

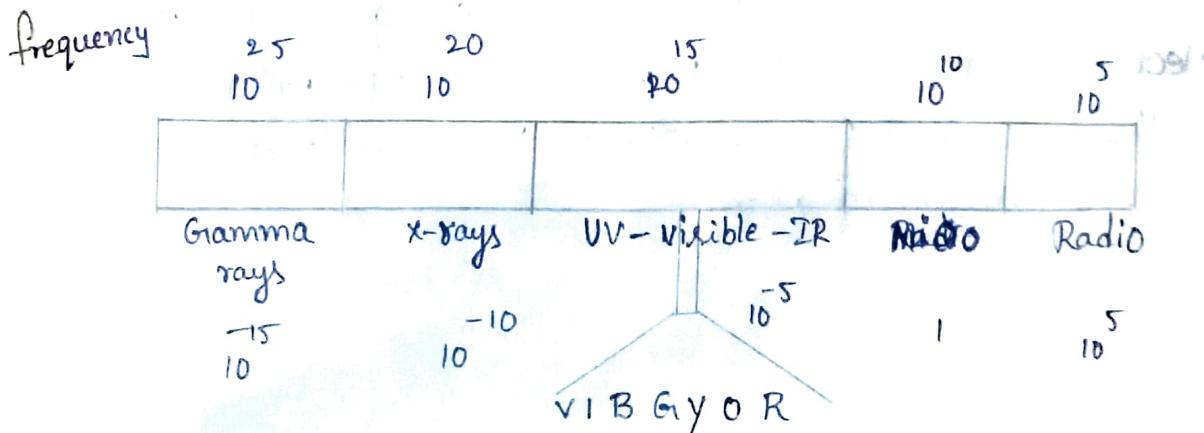
#### 4. Instrumental methods and applications.

##### → Electromagnetic spectrum:

###### Introduction:

Electromagnetic radiation is a form of energy that transmits through space at an enormous velocity. Electromagnetic radiation requires no supporting medium such as air. In contrast to this, other waves, such as sound, do require supporting medium. The most obvious example of electromagnetic radiation is light which occupies only a small region in a spectrum of electromagnetic radiation.

- The entire range over which electromagnetic radiation exists is known as the electromagnetic spectrum.
- The electromagnetic spectrum covers a immense range of wavelength.
- The mercury is inside the quartz - is a source for the IR radiations. Most of the radiations being generated by the hot quartz.
- for near IR, the source is nearest filament, ceramic filament containing rare-earth oxides.
- Tungsten-iodine lamp is used for the visible region spectrum near UV region, deuterium or xenon in quartz
- Microwaves are generated by klystron (a device which is used in radar station).



## Spectroscopy:

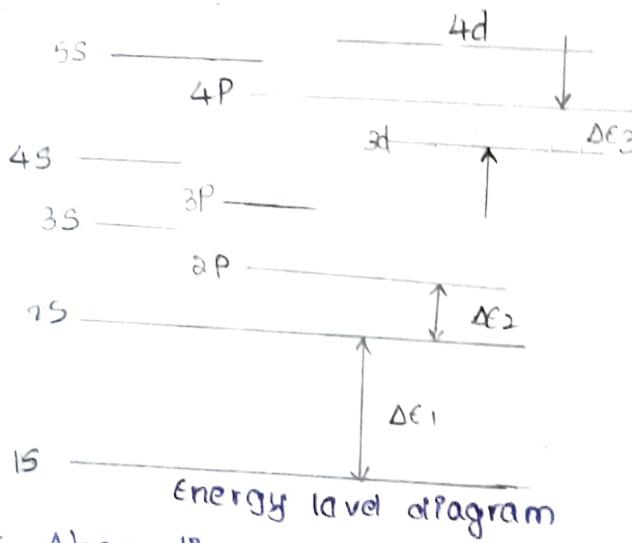
- Spectroscopy is the study of the interaction between matter and electromagnetic radiation.
- After the interaction, there may occur variation in the intensity of electromagnetic radiation with frequency. The instrument which records this variation in the intensity of radiation is known as spectrometer.
- In general, we are observing two types of interaction.
- The sample itself emits radiation and sample absorbs radiation from a source.
- If sample emits radiation, it is known as emission spectrum whereas sample absorbing radiation is known as absorptio spectrum.
- The spectra can be broadly classified into two categories: atomic and molecular spectra.
- Atomic spectra are the spectrum of frequencies of electromagnetic radiation emitted or absorbed during transitions of electrons between energy levels within an atom.
- Molecular spectra arise from the transition of electron between the molecular energy levels.

## Atomic absorption:

- When atoms absorb electromagnetic radiation, it results in an increase in their electronic energy.
- The absorption only occurs if the attacking photon of electromagnetic radiation possesses energy which is equal to the energy difference between two quantized energy levels of absorbing atom.

- If the valence electron moves or jumps from its lower energy to higher energy by the absorption of a photon, it is known as an excited state.
- When the excited atom reverts to the ground state (lower energy) and when it happens for a large number of atoms, the total effects are shown in the light spectrum as a spectral line.
- In the case of polyelectron atom, the multiplicity of absorptions is possible.

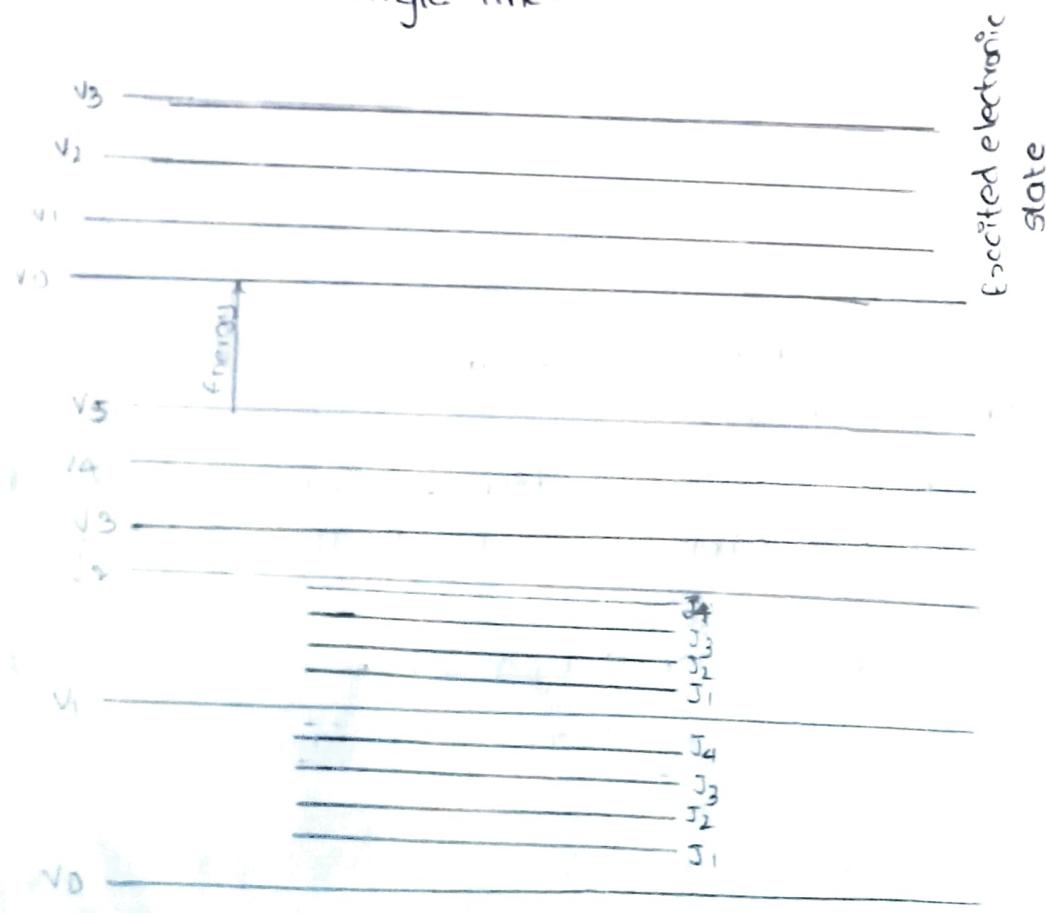
1s → electron transition  $\rightarrow (\Delta E_1) \rightarrow$  x-ray radiations  
 2s → 2p electron transition  $\rightarrow (\Delta E_2) \rightarrow$  far-ultraviolet radiation  
 3d → 4p electron transition  $\rightarrow (\Delta E_3) \rightarrow$  visible radiation.



### Molecular Absorption:-

- When a molecule absorbs electron electromagnetic radiation, it gets excited and when it returns to the ground state, it emits bands.
- When each band is observed by a high resolving power apparatus each band is seen to be a compound large number of very fine lines.
- The total energy state of a molecule includes electronic vibrational and rotational components. All these energies are required for pure rotational transitions.

- There is a small energy gap observed between the rotational transitions, therefore, less energetic photons are required.
- But more energetic photons are required for vibrational transition because of the large energy gap between existing in the molecule.
- When the molecule absorbs photons from electromagnetic radiation in the region of visible light and ultraviolet radiation, electronic energy of photons increases, resulting in the electrons to move out the new orbits of higher energy.
- A given electronic change is also accompanied by a vibration or rotational change.
- These changes introduce 'fine structure' into spectrum so that the molecular spectrum involves a band of wavelength rather than a single line.



Beer's Lambert's Law : The absorption of light by any material explained by two laws namely Lambert's and Beer's Law



If  $I_0$  is Incident light passing through the material  
If  $I$  is transmitted light from the material  
Absorption ( $A$ ) =  $\frac{I_0 - I}{I_0}$ ; Transmission ( $T$ ) =  $\frac{I}{I_0}$   
If the sample is non-absorbing sample, Incident light completely transmitted

$$\therefore I = I_0$$

$$A = 0\%$$

$$T = 100\%$$

i) If the sample is absorbing sample,  $I_0$  light is absorbed passing through the medium, some of the light is absorbed by the sample remaining light is transmitted

The absorption is calculated by using the formula

$$A = \log_{10}\left(\frac{I_0}{I}\right) \quad \therefore T = \frac{I}{I_0}$$

$$A = \log_{10}\left(\frac{I_0}{I}\right) \Rightarrow A = \log_{10}\left(\frac{I_0}{I}\right)$$

$A$  = absorption

$I_0$  = incident light

$I$  = Transmitted light

Lambert's Law :

when a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity of light is directly proportional to the thickness (length) of the medium

(or)

The absorption of light by the sample is directly proportional to the length of medium

$$A = \log_{10} \frac{I_0}{I} \propto l$$

$$A = \epsilon l$$

A = absorption

$\epsilon$  = molar extinction coefficient  
 $l$  = length of the medium or thickness of medium

Beer's Law :- When a beam of light is allowed to pass through a transparent medium, the rate of absorption is directly proportional to the concentration of sample  
(or)

When the intensity of incident light decreases exponentially concentration of absorbing medium increases and thus

$$A = \log_{10} \frac{I_0}{I} \propto C$$

$$[A = \epsilon C]$$

C = concentration of sample

→ combination of Lambert's - beer's law

$$A = \epsilon l$$

$$A = \epsilon C$$

$$\therefore A = \epsilon l C$$

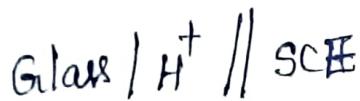
## pH meter :

- The term pH was introduced by Sorenson.
- The pH scale is a series of numbers. These numbers express the degree of acidity or alkalinity of solution.
- It is the negative logarithm of hydrogen ion concentration expressed in molarity.

$$pH = -\log [H^+]$$

## principle :

- A glass electrode is used to determine pH. The electrode consists glass membrane which separates two solutions varying in pH.
- The glass bulb is filled with normal hydrochloric acid and saturated with quinhydrone.
- The Ag wire dipping in the electrolytic passes out of the glass tube.
- The glass bulb is kept in the solution of which pH is to be measured which is contained in the beaker. The potential differences are measured against a standard calomel electrode.



where SCE is standard calomel electrode.

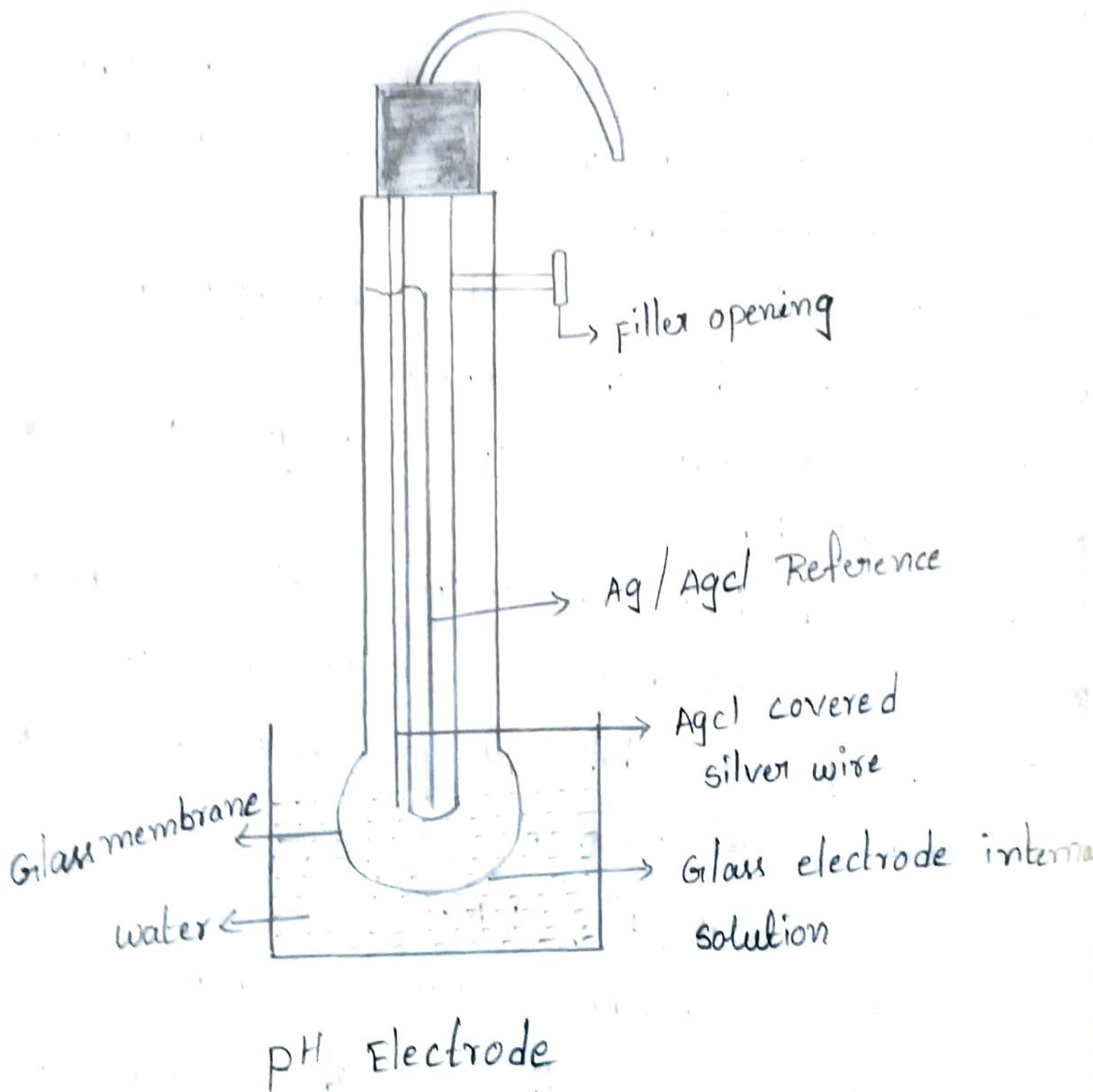
- The relation by which the emf of a suitable pH measuring electrode is related to the hydrogen ion

concentration based on Nernst equation.

$$E = E^{\circ} - \frac{2.303 RT}{nF} \log [H^+]$$

$$E = E^{\circ} + \frac{2.303 RT}{nF} \text{ pH}$$

$$E = E^{\circ} + 0.0591 \text{ pH}$$



- The standard value of  $E^\circ$  is evaluated by calibrating the apparatus with the help of a buffer solution of known pH value.
- The potential of standard calomel electrode is known, that of the glass electrode can easily be calculated from the assumed emf of the complete cell and the values of pH evaluated with the help of the above relationship.

### Applications :

- the measurement of pH reflects the effective concentration and activity of  $H^+$  and other ions in solution.
- these meters find major application to correct the hypochlorite concentration for an oxidation - Reduction potential (ORP).

Water treatment plants, micro-electronic laboratories and pharmaceutical laboratories are in constant need of pH level monitoring and control of their very accurate and precise applications.

In the agricultural field, it is used to determine the soil pH.

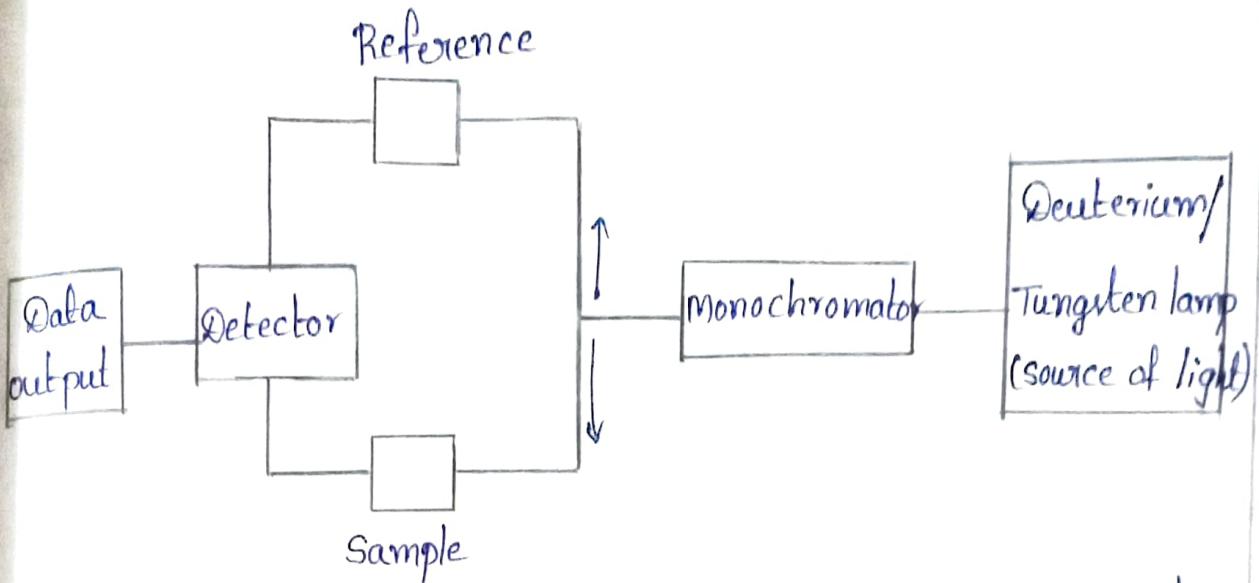
- The cosmetic products like shampoo and soap testing with pH meter to know the acidic nature or basic nature by following proper procedure.
- It is also used in the chemical and food industry, manufacturers of paints and textile industry.
- pH meter is used in almost all chemical laboratories.

### spectroscopy

The study of interaction between the matter and electromagnetic radiation.

#### UV-visible spectroscopy :

- The absorption of electromagnetic radiation by the substance in the visible and ultraviolet region.
- visible light are energetic enough to promote outer electrons from lower energy to higher energy orbital.
- The UV-visible Spectroscopy is usually applied to organic molecules or inorganic ions or complexes in solution.



A block diagram of double beam UV-visible spectrophotometer.

### Instrumentation :

- There are two types of instrumentation for these techniques as a single beam and double beam spectrophotometers.
- In single beam spectrophotometer, we can measure only either sample or reference but the double beam spectrometer measures reference and sample both.
- The uv-visible spectrometer is made up of the following components.

- (i) Source      (ii) mono chromator      (iii) sample holder
- (iv) Detector

### Source of UV-radiation :

The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum.



Both deuterium and hydrogen lamp emit radiation in the range of 160-375 nm. In the UV region, the sample must be taken in quartz cuvettes because the glass cuvettes absorb wavelength up to 340 nm.

#### Source of visible radiation:-

For the visible region, a tungsten filament lamp is used and produced wavelength range of 350-~~500~~<sup>900</sup> nm.

#### Monochromator :-

The monochromators contain different components such as an entrance slit, a collimating lens, a dispersing device, focusing lens and exit slit.

Poly chromatic radiation enters the monochromator through the entrance slit.

The beam is collimated and then strikes the dispersing elements in the dispersing device, and focus the wavelength. Radiation of only a particular wavelength leaves the monochromator through the exit slit.

#### Sample holders or sample cells:-

The cell holding the sample should be transparent to the wavelength region to be recorded.

Quartz or fused silica cuvettes are required for spectroscopy in the UV region.

The thickness of the cell is generally 1 cm. The shape of the cells may be rectangular or cylindrical with flat ends.

#### Detectors:-

Detectors are detect the radiation of transmitted radiation.

There are three types of photosensitive devices are used,

(a) A photovoltaic cell or barrier-layer cell.

(b) photo tubes

(c) photo multiplier

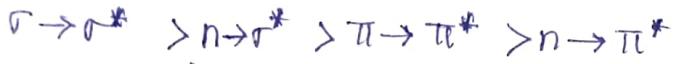
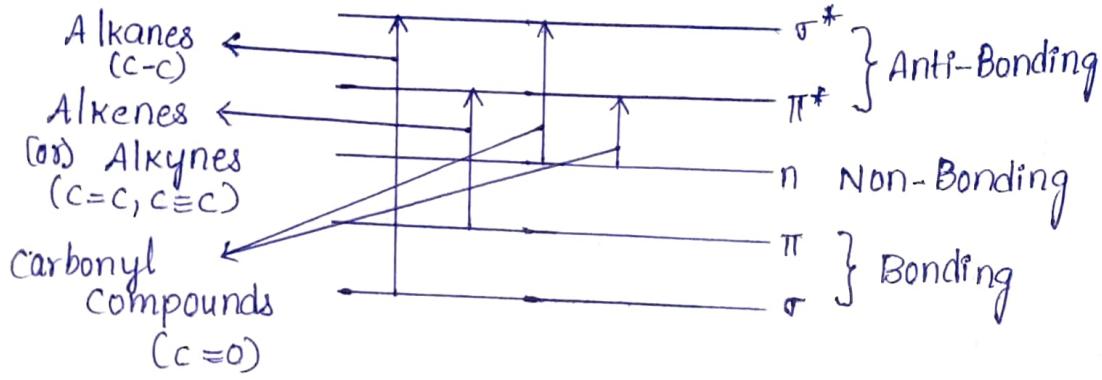
- The photo multiplier tube is a commonly used detector in UV-visible spectroscopy. It consists of anode, cathode and several dynodes.
- Dynodes emit several electrons for each electron striking.

Mechanism: —

Absorption of radiation in this region causes transition between electronic levels. So this is also known as Electronic Spectroscopy. Three types of electronic transitions observed in the molecule.

They are:-

- (1) The transition involving bonding, antibonding and non-bonding electrons ( $p, s$  and  $n e^-$ ).
  - (2) The transition involving charged-transfer electrons.
  - (3) The transition involving  $d$  and  $f$  electrons.
- (1) Transition involving  $p, s$  and  $n$  electrons: —



(Highest energy)

(lower wavelength)

(lowest energy)

(highest wavelength)

- (a)  $\sigma \rightarrow \sigma^*$  : An electron in a bonding  $\sigma$  orbital of a molecule is excited to the corresponding  $\sigma^*$  orbital by absorption of radiation.

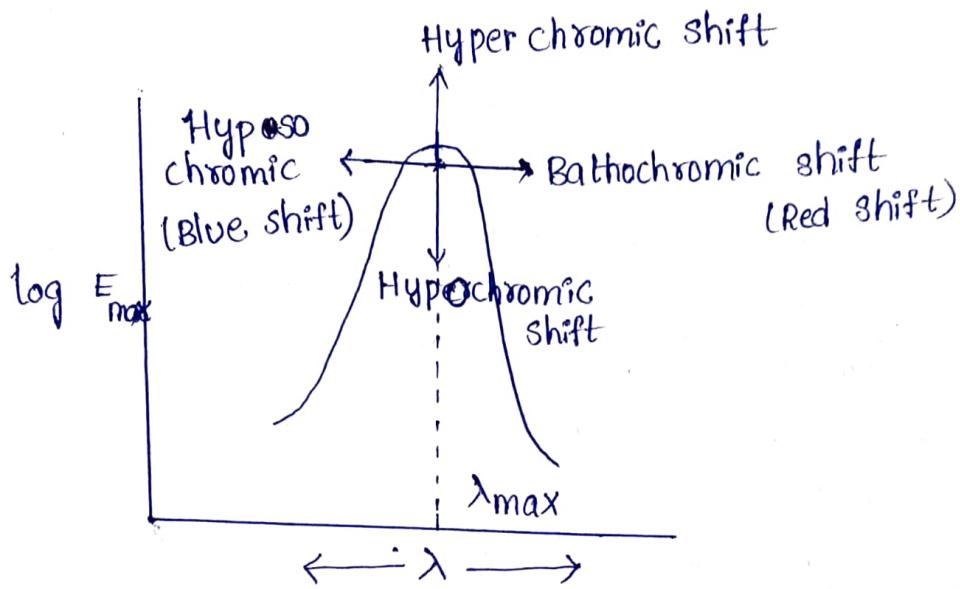
Ex:—  $CH_4$  etc.

- (b)  $n \rightarrow \sigma^*$  : Saturated atoms containing unsaturated (non-bonding electron) electron pair undergo  $n \rightarrow \sigma^*$  transition.

Ex:—  $H_2O, C_2H_5OH$  etc.

$n \rightarrow \pi^*$ ,  $\pi \rightarrow \pi^*$  :- Most organic compounds undergo <sup>6</sup> this transition.

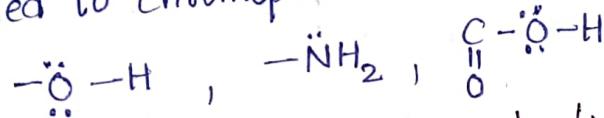
Electronic Spectra is a plot of Transmittance vs wavelength



Increasing the wavelength is Bathochromic shift, decreasing wavelength is Hypsochromic shift.

chromophores :- unsaturated groups are chromophores  $C=C$ ,  $C=O$

auxochromes :- A saturated group containing non-bonding  $e^-$  attached to chromophores.



) Transition involving charged - transfer electrons:

→ Many inorganic species show charge transfer absorption

and are called charge transfer complexes.

→ electrons transfer from acceptor to donor. donor to acceptor

e) Transition involving d and f electrons:

In this transition, the electron is promoted from lower energy to higher energy by absorption of photons in metal complexes.

Disadvantage :-

The major disadvantage of uv-visible Spectroscopy is no single lamp emits all the light wavelengths necessary for analysis. For example, a deuterium lamp emits wavelengths from 180 nm to 370 nm,

and a tungsten lamp emits wavelengths from 315 nm to 2500 nm. Changing the lamp is a time-consuming process.

### Applications:-

- It is the most widely used technique for quantitative analysis, for this Beer-Lambert law is applied.
- The UV-vis Spectroscopy is used to analyse food colouring, also used to test the quality of wine.
- UV-vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, biological macromolecules.
- UV-vis can be applied to determine the kinetics or rate constants of a chemical reaction. For example, mercury dithizonate.
- The UV-vis spectrometry plays a very important role in life sciences. It is used in DNA/protein estimation, kinetics and high-level multi-component quantification.
- In kinetics mode measures, absorbance changes as function of time and gets the enzymatic activity values.
- A UV-vis spectrophotometer may be used as a detector for HPLC.
- In cement industries, determine the metal ions such as Fe, Mn, etc.

### INFRARED SPECTROSCOPY (IR SPECTROSCOPY)

The term infrared covers the range of electromagnetic spectrum between 0.78 and 1000 nm. In infrared spectroscopy, the wavelength is measured in "wave numbers" which have the unit  $\text{cm}^{-1}$ .

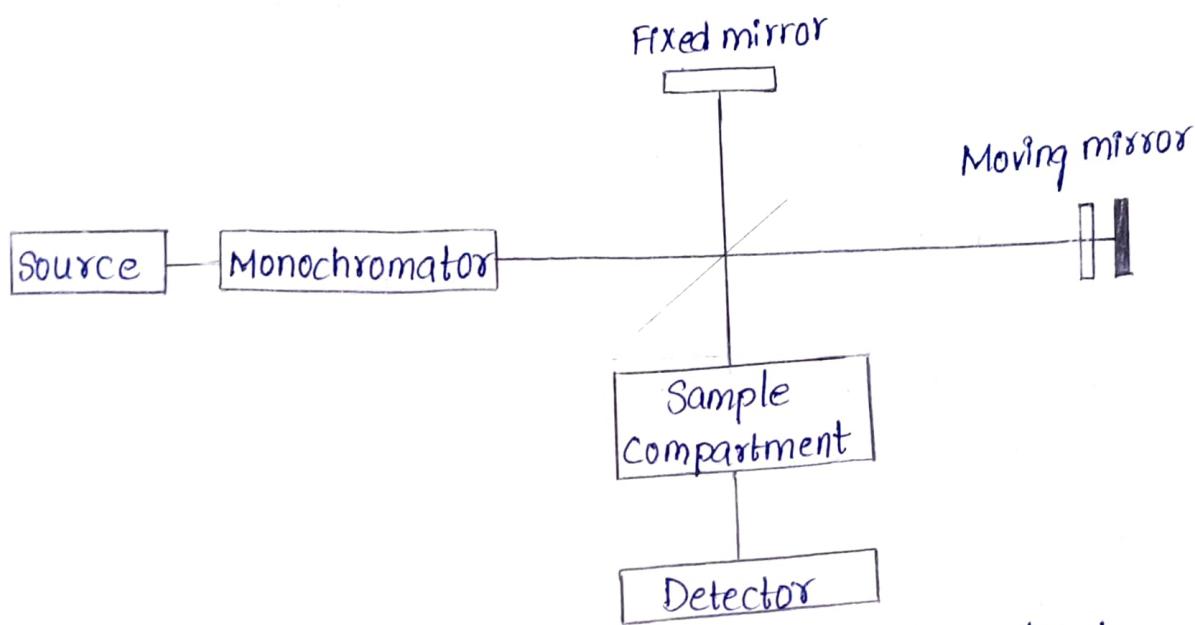
$$\text{Wave number} = 1/\text{wavelength (in cm)}$$

IR is mainly divided into three sections such as near, middle and far IR.

Region	Wavelength range ( $\text{cm}^{-1}$ )
Near	12800 - 4000
Middle	4000 - 200
Far	200 - 10

## Instrumentations:-

A double beam IR spectrometer consists of different components namely source, Monochromator, and Detector.



Source: IR instruments require a source of radiant energy which must be steady, intense enough for detection and extend over the desired wavelengths of various sources of IR radiations such as Nernst glower, Globex sources, tungsten lamp, mercury arc and nichrome halides.

Monochromators: Various types of monochromators are prisms, gratings and filters. Prisms are made of potassium bromide (KBr), sodium chloride (NaCl) or cesium Iodide (CsI); filters are made up of lithium fluoride.

Sample cell: A variety of sample cells are available for IR region. The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz & silica Cuvettes are used in IR Spectroscopy.

Detectors: Detectors are used to measure the intensity of unabsorbed infrared radiation. Detectors like thermo-couples, bolometers and pyroelectric detectors.

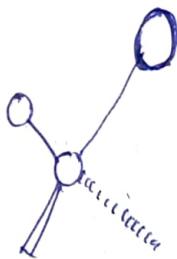
## Mechanism :

- IR spectroscopy is concerned with the study of absorption of infrared radiation, which causes a vibrational transition in the molecule. Therefore IR spectroscopy is also known as vibrational spectroscopy.
- The vibrational energy of molecule changes when the molecule absorb electromagnetic radiation in the infrared region of the spectrum.
- All the molecules have vibrations in the form of stretching, bending etc. It is useful for identification of functional groups.
- Molecular Vibrations and Rotations :
- Rotational levels are quantized and absorption of IR by gases yield line spectra, in liquids or solids, these lines broaden into a continuum due to molecular collision and other interactions.
- The positions of atoms in a molecule are not fixed, if they absorb IR radiation they change vibrations.

### ii) Stretching :



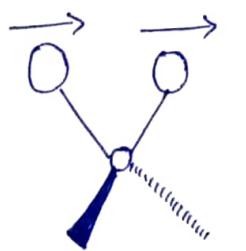
symmetry



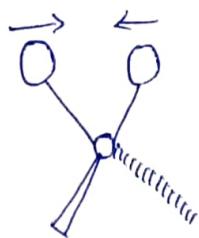
Asymmetric

- Change in the inter-atomic (Bond length) distance along the bond axis.

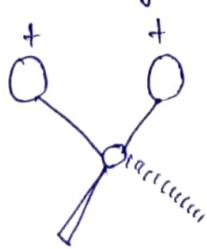
Bending : change in the angle between two bonds, there are four types of bends.



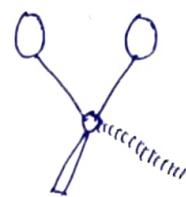
Rocking



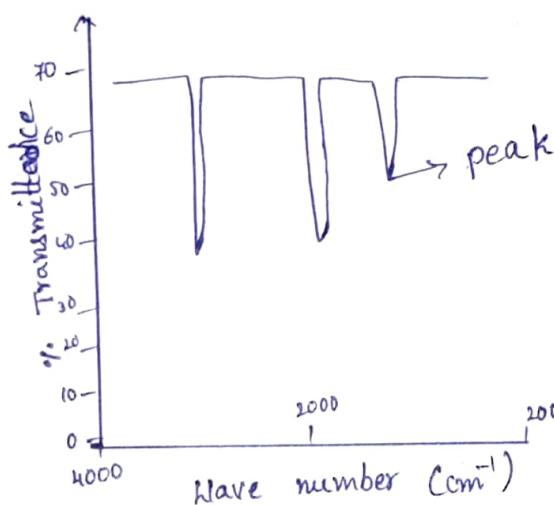
scissoring



wagging



Twisting



IR spectra is a plot plot of % transmittance vs frequency in wave no. ( $\text{cm}^{-1}$ )

$$T = \frac{I}{I_0}$$

IR spectroscopy is very useful for identifying the various functional groups present in molecule.

Alkanes  $\rightarrow$  C-C stretching  $\bar{\nu} \sim 1000 - 1200 \text{ cm}^{-1}$

C-H stretch  $\bar{\nu} \sim 2900 \text{ cm}^{-1}$

Alkenes  $\rightarrow$  C=C stretching  $\bar{\nu} \sim 1600 - 1680 \text{ cm}^{-1}$

Terminal alkenes  $\swarrow\searrow =$  C-H stretch  $\sim 3000 - 3100 \text{ cm}^{-1}$

Alkynes  $\rightarrow$  C≡C stretching  $\bar{\nu} \sim 2100 - 2200 \text{ cm}^{-1}$

Terminal alkynes  $\swarrow\searrow\equiv =$  C-H stretch  $\sim 3100 - 3300 \text{ cm}^{-1}$

Aliphatic aldehyde  $- R-\overset{\text{H}}{\underset{\parallel}{\text{C}}}-\text{H} \sim 1700 \text{ cm}^{-1}$

Ketones  $- \begin{array}{c} \text{R}_1 \\ | \\ \text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{R}_2 \end{array} \sim 1680 - 1700 \text{ cm}^{-1}$

Aldehyde  $- \text{C}-\text{H} \text{ stretch} \sim 2700 - 2800 \text{ cm}^{-1}$

Amide  $- \text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{NH}_2 - \text{C}=\text{O} \text{ stretch} \sim 1630 - 1690 \text{ cm}^{-1}$

Ester  $- \text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{OR} \rightarrow \text{C}=\text{O} \text{ stretch} \sim 1720 - 1750 \text{ cm}^{-1}$

Anhydride  $- \text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{O}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{R} - 1740 - 1790 \text{ and } 1800 - 1850 \text{ cm}^{-1}$

Acid chloride  $- \text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{Cl} - 1700 - 1815 \text{ cm}^{-1}$

$\bar{\nu}$  withdrawing group increases  $\bar{\nu}$

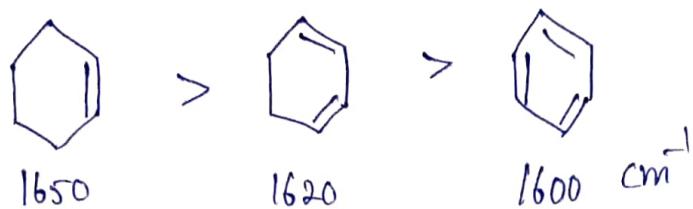
$\bar{\nu}$  donating group decreases  $\bar{\nu}$

Aromatic compounds

C=C stretch  $\bar{\nu} \sim 1600 \text{ cm}^{-1}$

= C-H stretch  $\bar{\nu} \sim 3000 - 3100 \text{ cm}^{-1}$

- conjugation increases  $\nu$  decreases.



### Disadvantage:

- The disadvantage arises from the fact that if noise occurs in one part of the radiation from the IR source, it will spread throughout the spectrum.

### Application:

- The identification and structural analysis of organic compounds such as natural products, polymers and synthesized products.
- It is used to find the food contaminants in food items.
- In biology it is used to find bacterial and fungal identification.
- It is used in pharmaceutical drugs in pharmaceutical companies.
- In agriculture fields, it is used to detect the humic acid or humus in soil.
- Analysis of petroleum hydrocarbons, oils and grease contents.
- Determine the atmospheric pollutants from the atmosphere itself.
- Bio-medical studies of human hair to identify disease states.

## Solid-Liquid chromatography:

Liquid mixtures can be separated using a variety of techniques. Chromatography involves solvents separation on a solid medium.

## Chromatography:

It is a separation technique employed to separate the different components in a liquid mixture. Russian scientist Michael Tswett introduced it. Chromatography involves the sample being dissolved in a particular solvent called mobile phase. The mobile phase may be a gas or liquid. The mobile phase is then passed through another phase called the stationary phase. The stationary phase may be a solid packed in a glass plate or piece of chromatography paper.

The various constituent / components in the mixture travel at different speeds, causing them to separate. There are different types of chromatographic techniques such as column chromatography, TLC, paper chromatography, liquid chromatography and gas chromatography.

## TLC (Thin Layer chromatography) :

Thin Layer chromatography is one of the important chromatographic techniques. where in silica coated glass plate is used as the stationary phase and a liquid solvent as the mobile phase (acetone & Ether). In TLC, the sample is placed in the form of a spot on the silica coated glass plate and the glass plate is carefully dipped into a solvent containing in the TLC chamber.

- The solvent rises up the glass plate due to capillary action and the components of the mixture rise up at different rates and thus are separated from one another.
- If the molecular weight of the constituent is less, it can travel in the glass plate for more distance then the molecular weight of the constituent at higher, it travel in the glass plate for less distance.
- The mobility or separation of the components in glass thin layer chromatography is identified in the form of spots.

## Applications :

- To separate drugs from the blood
- To separate pigments from natural colours.

- To separate colors in a dye.
- To separate pigments in the leaf.

### Retention Time:

Retention time is the amount of time a compound spends on the column after it has been injected. If a sample containing several compounds, each compound in the sample will spend a different amount of time on the column according to its chemical composition. i.e. each will have a different retention time. Retention times are usually quoted in units of seconds or minutes.

$$\text{Retention time (R}_f\text{)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}} \times 100$$

### a) finger print region :

The region between  $400 \text{ cm}^{-1}$  and  $1500 \text{ cm}^{-1}$  in an IR spectrum is known as the fingerprint region. It usually contains a large number of peaks, making it difficult to identify individual peaks.

