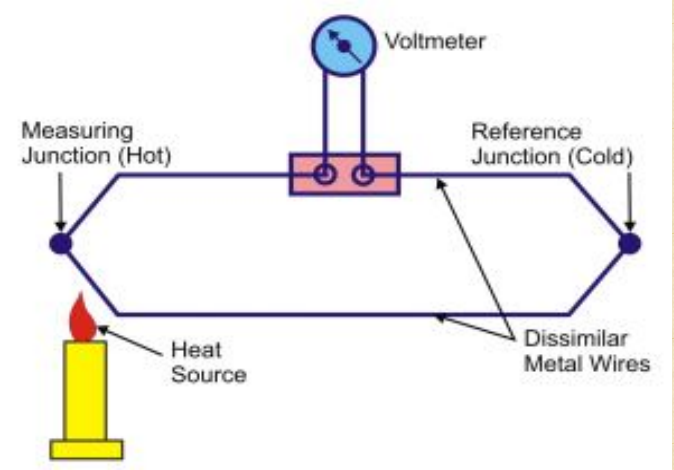
Thermocouples



- **The hot junction is exposed to the IR radiation, which increases the temperature of the junction.**
- **The potential difference generated in the wires is a function of the temperature difference between the junctions and, therefore, of the intensity of IR radiation falling on the hot junction.**
- **Response time ~60m sec.**

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(b) Bolometer:

- A bolometer is a type of resistance thermometer.
- It consist of thin metal conductor.

- A bolometer based upon the fact that the electrical resistance of a metal increases approximately 0-4% for every Celsius degree increases of temperature.

- When IR falls on conductor ,Its temperature changes.
- As the resistance of a metallic conductor changes with temperature ,the degree of change in the resistance is regarded as a measure of the amount of radiation fallen on the bolometer.
- Response time ~4m sec.

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(c)Thermistor :

- It is made up of a fused mixture of metal oxide.
- As the temperature of the mixture increases, its electrical resistance decreases (as opposed to bolometer).
- This relationship between temperature and electrical resistance allows thermistor to be used as IR detector.
- The thermistor typically changes resistance by about 5 % per °C.
- Its response time is also slow.

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APPLICATIONS OF IR SPECTROSCOPY

- Identification of functional groups & structure elucidation of organic compounds.
- Quantitative analysis of a number of organic compounds.
- Study of covalent bonds in molecules.
- Studying the progress of reactions.
- Detection of impurities in a compound.
- Ratio of cis-trans isomers in a mixture of compounds.
- Shape of symmetry of an inorganic molecule.
- Study the presence of water in a sample.
- Measurement of paints and varnishes.

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