SRI CHANDRASEKHARENDRA SARASWATHI VISWA MAHAVIDYALAYA
(University U/S 3 of UGC Act 1956)
Accredited with "A" Grade by NAAC
ENATHUR, KANCHIPURAM - 631561

Course Material

SUBJECT	**:**	**ANALYTICAL INSTRUMENTATION**
BRANCH	**:**	**EIE/MECHATRONICS**
YEAR/SEM	**:**	**THIRD/FIFTH**

Prepared by

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PROGRAMME: B.E. BRANCH: Electronics and Instrumentation / Mechatronics Engineering

Semester	Subject Code	Subject Name	Total Contact Hours	Weekly Hours			Credit
				L	T	P	
VI	**BEIF185EA0/ BMTF185T20**	**Analytical Instrumentation**	**45**	**3**	**0**	**0**	**3**

(For Students admitted from 2018 onwards)

Prerequisite: Sensors & Actuators and Industrial Instrumentation.

Course Objectives

- To understand various techniques and methods of different types of Spectrometer.
- To understand the working Principle and application of Chromatography.
- To study important methods of analysis of industrial gases.
- To understand the important radio chemical methods of analysis.
- To impart knowledge on NMR and Mass spectrometry.

UNIT- I SPECTROPHOTOMETRY

Spectral methods of analysis– Beer-Lambert law – UV-Visible spectrophotometers – Single and double beam instruments , Sources and detectors – IR Spectrophotometers – Types – Attenuated total reflectance flame photometers – Atomic absorption spectrophotometers – Sources and detectors – FTIR spectrophotometers – Flame emission photometers – Fluorescence spectrophotometer.

UNIT- II CHROMATOGRAPHY

Different techniques – Techniques by chromatographic bed shape: Column chromatography-Planer Chromatography-Paper Chromatography-Thin layer Chromatography-Applications - Techniques by physical state of mobile phase: Gas chromatography – Sources- Detectors – Liquid chromatographs – sources- detectors- Applications – High-pressure liquid chromatographs – sources-detectors- Applications- Techniques by separation mechanism: Ion exchange chromatography-size-exclusion chromatography-Applications

UNIT -III INDUSTRIAL GAS ANALYZERS AND POLLUTION MONITORING INSTRUMENTS

Types of gas analyzers: Oxygen, NO2 and H2S types, IR analyzers, thermal conductivity analyzers, analysis based on ionization of gases. Air pollution due to carbon monoxide, hydrocarbons, nitrogen oxides, sulphur dioxide estimation - Dust and smoke measurements.

UNIT –IV pH METERS AND DISSOLVED COMPONENT ANALYZERS

Selective ion electrodes- Principle of pH measurement and conductivity measurements-dissolved oxygen analyzer – Sodium analyzer – Silicon analyzer – Water quality Analyzer.

UNIT-V NUCLEAR MAGNETIC RESONANCE AND RADIATION TECHNIQUES

NMR: – Basic principles, Continuous and Pulsed Fourier Transform NMR spectrometer and Applications -.Mass spectrometry and Applications, Nuclear radiation detectors, GM counter, proportional counter, solid state detectors, Scintillation counter.

TEXT BOOKS:

1. R.S. Khandpur, Handbook of Analytical Instruments, Tata McGraw Hill publishing Co. Ltd., 5 edition, 2018.
2. G.W. Ewing, Instrumental Methods of Analysis, Mc Graw Hill, 2004.
3. Liptak, B.G., Process Measurement and Analysis, CRC Press, 5 edition, 2016.

REFERENCES:

1. Braun, R.D., Introduction to Instrumental Analysis, Mc Graw – Hill, Singapore, 2006.
2. H.H.Willard, L.L.Merritt, J.A.Dean, F.A.Settle, Instrumental methods of analysis, CBS publishing & distribution, 1995.
3. James keeler ; Understanding NMR Spectroscopy, Second Edition John Wiley & Sons, 2010.
4. John H.Nelson , Nuclear Magnetic Resonance Spectroscopy, Prentice Hall/Pearson Education,2003.
5. Frank G. Kerry Industrial Gas Handbook: Gas Separation and Purification, Taylor and Francis group, 2007.
6. NPTEL Lecture Notes on, "Modern Instrumental Methods of Analysis" by Dr.J.R.Mudakavi, IISC, Bangalore.

Course Outcomes

At the end of the course the students will be able to

CO1. Understand various techniques and methods of Spectral analysis.
CO2. Apply the knowledge of chromatography to separate the constituents from a complex mixture.
CO3. Able to get adequate knowledge about Gas sensor and pollution monitoring instruments.
CO4. Able to select an appropriate analyzer for an Industrial requirement.
CO5. Able to understand the working principle of NMR and Mass spectroscopy.

Pre-Test:

1. What is the unit of absorbance which can be derived from Beer Lambert's law?
   a) L mol^{-1} cm^{-1}
   b) L gm^{-1} cm^{-1}
   c) Cm
   d) **No unit**
2. Beer Lambert's law relates between which of the following?
   a) Reflected radiation and concentration
   b) Scattered radiation and concentration
   c) **Energy absorption and concentration**
   d) Energy absorption and reflected radiation
3. In chromatography, which cannot act as the stationary phase and mobile phase respectively?
   a) Solid and liquid
   b) **Gas and Solid**

c) Solid and gas
d) Liquid and Gas

4. In Thin layer chromatography, the stationary phase is made of _____ and the mobile phase is made of _____
   a) **Solid, liquid**
   b) Liquid, liquid
   c) Liquid, gas
   d) Solid, gas
5. Which among the following gases have diamagnetic property?
   a) Oxygen
   b) Nitrogen
   c) Nitrogen dioxide
   d) Nitric oxide
6. How does solubility of oxygen in water change with respect to temperature?
   a) **It decreases with increase in temperature**
   b) It increases with increase in temperature
   c) It decreases with decrease in temperature
   d) It does not depend on temperature
7. Which of the following is the relationship between the density of ideal gas and its molecular weight?
   a) Not equal
   b) Inversely proportional
   c) **Linear**
   d) No relation
8. Which of the following transducers must be used for dissolved oxygen analyzer?
   a) Potentiometric
   b) **Polarographic**
   c) Ion-selective electrode
   d) Optical transducer
9. Which of the following acts as quenching gas in Geiger Muller counter?
   a) **Alcohol**
   b) Argon gas
   c) Krypton
   d) Hydrogen
10. Which of the following is not a type of radiation detector?
    a) Geiger Muller counter
    b) Proportional counter
    c) Semiconductor detector
    d) **Flame emission detector**

Analytical Instrumentation

Analytical instruments are a large class of instruments used for analytical applications in chemical, pharmaceutical, clinical, food-processing laboratories, and oil refineries.

- The instruments help in analyzing materials and establishing the composition.
- Instrumental and Non Instrumental methods.

Instrumental methods may be used to separate samples using chromatography, electrophoresis or field flow fractionation. Then qualitative and quantitative analysis can be performed, often with the same instrument and may use light interaction, heat interaction, electric fields or magnetic field. The general Analytical methods are

1. Spectrophotometer
2. Chromatography
3. Gas analyzers
4. Dissolved component Analyzer
5. NMR Spectrometer

Unit-1

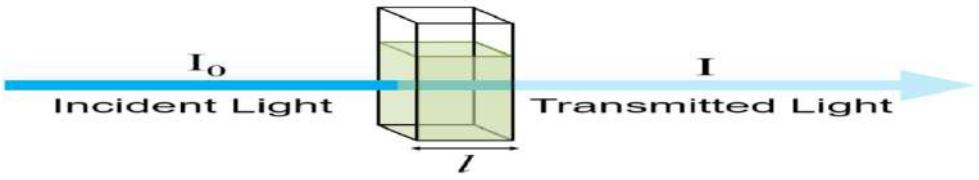
SPECTROPHOTOMETRY

Spectrophotometer

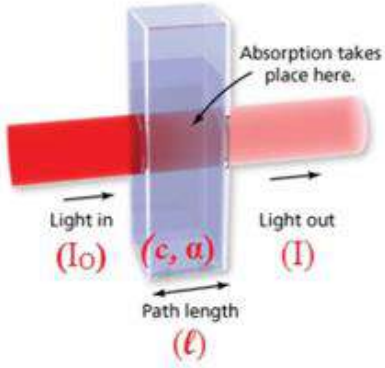
- Spectrophotometer is a method used to estimate the quantity of an analyte in solution.
- Spectrophotometer is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.
- Absorption Spectrophotometer works with the principle of **Beer–Lambert law.**
- The **Beer–Lambert law** states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm



Beer-Lambert Equation



$$A = log_{10}(\frac{I_0}{I}) = \varepsilon c l$$

$$A = \varepsilon c l$$

Classification of Spectrophotometer

- Based on Instrumental setup
  - Single beam method
  - Double beam method
- Based on Radiation source
  - UV spectrophotometer
  - VIS spectrophotometer
  - Infrared spectrophotometer

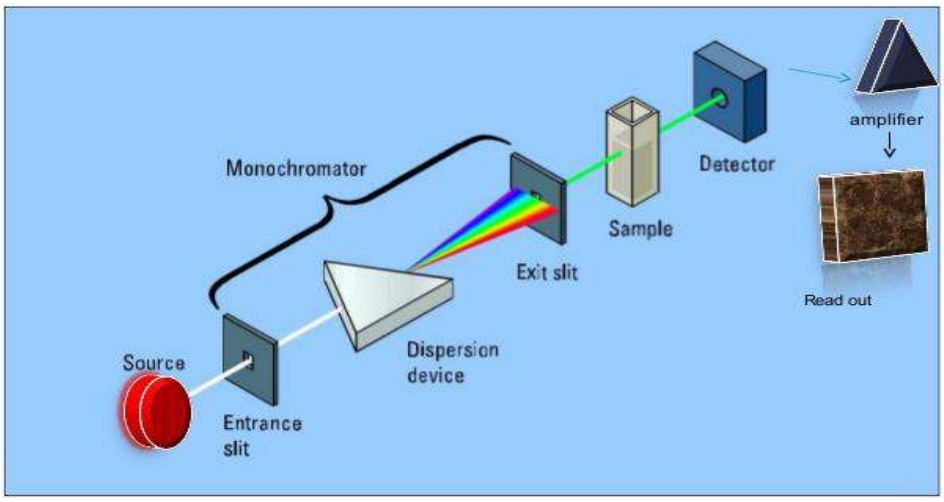
- Based on Interaction with the Analyte
  - Absorption Spectroscopy (UVS)
  - Emission Spectroscopy (Fluorescence)
  - Scattering Spectroscopy (Raman Spectroscopy)

Spectrophotometer Types

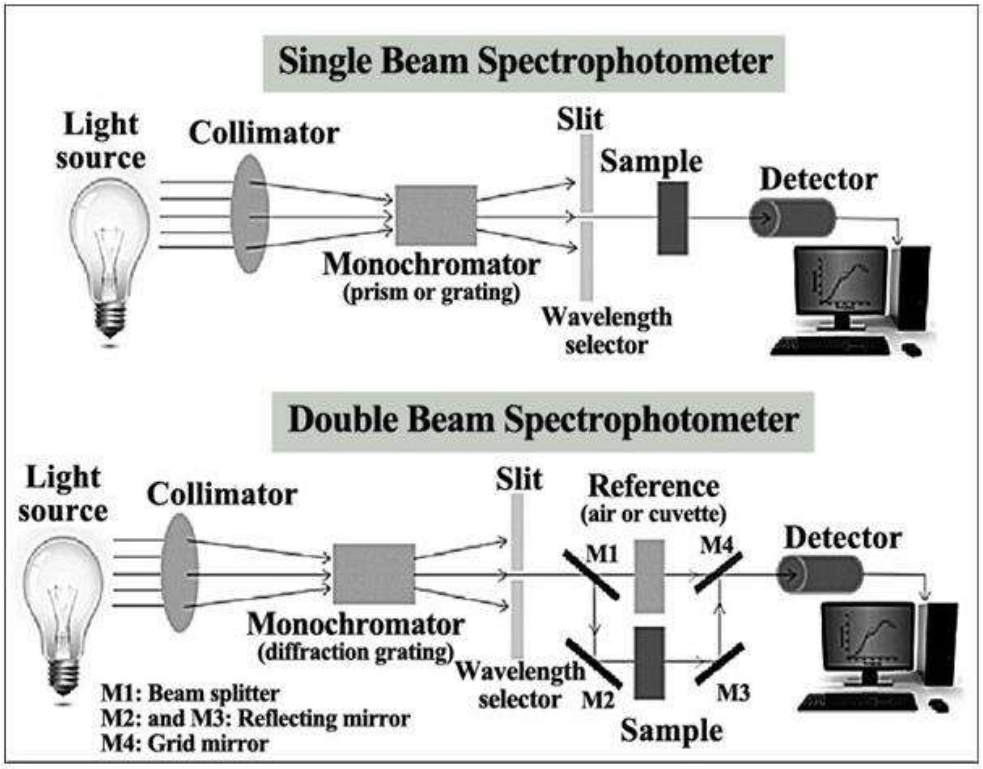
- UV spectrophotometer (180nm to 400nm).
- Vis spectrophotometer (400nm to 700nm).
- Infrared spectrophotometer (700nm to 1500nm).
- Fourier Transform Infra Red spectrophotometer (FTIR).
- Attenuated Total Reflection Flame Photometer (ATR).
- Fluorescence spectrophotometer.
- Atomic Absorption Spectrophotometer (AAS).
- Flame Emission Spectrophotometer (FES).

Instrumental Setup

- A spectrophotometer consists of three primary components: a light source, Monochromator/ wavelength selector, and a detector.



Single and Double Beam Spectrophotometer



Radiation Source

The best light source would be one that give good intensity with low noise across all ultraviolet and visible wavelengths and also provided stability over a long period of time.

UV Radiation Source

Hydrogen/Deuterium Lamps: (UV)

- For the ultraviolet region, hydrogen and deuterium lamps are frequently used.
- Range is approximately 200 to 450 nm.
- Deuterium lamps are generally more stable and have a life time of about 500 hr.
- This lamp can generate continuous or discontinuous spectral.

IR Radiation Source

An inert solid is electrically heated to a temperature in the range 1500-2000 K. The heated material will then emit infra red radiation.

1. Nernst glower
2. Incandescent lamp
3. Xenon flash Lamps / Mercury arc
4. Tungsten lamp
5. Globar source/rod
6. Nichrome wire

Tungsten Lamp: (IR)

- It is most commonly light source used in SPM Wavelength
- Range of about 330 to 900 nm
- It has long life of about 1200 hr.

Xenon flash Lamps:

- Their range is 190 to 1000nm.
- Emit both UV and Visible wavelength
- Long life
- Do not heat up the instrument
- Reduce warm up time.

Nernst glower is a cylinder (1-2 mm diameter, approximately 20 mm long) of rare earth oxides. Platinum wires are sealed to the ends, and a current passed through the cylinder. The Nernst glower can reach temperatures of 2200 K. Preheat required and as well as ballast System to prevent overheating. Radiation Intensity is twice that of Nichrome and Globar rod.

Globar source is a silicon carbide rod (5mm diameter, 50mm long) which is electrically heated to about 1500 K. Water cooling of the electrical contacts is needed to prevent

arcing. The spectral output is comparable with the Nernst glower, except at short wavelengths (less than 5 mm) where it's output becomes larger.

The incandescent wire source is a tightly wound coil of nichrome wire, electrically heated to 1100 K. It produces a lower intensity of radiation than the Nernst or Globar sources, but has a longer working life.

Wavelength selector

Wavelength selectors limit the radiation absorbed by a sample to a certain wavelength or a narrow band of wavelengths.

1. **Filters**
2. **Monochromator**

Filters

Filters are used to remove the unwanted wavelength or it will allow only a particular wavelength to pass through the sample.

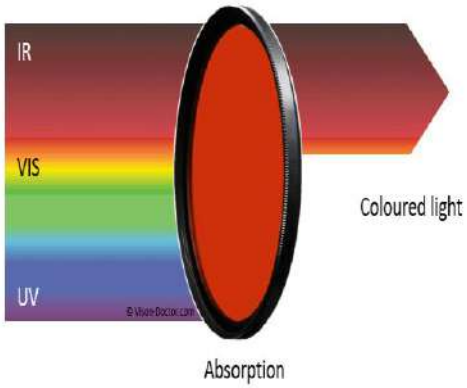
- It should have high transmittance at the desired wavelength.
- It should have low transmittance at other wavelength.
    - Absorption filter
    - Interference filter

Monochromator

- Prism monochromator
- Grating monochromator

Absorption Filter:

- Commonly used in visible Spectrophotometer.
- Normally constructed of colored glass, colored films or a dye in a gelatin.
- Often two absorption filters are paired to give a narrower band of transmittance.
- Selectivity depends on the thickness of the glass.
- Less degree of monochromaticity, hence used only for simple photometers.



Glass Filters

- Made from piece of colored glass which transmits limited wavelength range of spectrum.
- Color produced by incorporation of oxides of vanadium, chromium, iron, nickel, copper.

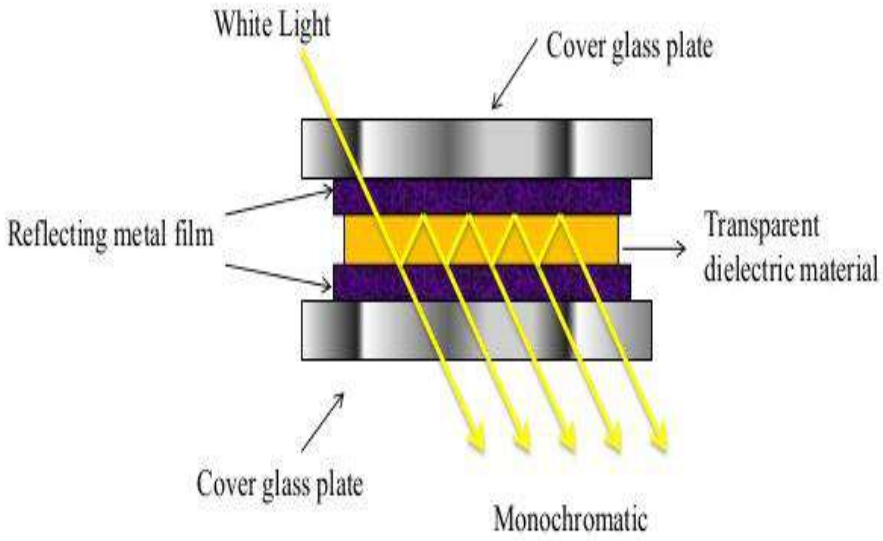
Gelatin Filters

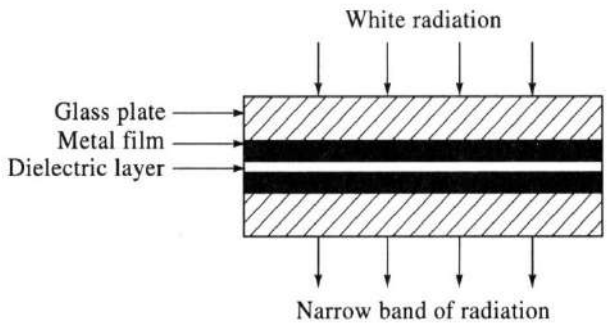
- Consist of mixture of dyes placed in gelatin & sandwiched between glass plates.
- Band width 25nm.

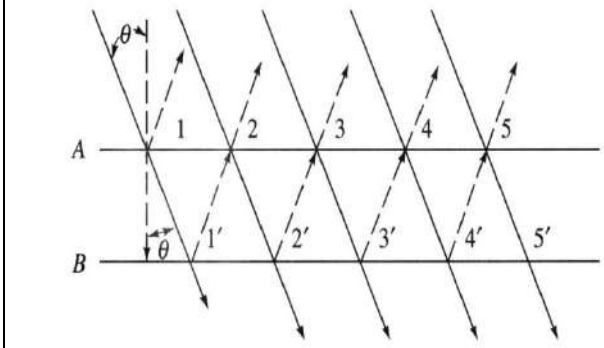
Interference Filter:

- It consists of two semi transparent layers of silver, deposited on glass by the process of evaporation in vacuum and they are separated by a layer of dielectric.
- Useful in the UV, visible, and IR regions of the EM spectrum.
- The wavelength output
    - **n** = order of interference (small integer)
    - η = refractive index of the dielectric
    - t = thickness of dielectric layer.

$$\lambda = \frac{2t\eta}{\mathbf{n}}$$

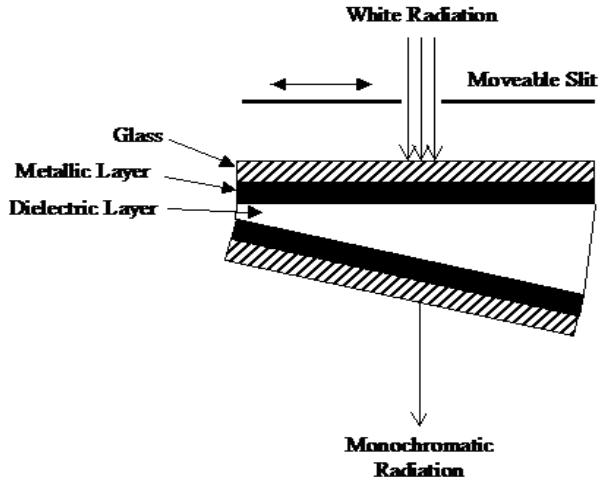






Interference Wedges

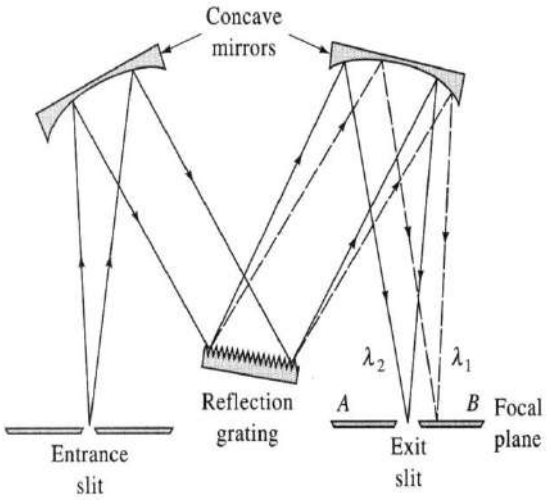
If two glass plates are placed face to face with one end separated by a piece of tissue paper or thin metal foil an air wedge will be formed between them. If monochromatic light is directed on the plates a series of straight-line fringes will be seen parallel to the line along which they touch. This is due to interference by division of amplitude, as with Newton's rings. Some light is reflected from the bottom surface of the top plate and some from the top surface of the bottom plate.



Monochromator

Components of a Monochromator:

1) Entrance Slit – provides a rectangular optical image of the incoming polychromatic radiation.
2) Colliminating Lens or Mirror -provides a parallel beam of radiation that impinges upon the dispersive element.
3) Prism or Grating - (dispersive element) disperses the polychromatic radiation by the process of diffraction.
4) Focusing Lens or Mirror - Focuses the dispersed radiation on the exit slit.
5) Exit Slit - Isolate the wavelength band of interest.

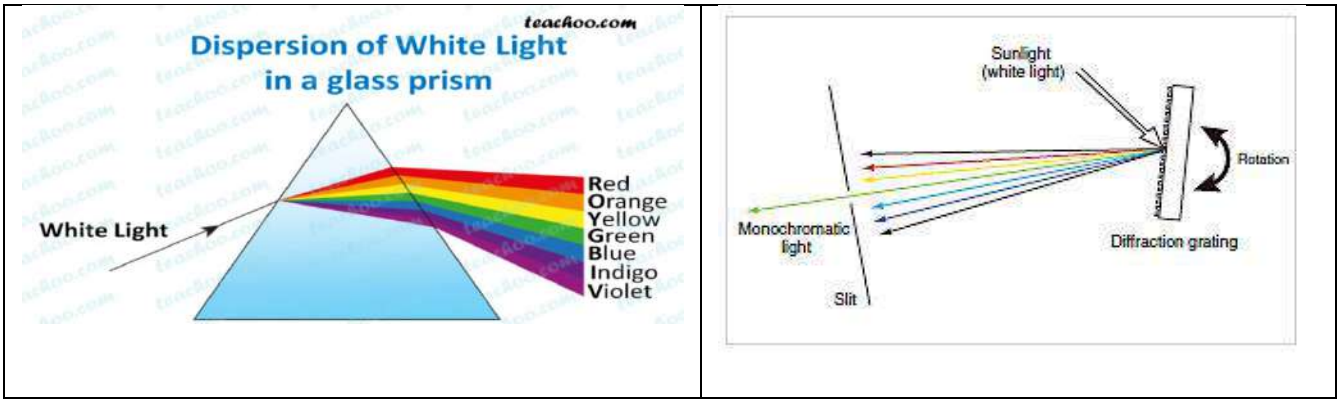


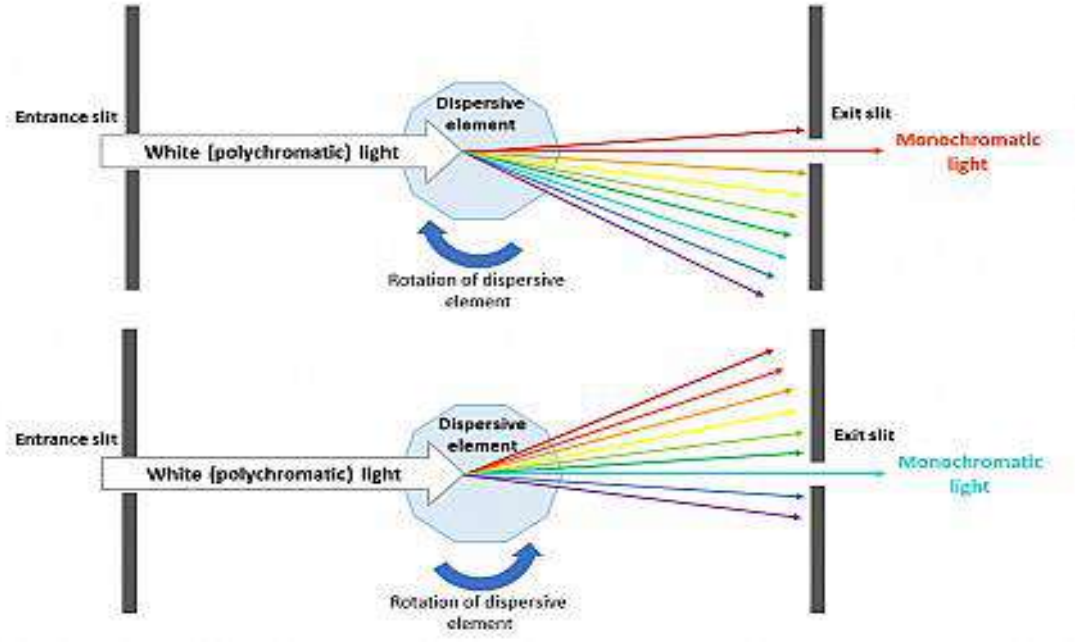
Monochromator

- Prism
- Diffraction Grating

Prism Monochromator

The **prism** refracts light into its different colours (wavelengths). The dispersion occurs because the angle of refraction is dependent on the refractive index of the **prism's** material, which in turn is slightly dependent on the wavelength of light that is travelling through it.





Grating Monochromator

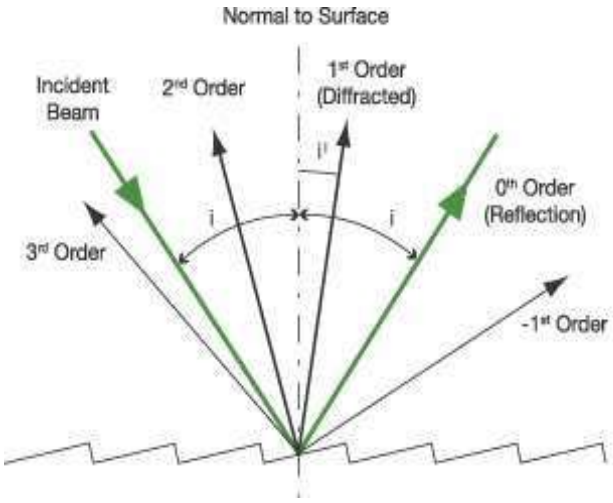
A monochromator is an optical instrument which measures the light spectrum. Light is focused in the input slit and diffracted by a grating. In this way, only one color is transmitted through the output slit at a given time. Spectra are then recorded wavelength by wavelength, rotating the grating.

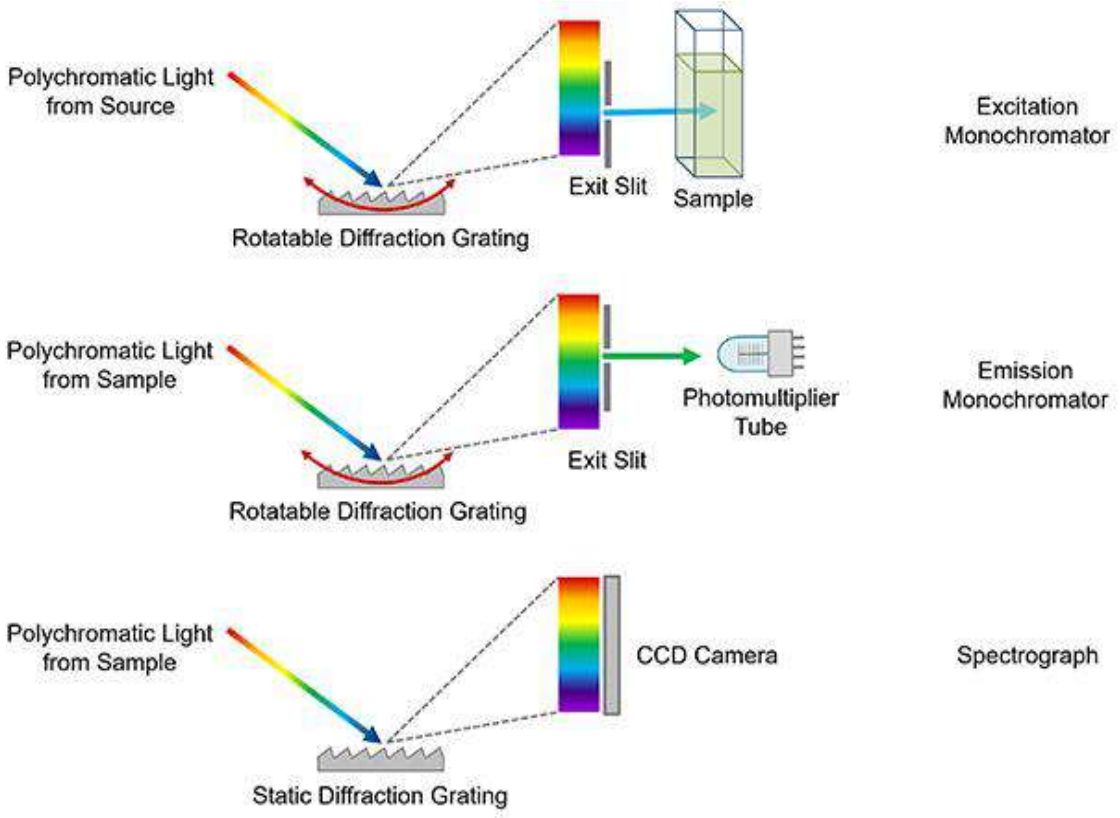
Gratings can be reflective or transmissive, and the surface of a grating can either be planar or concave. Planar gratings generally give higher resolution over a wide wavelength range while concave gratings can function as both a dispersing and focusing element in a spectrometer. A grooved or blazed mirror that has relatively broad faces upon which reflection occurs and narrow unused faces. Grating has higher dispersion than Prism. It follows law of diffraction

$$n\lambda = d^*(\sin I \pm \text{Sin } \theta)$$

Where,

n = order of grating
ʎ = wavelength of radiation
d = distance between the grooves
I = angle of incidence
θ = angle of dispersion





Difference between Prism and Grating

Difference between Prism and Grating		
Comparison	Prism	Grating
Made of	Glass: Visible Quartz/fused silica:UV Alkali halide:IR	Grooved on highly polished surface like alumina.
Working Principle	Angle of Incident	Law of diffraction
Merits/Demerits	• Prism gives non-linear dispersion hence no overlap of spectral order. • It can't be used over consideration wavelength ranges. • Prisms are not sturdy and long lasting.	• Grating gives linear dispersion hence overlap of spectral order. • It can be used over considerable wavelength ranges. • Gratings are sturdy and long lasting.

Difference between Monochromator and Filter

Difference between Monochromator and Filter		
Comparison	Monochromator	Filter
Means of selection	Diffraction	Absorption
Wavelength width	Narrow	Wide
Cost	Expensive	Cheap
Portability	None	Very
Capable of recording Spectra	Yes	No
Suitable for quantitative analysis	Yes	Yes

Detectors

Detectors are the devices that convert the radiation to an electrical signal.

Properties of an ideal detector:

1. High sensitivity
2. High signal-to-noise ratio
3. Constant response over a large range of wavelengths
4. Fast response time
5. Electrical signal (S) produced is proportional to the radiant power (P)

$$S = KP$$

K = calibration sensitivity

UV-VIS Spectrophotometer – detectors

Photo Detectors

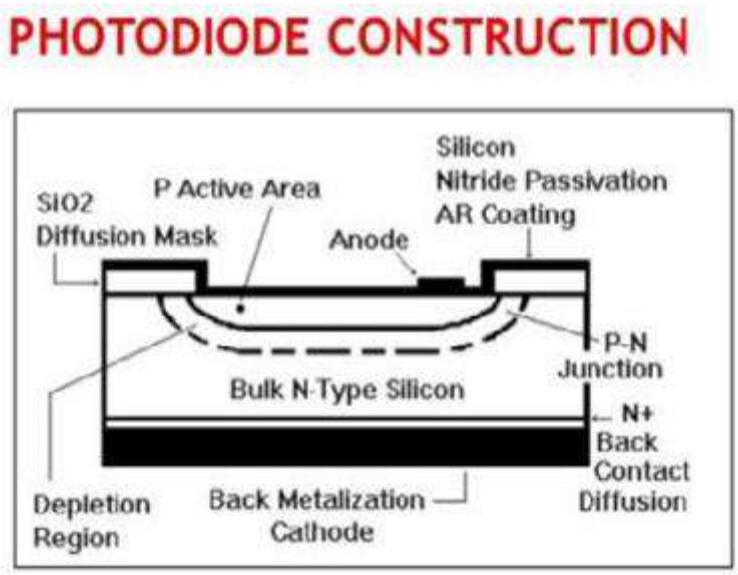
1. Photodiode
2. Photomultiplier tube
3. Photo Emissive Tube

Photodiode

The linear photodiode array is an example of a multichannel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously.

A linear photodiode array comprises many small silicon photodiodes formed on a single silicon chip.

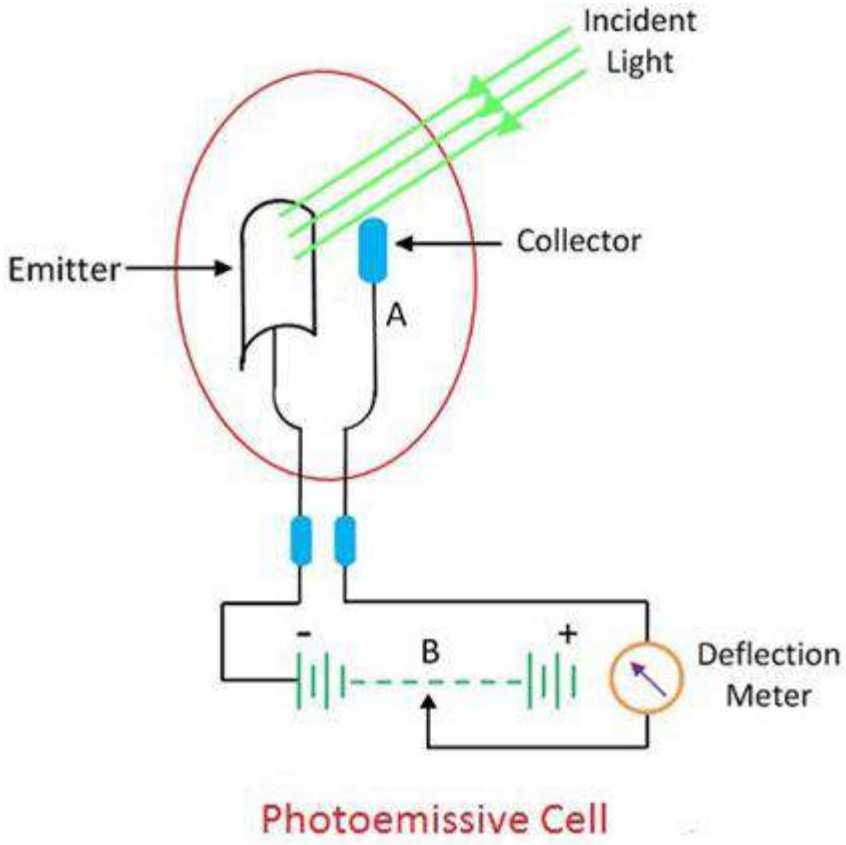
In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) such that the spectrum falls on the diode array. They are useful for recording UV-Vis. absorption spectra of samples that are rapidly passing through a sample flow cell.



CROSS SECTION OF THE SILICON PHOTODIODE

Photo Emissive Tubes

Photo emissive detectors (also called photoelectric detectors) are photo detectors which are based on the external Photoelectric effect. Such a device contains some kind of Photocathode, where incident light is partially absorbed and generates photoelectrons, i.e., electrons which are released into free space. Using another electrode, called the anode, which is held at a substantially more positive electric potential, one can pull the photoelectrons away from the photocathode and obtain a photocurrent. This is the operation principle of a phototube.



Photomultiplier Tube (PMT)

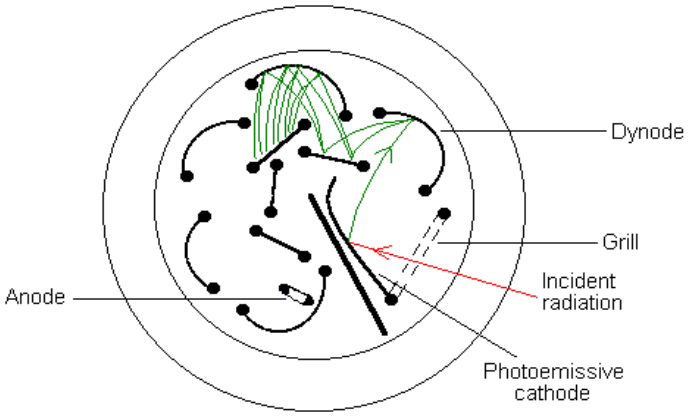
The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy.

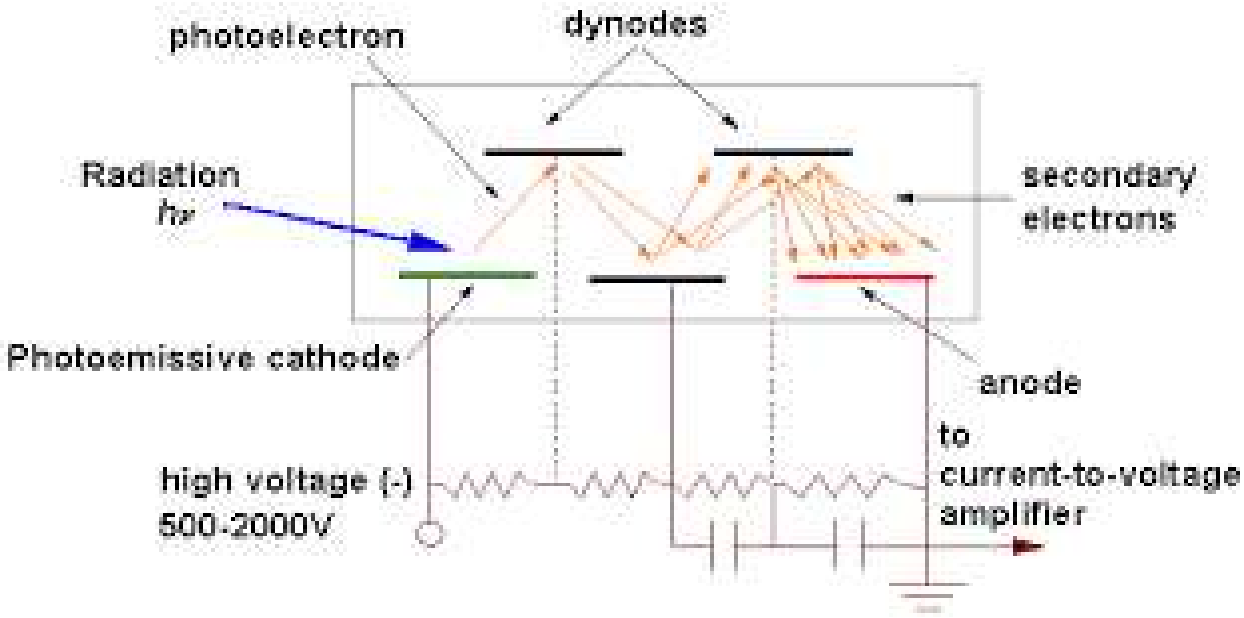
It consists of a *photo emissive cathode* (a cathode which emits electrons when struck by photons of radiation), several *dynodes* (which emit several electrons for each electron striking them) and an *anode*.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode). The electrons strike the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced 10^6 - 10^7 electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and visible radiation.

They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.





IR Spectrophotometer Detector

Detectors are used to measure the intensity of unabsorbed infrared radiation.

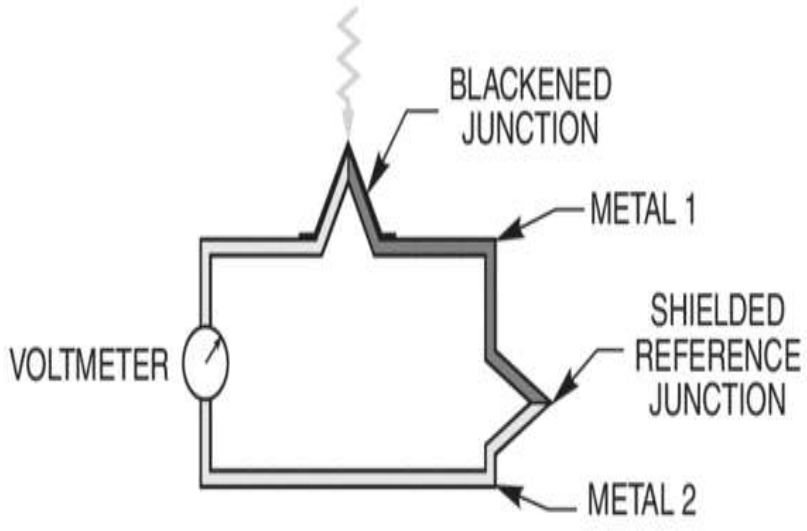
There are three categories of IR detector:

- **Thermal Detectors**
  - **Thermocouple**
  - **Thermistor**

  - **Bolometer**
  - **Pyroelectric Transducers**
- **Golay Cell / Pneumatic detector**
- **Photo-conducting detectors**

Thermal Detector (Thermocouple)

Thermocouples consist of a pair of junctions of different metals; for example, two pieces of bismuth fused to either end of a piece of antimony. The potential difference (voltage) between the junctions changes according to the difference in temperature between the junctions.



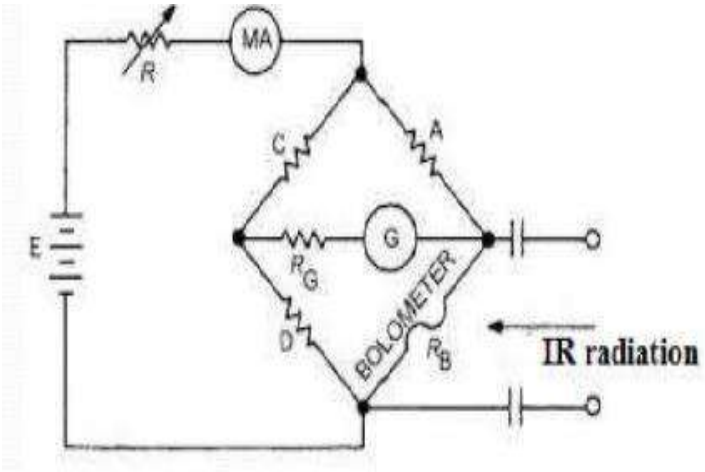
Bolometer & Thermistor

- **Bolometer**

A bolometer functions by changing resistance when heated. It is constructed of strips of metals such as platinum or nickel or from a semiconductor.

- **Thermistor**

Thermistor is a resistance thermometer, or a resistor whose resistance is dependent on temperature.



Pyroelectric detectors:

Pyroelectric detectors are thermal detectors: Temperature fluctuations produce a charge change on the surface of Pyroelectric crystals, which produces a corresponding electrical signal. This temperature gradient can be created by the absorption of light.. Triglycine sulphate is the most common material for Pyroelectric infrared detectors. Unlike other thermal detectors the Pyroelectric effect depends on the rate of change of the detector temperature rather than on the temperature itself. This allows the Pyroelectric detector to operate with a much faster response time and makes these detectors the choice for Fourier transform spectrometers where rapid response is essential.

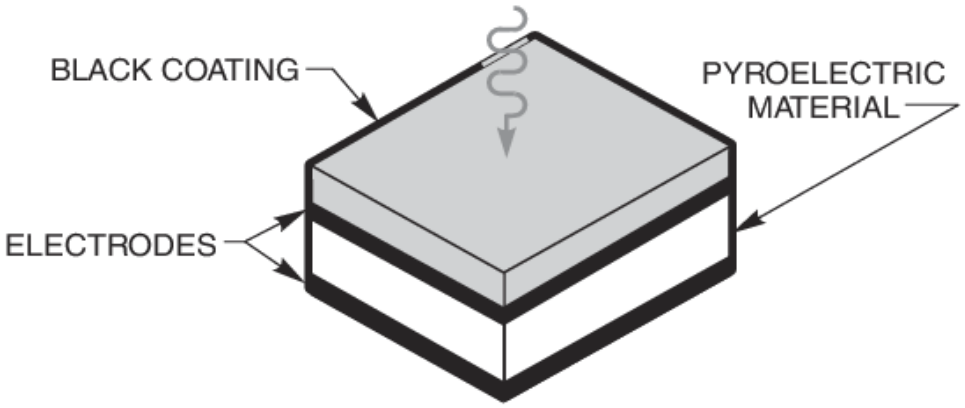


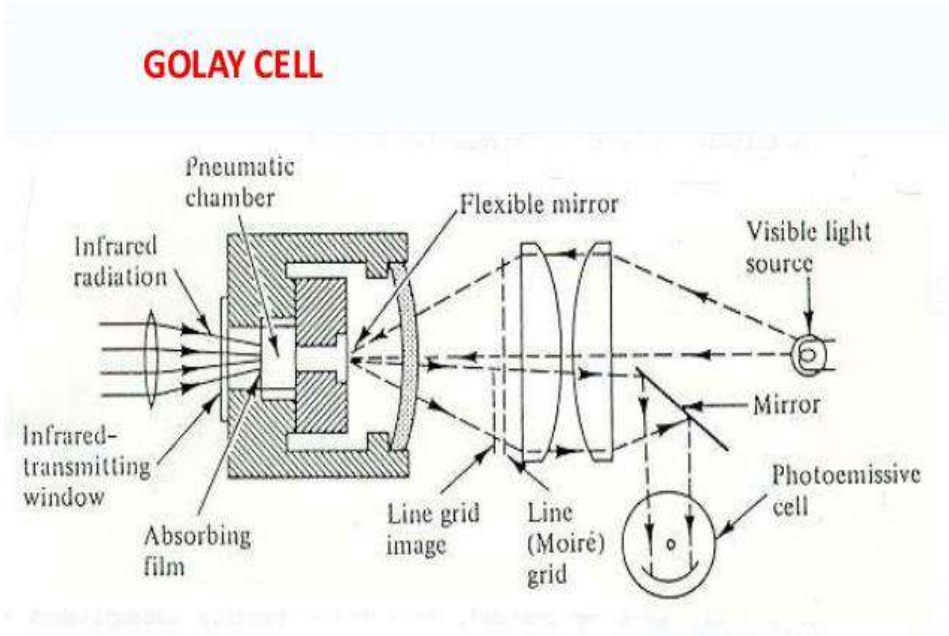
Photo-conducting Detectors

Photo-conducting detectors are the most sensitive detectors. They rely on interactions between photons and a semiconductor. The detector consists of a thin film of a semiconductor material such as lead sulphide, mercury cadmium telluride or indium antimonite deposited on a no conducting glass surface and sealed into an evacuated

envelope to protect the semiconductor from the atmosphere. The lead sulphide detector is used for the near-infrared region of the spectrum. For mid- and far-infrared radiation the mercury cadmium telluride detector is used. It must be cooled with liquid nitrogen to minimize disturbances.

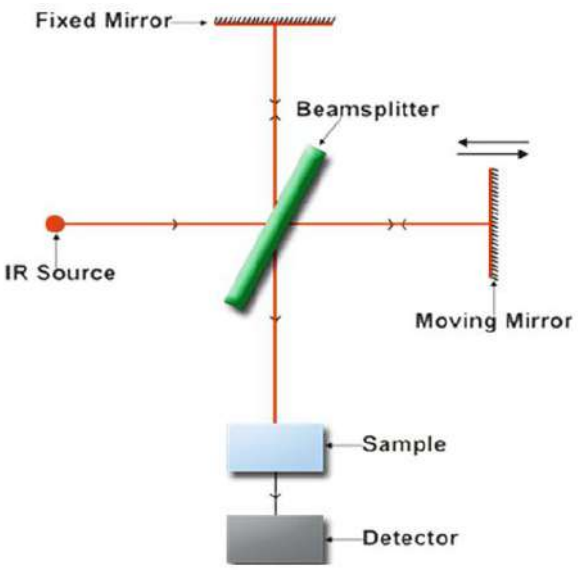
Golay cell

The Golay cell is a type of opto-electric detector mainly used for infrared spectroscopy. It consists of a gas-filled enclosure with an infrared absorbing material and a flexible diaphragm or membrane. When infrared radiation is absorbed, it heats the gas, causing it to expand. The resulting increase in pressure deforms the membrane. Light reflected off the membrane is detected by a photodiode, and motion of the membrane produces a change in the signal on the photodiode.

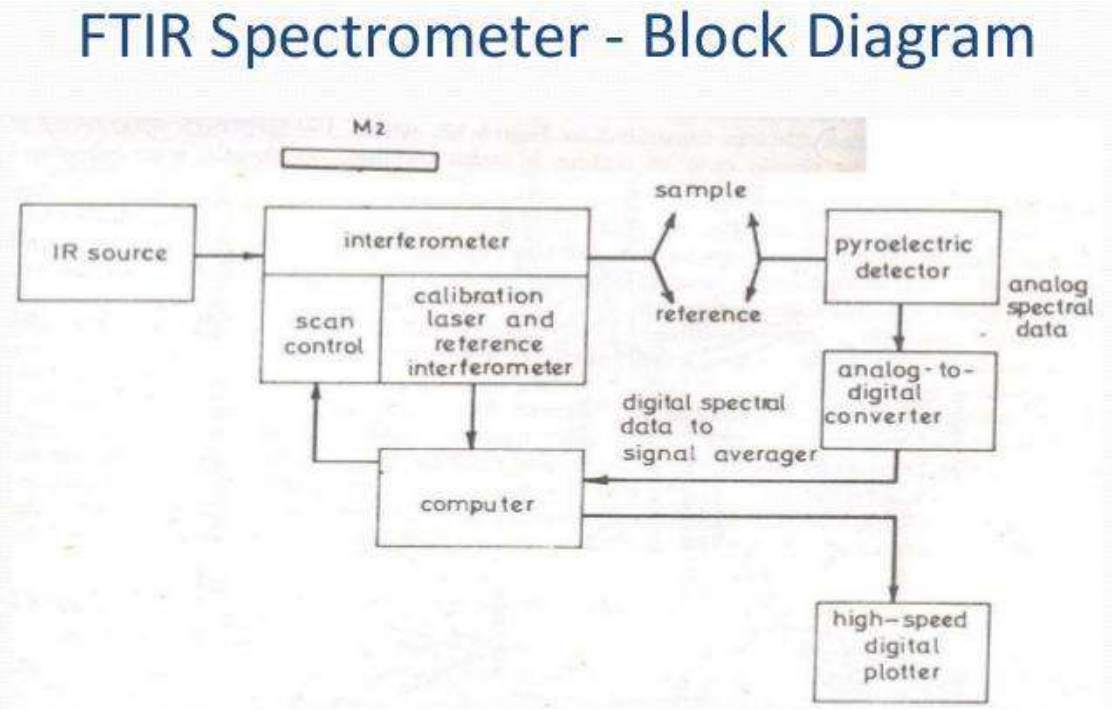


FTIR Spectrophotometer

- FTIR (Fourier Transform Infrared) spectrometer is a obtains an infrared spectra by first collecting an interferogram of a sample signal using an interferometer, then performs a Fourier Transform on the interferogram to obtain the spectrum.
- An interferometer is an instrument that uses the technique of superimposing (interfering) two or more waves, to detect differences between them. The FTIR spectrometer uses a Michelson interferometer.



- The heart of the FTIR is a Michelson interferometer.
- The mirror moves at a fixed rate. Its position is determined accurately by counting the interference fringes of a collocated Helium-Neon laser.
- The Michelson interferometer splits a beam of radiation into two paths having different lengths, and then recombines them.
- A detector measures the intensity variations of the exit beam as a function of path difference.
- A monochromatic source would show a simple sine wave of intensity at the detector due to constructive and destructive interference as the path length changes.
- In the general case, superpositions of wavelengths enter spectrometer, and the detector indicates the sum of the sine waves added together.
- Some idealized light sources, and the interferogram that they would theoretically produce.
- The difference in path length for the radiation is known as the retardation.
- When the retardation is zero, the detector sees a maximum because all wave numbers of radiation add constructively.
- When the retardation is l/2, the detector sees a minimum for the wavelength l.
- An interferogram is the sum of all of the wave number intensities.



Advantages of Fourier transform IR over dispersive IR;

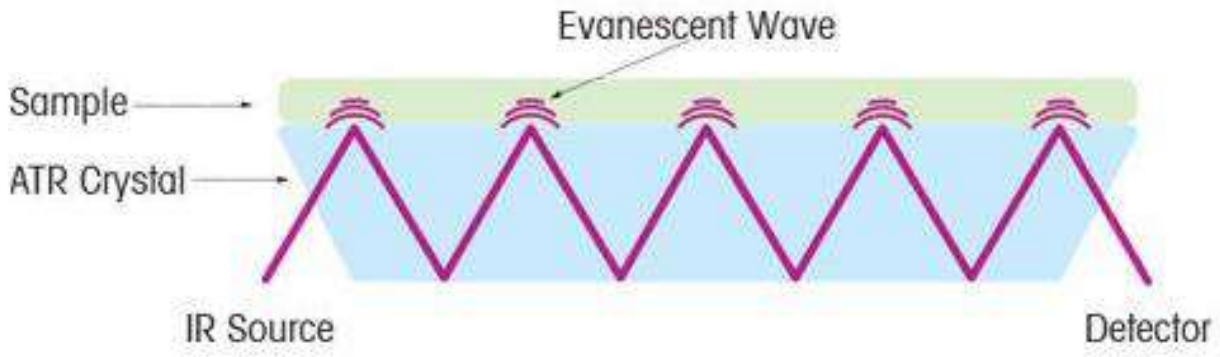
1. Improved frequency resolution
2. Improved frequency reproducibility (older dispersive instruments must be recalibrated for each session of use)
3. Higher energy throughput
4. Faster operation
5. Computer based (allowing storage of spectra and facilities for processing spectra)
6. Easily adapted for remote use (such as diverting the beam to pass through an external cell and detector, as in GC - FT-IR)

Attenuated Total Reflectance (ATR)

- Attenuated Total Reflectance (ATR) is a sampling method that introduces light onto a sample in order to acquire structural and compositional information. ATR is one of the most used sampling technologies for FTIR Spectroscopy. The reason for use of ATR is that it enables solids and liquid samples to be analyzed simply.
- In Attenuated Total Reflectance (ATR), light energy is passed though an optical material (i.e., the ATR sensor) that has two major characteristics:
  - It must be optically transparent to the frequency of the energy, so that sensor material absorbs little or none of the radiation;

  - The ATR sensor material has an index of refraction that is higher than that of the surrounding media so that the ATR acts as a waveguide, internally reflecting the light energy.

- ATR uses a property of total internal reflection resulting in an evanescent wave. A beam of infrared light is passed through the ATR crystal in such a way that it reflects at least once off the internal surface in contact with the sample. This reflection forms the evanescent wave which extends into the sample.
- The penetration depth into the sample is typically between 0.5 and 2 micrometres, with the exact value determined by the wavelength of light, the angle of incidence and the indices of refraction for the ATR crystal and the medium being probed.
- The number of reflections may be varied by varying the angle of incidence. The beam is then collected by a detector as it exits the crystal.
- Most modern infrared spectrometers can be converted to characterise samples via ATR by mounting the ATR accessory in the spectrometer's sample compartment.
- This evanescent effect only works if the crystal is made of an optical material with a higher refractive index than the sample being studied. Otherwise light is lost to the sample.
- **Evanescent Waves**
- The infrared radiation reacts with the sample through a series of standing waves, called Evanescent Waves.
- An Evanescent Waves is penetrating electromagnetic field whose intensity quickly decay as it moves away from the source.



Attenuated Total Reflection (ATR)

- In the case of a liquid sample, pouring a shallow amount over the surface of the crystal is sufficient. In the case of a solid sample, samples are firmly clamped to ensure good contact is made and to remove trapped air that would reduce signal intensity.
- The signal to noise ratio obtained depends on the number of reflections but also on the total length of the optical light path which dampens the intensity.
- Typical materials for ATR crystals include germanium, KRS-5 and zinc selenide, while silicon is ideal for use in the Far-IR region of the electromagnetic spectrum.
- The excellent mechanical properties of diamond make it an ideal material for ATR, particularly when studying very hard solids, although the broad diamond phonon band between 2600 and 1900 cm^{-1} significantly decreases signal to noise in this region.
- The shape of the crystal depends on the type of spectrometer and nature of the sample. With dispersive spectrometers, the crystal is a rectangular slab with chamfered edges, seen in cross-section in the illustrations. Other geometries use prisms, half-spheres, or thin sheets.

ATR – Advantage & Disadvantage

Advantage:

- ATR is an easy to use, fast and versatile technique for IR sampling.
- Solid, pastes, gels, powders, liquid can be analysed with little or no preparation.

Disadvantage:

- The ATR Crystals absorb energy at lower energy level.
- If the sample does not have good contact with the crystal, the data will not be accurate.
- Most of ATR crystals have pH limitations.

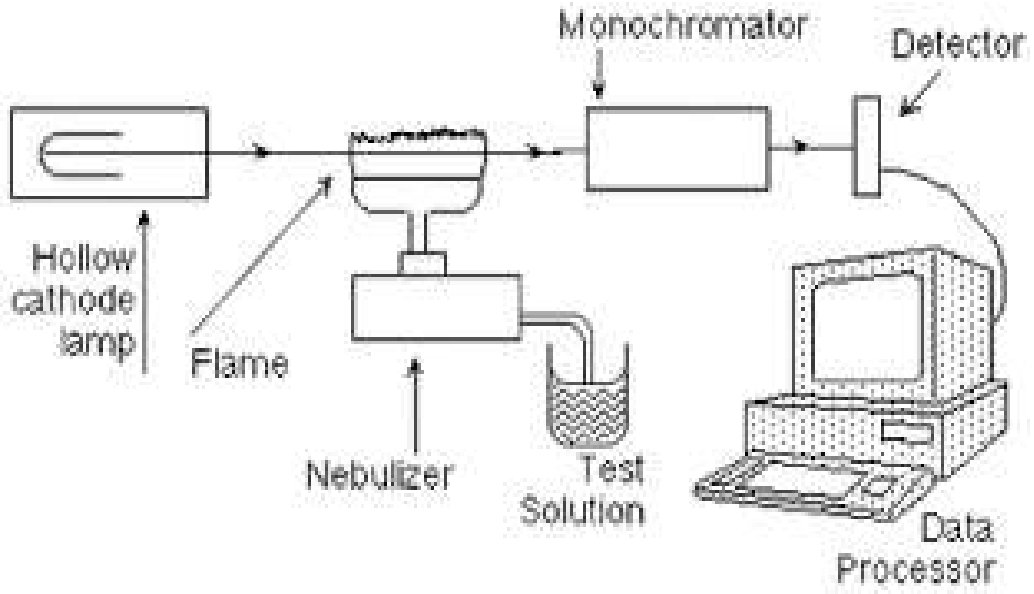
ATR – Application

- Biological industries
- Pharmaceutical industries
- Medicinal industries

- Chemical industries include measurement of drugs during manufactured by fermentation methods, following the kinematics of metabolism of dietary constituents, investigation of detergents and cosmetics, or identification of contaminants in waste water, etc.
- Using ATR combined with spectral summation the infrared spectra of solutes, very dilute aqueous solution can be measured.

Atomic Absorption Spectrophotometer (AAS)

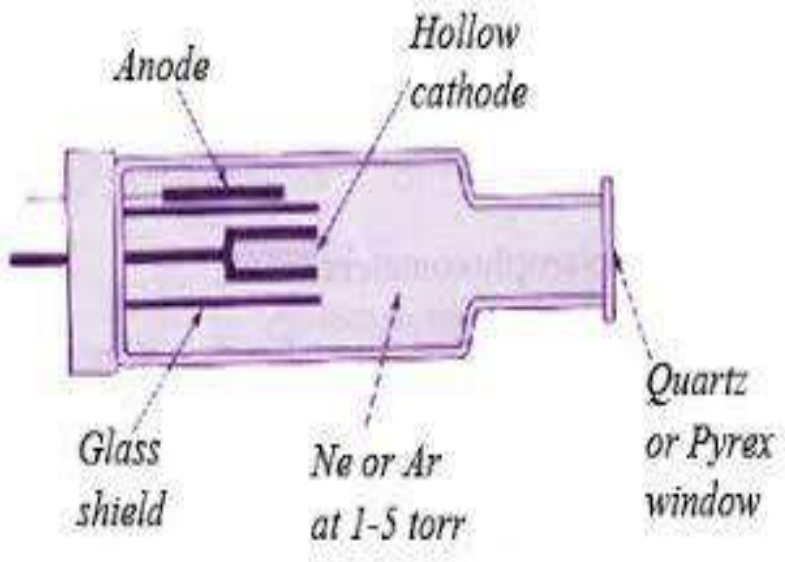
- In atomic absorption spectroscopy, the concentration of the analyte present in sample is measured by absorbance relating to the signal by Beer-Lambert's law.
- The technique uses basically the principle that free atoms generated in an atomizer can absorb radiation at specific frequency.
- Atomic Absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state.
- The atoms absorb UV or Visible light and mark transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption.
- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.



AAS- Sources

HOLLOW CATHODE LAMPS:

A HCL is composed of a silica envelope that contains 1–5 Torr of argon or neon and two metal electrodes. HCLs are almost ideal line sources for AAS because of their high stability and narrow line width (0.002 nm), but their relatively low intensity is a disadvantage for AFS. High-intensity hollow cathode lamps (HI-HCLs) provide increased intensity by use of an additional electrode to separate the atomization and excitation processes. The irradiance of the HI-HCLs is a factor of 20–100 times greater than that of conventional HCLs, and provides better sensitivity for AFS.



AAS-Flame Photometer

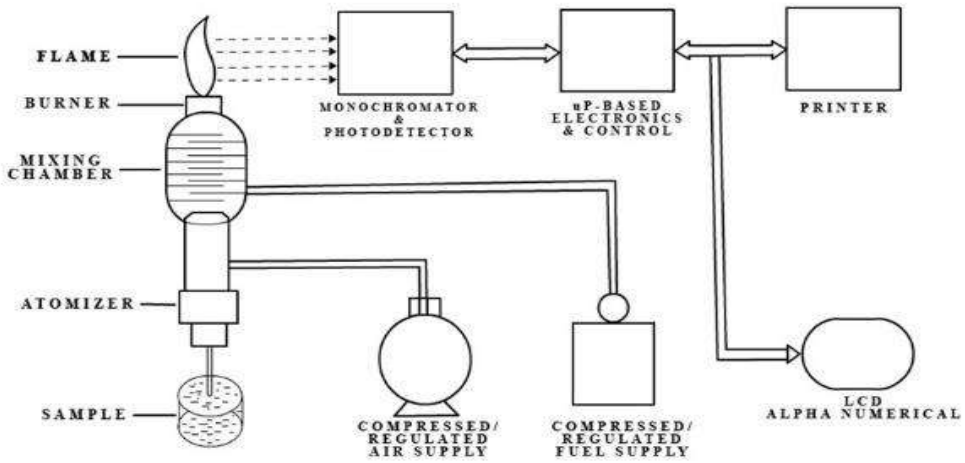
Production of Atomic Vapour by Flame

Pressure Regulator:

For generation of the flame the air or oxygen and fuel are required with some specified pressure level. These pressure maintenances could be done with the help of pressure regulators. Regulators are required to have a steady flame which is free from flickers.

Flow meters:

There are many types of flames are available. The selection of a particular type of flame depends on the ratio between the fuel flow rate and oxygen flow rate. The maintenance of this ratio could be done with the help of flow meters which are connected in the path of oxygen and fuel. These are also used for the detection of clogging in the orifice.



Supply of Fuel and Oxygen:

Normally used fuel gas in flame photometry is acetylene gas, which is commercially available; the other fuels used in flame photometry are propane, butane and hydrogen. Oxygen supply could also be done with the help of regulator.

Atomizer:

Atomizer is used for the purpose of introducing the liquid samples into the flame by breaking a large mass of liquid into small drops. This device is also responsible for introducing the liquid sample into the flame at a stable and reproducible rate and this device must not be attacked by corrosive liquid solutions.

Types of Atomizer:

a) Atomizers which introduces the sample directly into the flame.

b) Atomizers which introduces the small droplets of the sample into the flame.

Burner:

Burner is the place to get flame by mixing the fuel, oxidant and sample together. The requirements of a good flame are:

- It should posses the ability to evaporate liquid sample and to form the solid residue.

- Solid residue should be decomposed completely to form the atoms.
- Atoms should be excited to higher energy states to emit the radiant energy and this radiation should be steady for over the period of analysis.

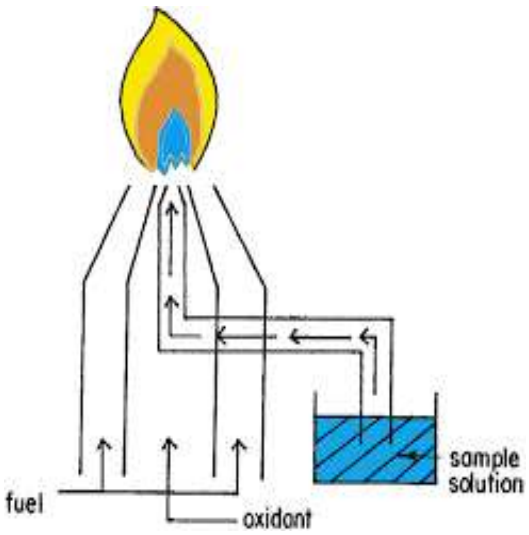
Types of Burners

i) Total consumption burner

ii) Laminar flow burner

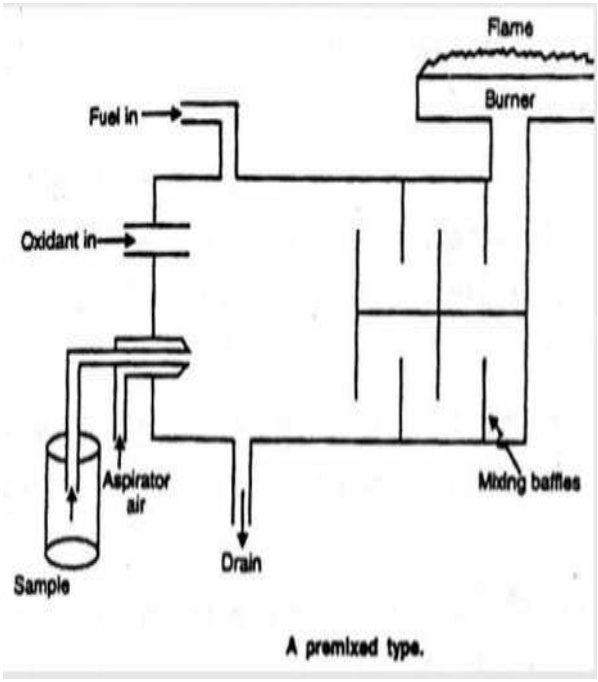
Total Combustion Burner

- In total-consumption burner, the fuel and oxidant (support) gases are mixed and combust at the tip of the burner. The fuel (usually acetylene), oxidant (usually air) and sample all meet at the base of flame. The sample is drawn up into the flame by the 'Venturi Effect' (The Venturi Effect is the reduction in fluid pressure that results when a fluid flows through a constricted section of pipe), by the support gas. The gas creates a partial vacuum above the capillary barrel, causing the sample to be forced up the capillary. It is broken into a fine spray at the tip where the gases are turbulently mixed and burned. This is the usual process of 'nebulisation'.
- The burner is called total consumption because the entire aspirated sample enters the flame or in other words the sample solution is directly aspirated into the flame. All desolations, atomization, and excitation occur in the flame.
- The total consumption burner can be used to aspirate viscous and 'high solids' samples with more ease, such as undiluted serum and urine. Also, this burner can be used for most types of flames, both low- and high-burning velocity flames.



Premix Chamber Burner/ Laminar-flow Burner

Laminar Flow Burner



The second type of burner, most commonly used now, is the premix chamber burner, sometimes called laminar-flow chamber. A premix burner system really consists of two key components, the burner head or nozzle, and the gas-air mixing device that feeds it. The fuel and support gases are mixed in a chamber before they enter the burner head (through a slot) where they combust. The sample solution is again aspirated through a capillary by the 'Venturi effect' using the support gas for the aspiration. Large droplets of the sample condense and drain out of the chamber. The remaining fine droplets mix with the gases and enter the flame. As much as 90% of the droplets condense out, leaving only 10% to enter the flame. The 90% of the sample that does not reach the flame will travels back through the mixing chamber and out as waste drain.

The premix burners are generally limited to relatively low-burning velocity flames. The most outstanding disadvantage of the premix burner is that only low burning-velocity flames can be used. A burning velocity which is higher than the rate of flow gases leaving the burner will cause the flame to travel down into the burner resulting in an explosion commonly known as flashback. Because of this limitation it is somewhat difficult to use high burning-velocity gases, which includes oxygen-based flames. Most commercial instrument use premix burners with the option of using total-consumption burner.

AAS – Instruments

Monochromator (Explanation in Page 9)

1. Prism
2. Grating

Detectors (Explanation in Page 16)

1. Photovoltaic cell
2. Photo-emissive Tube
3. Photomultiplier Tube

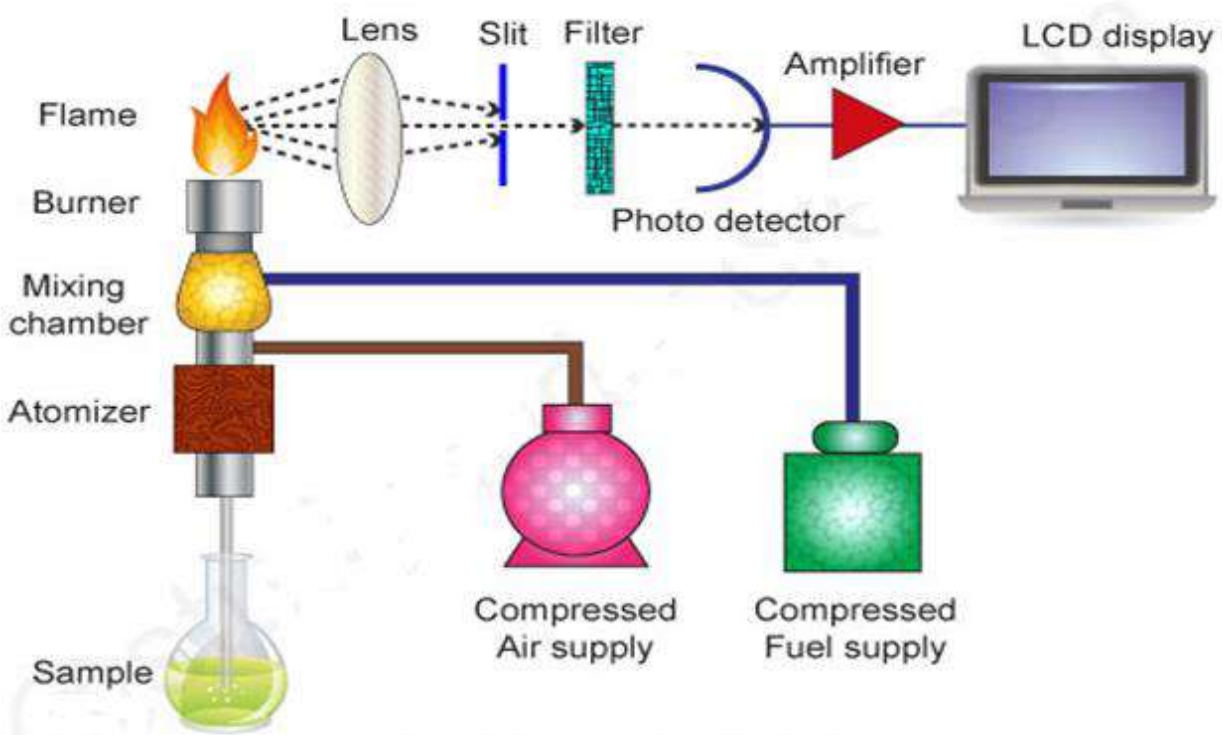
Application of AAS

1. **Quantitative and Qualitative** Analysis
2. **Clinical Analysis** –Blood samples: whole blood, plasma, serum; Ca, Mg, Li, Na, K, Fe.
3. **Environmental Analysis:** Monitoring our environment - find out the level of various elements in rivers, seawater, drinking water, air and in petrol.
4. **Mining:** By using AAS the amount of metals such as gold in rocks can be determined to see whether it is worth mining the rocks to extract the gold.

Flame Emission Spectroscopy (FES)

- The absorption and emission of radiant energy by atoms provide powerful analytical tools for both quantitative and qualitative analysis of substances. Flame emission and Atomic absorption spectroscopy is a method of element analysis.
- In Flame emission spectroscopy, the concentration of the analyte present in sample is proportional to the intensity of emitted radiation.
- In flame emission spectrometry, the sample solution is nebulised(convert into a fine aerosol) and introduced into the flame where it is desolated, vaporized, and atomized, all in rapid succession.
- Subsequently, atoms and molecules are raised to excited state via thermal collision with the constituents of the partially burned flame gases. Upon their return to lower or ground electronic state, the excited atoms and molecules emits radiation characteristics of the sample.
- The emitted radiation passes through a Monochromator that isolates the specific wavelength for desire analysis. A photo-detector measures the radiant power of the selected radiation, which is then amplified and sent to a readout device.

- Solution is introduced into the flame as a spray. This is normally achieved using an aspirator. Solution is drawn out of the sample holder using a pump and fed into the gas stream through a thin nozzle creating an aerosol spray.
- Solvent evaporate leaving the dehydrated salt.
- Salt dissociated into free gaseous atoms in the ground state.
- A certain fraction of atoms absorbs energy and are raised into excited state.
- These excited atoms on returning to ground state emit photons of characteristic wavelength.
- The emission from the flame passes through a conventional Monochromator which filters out all emitted light except the wavelength of our interest.
- A photoelectric detector measures the intensity of the filtered light.



FLAME PHOTOMETER

Basic components for flame photometer are:

- Burner(source)
- Atomizer
- Monochromator

- Detector
- Readout device

Types of burners (Explanation in Page 29)

- Total combustion burner
- Laminar flow burner

FES Monochromator (Explanation in Page 9)

- Prism
- Grating

FES Detector (Explanation in Page 16)

- Photovoltaic Cell
- Phototubes
- Photomultiplier tubes.

Application of FES:

- FES has found wide application in agricultural and environmental analysis, industrial analyses of ferrous metals and alloys as well as glasses and ceramics materials, and clinical analyses of body fluids.
- They are also used to determine the metals present in chemicals, soils, cements, plant materials, water, Air pollutants and Oceanography.

Luminescence

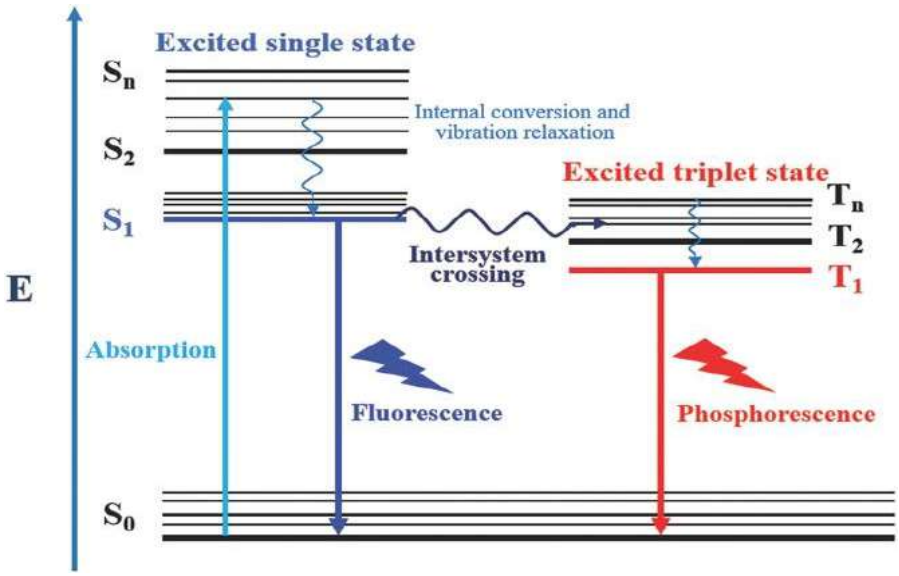
- Photoluminescence (due to Photons)
  - Fluorescence
  - Phosphorescence
- Chemiluminescence (due to chemical reactions)
- Electroluminescence (due to current)
- Incandescence (due to heat)
- Bioluminescence (by living organism)

Fluorescence spectroscopy

- **Atomic Fluorescence spectroscopy (AFS)** is a type of electromagnetic spectroscopy that analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain

compounds and causes them to emit light. A complementary technique is absorption spectroscopy.

- In fluorescence, the sample is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state.
- The molecule then drops down to one of the various vibrational levels of the ground electronic state again, emitting a photon in the process. As molecules may drop down into any of several vibrational levels in the ground state, the emitted photons will have different energies, and thus frequencies.



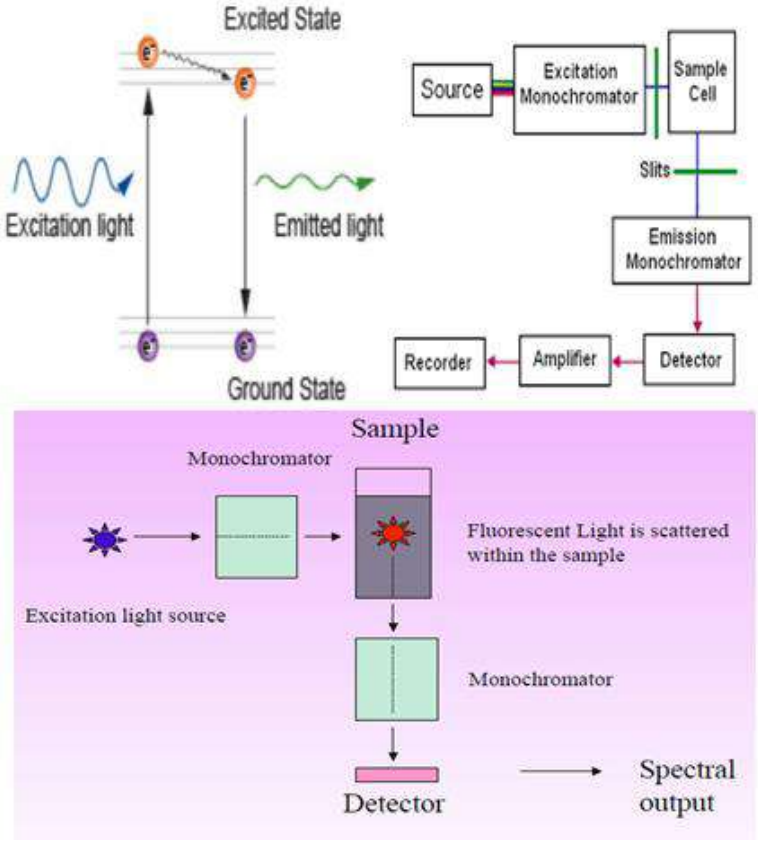
- Therefore, by analysing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined.
- Two general types of instruments exist: filter fluorometers that use filters to isolate the incident light and fluorescent light and spectrofluorometers that use a diffraction grating Monochromator to isolate the incident light and fluorescent light.
- Both types use the following scheme: the light from an excitation source passes through a filter or monochromator, and strikes the sample. A proportion of the incident light is absorbed by the sample, and some of the molecules in the sample fluoresce. The fluorescent light is emitted in all directions. Some of this fluorescent light passes through a second filter or monochromator and reaches a detector, which is usually placed at 90° to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the detector.
- **Sources:** Various light sources may be used as excitation sources, including lasers, LED, and lamps; xenon arcs and mercury-vapour lamps in particular. A mercury vapour lamp is a line lamp, meaning it emits light near peak wavelengths. By

contrast, a xenon arc has a continuous emission spectrum with nearly constant intensity in the range from 300-800 nm and a sufficient irradiance for measurements down to just above 200 nm.

- **Filters and/or Monochromator** may be used in fluorometers. A monochromator transmits light of an adjustable wavelength with an adjustable tolerance. The most common type of monochromator utilizes a diffraction grating, that is, collimated light illuminates a grating and exits with a different angle depending on the wavelength. The monochromator can then be adjusted to select which wavelengths to transmit. The addition of two polarization filters is necessary: One after the excitation monochromator or filter, and one before the emission monochromator or filter. As mentioned before, the fluorescence is most often measured at a 90° angle relative to the excitation light. This geometry is used instead of placing the sensor at the line of the excitation light at a 180° angle in order to avoid interference of the transmitted excitation light.

Detectors (Explanation in Page 16)

- Photodiode
- Photo-emissive Tube
- Photomultiplier Tube



APPLICATION:

- Fluorescence spectroscopy is used in biochemical, medical for analysing organic compounds. There has also been a report of its use in differentiating malignant, bashful skin tumours from benign.
- Fluorescence spectroscopy techniques are useful in other kind of analysis/ measurement of a compound present in air or water or other media, such as CVAFS which is used for heavy metal detection, such as mercury.
- Fluorescence detectors are used with HPLC.

Unit - 2

Chromatography

'Chromatography' is an analytical technique commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed.

Principle of separation

Principle of separation of different components: Differential affinities (strength of adhesion) of the various components of the analyte towards the stationary and mobile phase results in the differential separation of the components. Affinity, in turn, is dictated by two properties of the molecule: 'Adsorption' and 'Solubility'.

We can define adsorption as the property of how well a component of the mixture sticks to the stationary phase, while solubility is the property of how well a component of the mixture dissolves in the mobile phase.

- Higher the adsorption to the stationary phase, the slower the molecule will move through the column.
- Higher the solubility in the mobile phase, the faster the molecule will move through the column.

So, the interplay between the above two factors determines the differential rates at which the different components of the analyte will move through the column. Adsorption and solubility of a molecule can be manipulated by choosing the appropriate stationary phase and mobile phase.

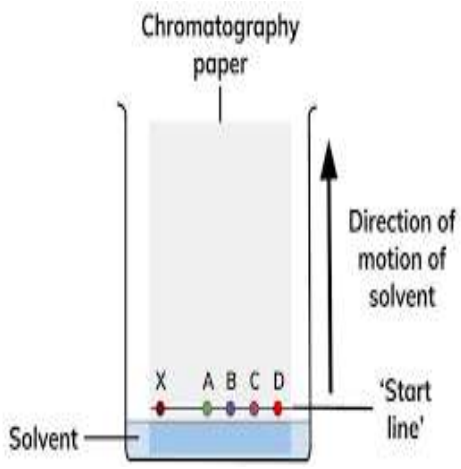
Classification of Chromatography

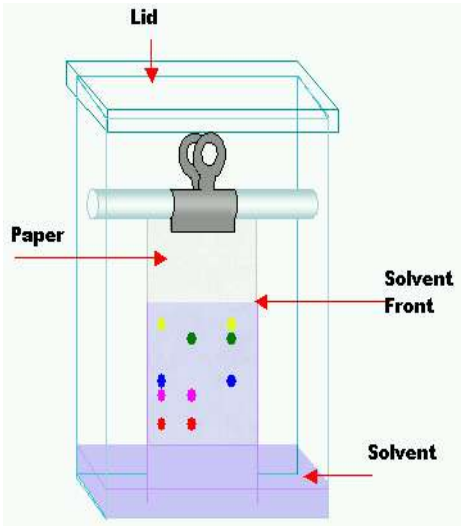
1. **Based on Mechanism of separation**
    1. Absorption Chromatography
    2. Partition Chromatography
    3. Size Exclusion Chromatography
    4. Ion Exchange Chromatography
    5. Affinity Chromatography
2. **Based on Type of mobile Phases**
    1. Gas Phase Chromatography
        1. Gas - Solid Chromatography(GSC)
        2. Gas - Liquid Chromatography(GLC)
    2. Liquid Phase Chromatography
        1. Liquid- liquid Chromatography(LSC)
        2. Liquid – Solid Chromatography(LLC)
3. **Based on Shape of Chromatography Bed**
    1. Planner Chromatography
        1. Paper Chromatography
        2. Thin Layer Chromatography
    2. Column Chromatography
        1. Packed column Chromatography
        2. Open Tubular column Chromatography

Paper Chromatography

Paper chromatography procedure runs on a piece of specialized paper. It is a planar chromatography system wherein a cellulose filter paper acts as a stationary phase on which the separation of compounds occurs. The principle of separation is mainly partition rather than absorption, cellulose layers in the filter paper contains moisture which acts as stationary phase. Organic solvents or buffers are used as mobile phases. Instead of water as stationary phase other organic solvents can be used by suitable modification.

- There are two types of Paper Chromatography
    - Paper Absorption Chromatography.
    - Paper Partition Chromatography.





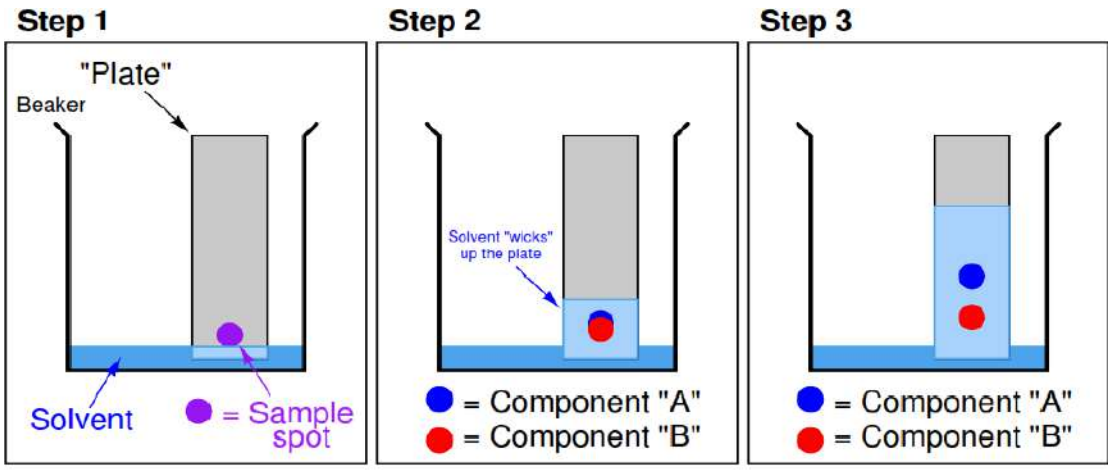


Figure 2

Principle of paper chromatography: The principle involved is partition chromatography wherein the substances are distributed or partitioned between liquid phases. One phase is the water, which is held in the pores of the filter paper used; and other is the mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase solvents during the movement of mobile phase under the capillary action of pores in the paper.

The principle can also be adsorption chromatography between solid and liquid phases, wherein the stationary phase is the solid surface of the paper and the liquid phase is of the mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography, i.e., partitioned between two liquid phases.

The Paper remaining after the experiment is known as the Chromatogram.

Modes of Paper Chromatography

Based on the way the development of chromatogram on paper is done in procedures, we have, broadly, five types of chromatography.

- Ascending chromatography
- Descending chromatography
- Circular/ radial chromatography(Horizontal)
- Two dimensional Chromatography

1. Ascending chromatography: As the name indicates, the chromatogram ascends. Here, the development of paper occurs due to the solvent movement or upward

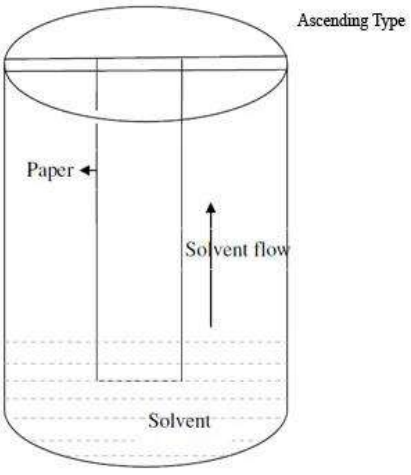
travel on the paper. The solvent reservoir is at the bottom of the beaker. The paper tip with sample spots just dips into the solvent at the bottom so that spots remain well above the solvent.

2. **Descending chromatography**: Here, the development of paper occurs due to solvent travel downwards on the paper. The solvent reservoir is at the top. The movement of the solvent is assisted by gravity besides the capillary action.

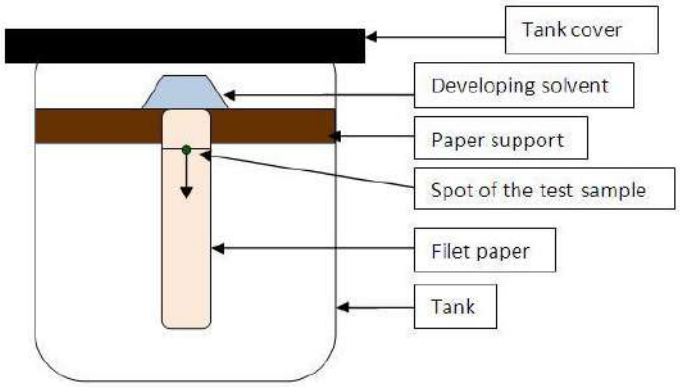
3. **Ascending- descending mode**: Here solvent first travels upwards and then downwards on the paper.

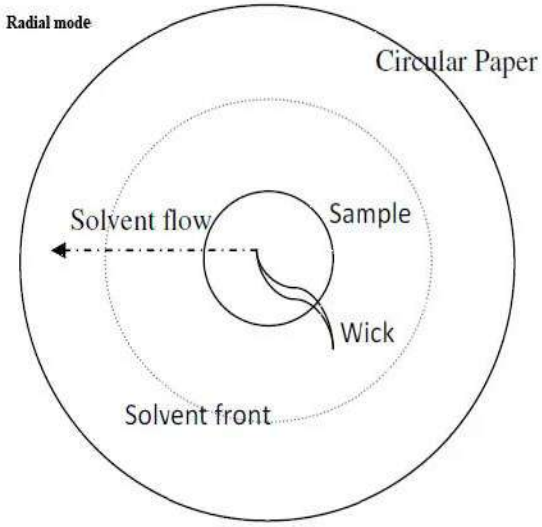
4. Radial mode: Here, the solvent moves from the center (mid-point) towards the periphery of circular chromatography paper. The entire system is kept in a covered Petri dish for the development of the chromatogram. The wick at the center of paper dips into the mobile phase in a Petri dish, by which the solvent drains on to the paper and moves the sample radially to form the sample spots of different compounds as concentric rings.

5. **Two-dimensional chromatography:** Here the chromatogram development occurs in two directions at right angles. In this mode, the samples are spotted to one corner of rectangular paper and allowed for first development. Then the paper is again immersed in the mobile phase at a right angle to the previous development for the second chromatogram.

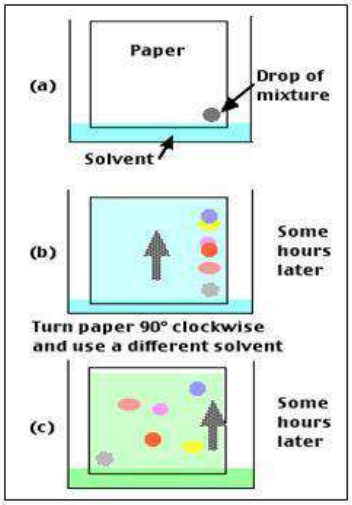


Descending chromatography





Two-dimensional Mode



Paper Chromatography Experiment Method

The experimental method involves:

a) **Selection of suitable type of development:** This depends on the complexity of the mixture, solvent, paper, etc. But in general ascending type or radial type chromatography are used as they are easy to perform, handle, less time-consuming and also give chromatogram faster.

b) **Selection of suitable filter paper**: Filter paper is selected based on pore size, the quality of the sample to be separated, and also the mode of development.

c) **Preparation of sample:** Preparation of the sample involves the dissolution of the sample in a suitable solvent used in making mobile phase. The solvent used should be inert with the sample under analysis.

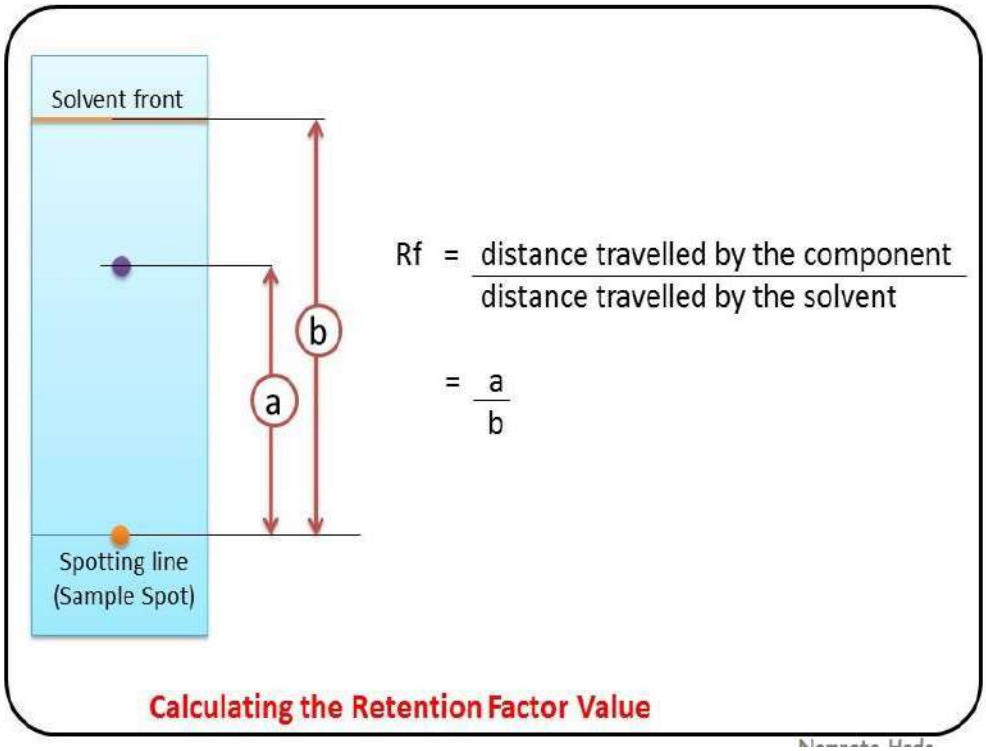
Spotting of sample on the paper: Samples are spotted at the proper position on the paper using a capillary tube.

e) **Development of chromatogram:** Sample spotted paper is subjected to development by immersing it in the mobile phase. The mobile phase moves over the sample on the paper under the capillary action of paper.

f) **Drying of the paper and detection of the compounds**: Once the development of chromatogram is over; the paper is held carefully at the borders to avoid touching the sample spots and dried using an air drier.

Different compounds in the sample mixture travel at different rates due to

- ✓ differences in solubility in the solvent
- ✓ Differences in their attraction to the fiber in the paper.



Advantages of Paper chromatography

- Paper chromatography requires very less quantitative material.
- Paper chromatography is cheaper compared to other chromatography methods.
- Both unknown inorganic as well as organic compounds can be identified by Paper chromatography method.

Disadvantages of Paper chromatography

- Large quantity of sample cannot be applied on Paper chromatography, hence in quantitative analysis Paper chromatography is not effective.
- Complex mixture cannot be separated by Paper chromatography.
- Less accurate compared to HPLC and HPTLC.

Paper Chromatography- Application

- Paper Chromatography is specially used for separation of mixtures having polar and non polar compounds.
- For separation of amino acids.
- It is used to determine organic compound, biochemical in urine, etc.
- Some time used for evolution of inorganic compound like salt and complex.

Thin Layer Chromatography

Thin Layer Chromatography is a technique used to separate mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel(stationary phase).

Like other chromatographic techniques, thin-layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

TLC system components consist of

TLC plates, preferably ready-made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in fine particle size.

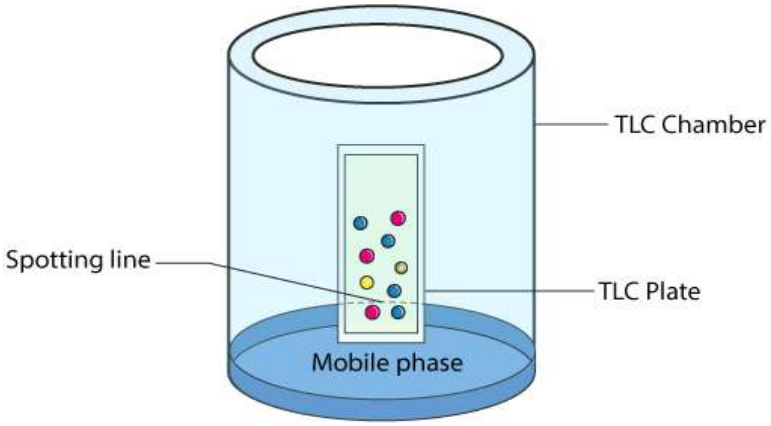
TLC chamber. This is used for the development of the TLC plate. The chamber maintains a stable environment inside for proper development of spots. It also prevents the evaporation of solvents and keeps the process dust-free.

Mobile phase. This comprises of a solvent or solvent mixture. The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

A filter paper. This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.

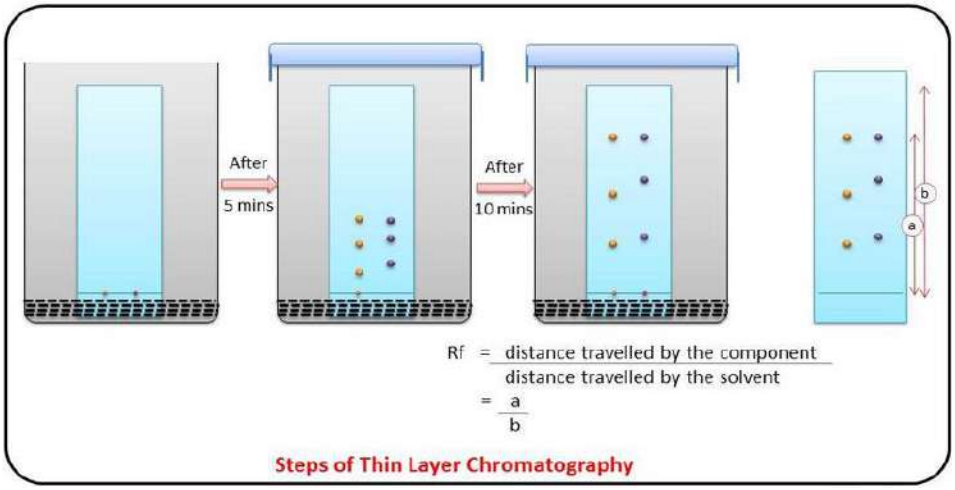
Procedure

- The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize. These days, however, ready-made plates are preferred.
- With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots.
- Then, samples solutions are applied on the spots marked on the line in equal distances.
- The mobile phase is poured into the TLC chamber to a levelled few centimetres above the chamber bottom.



- Now, the plate prepared with sample spotting is placed in the TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.
- The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development.

- Allow sufficient time for the development of spots. Then remove the plates and allow them to dry. The sample spots can now be seen in a suitable UV light chamber or any other methods as recommended for the said sample.



Advantages of Thin Layer Chromatography:

- It is a simple process with short development time.
- It helps with the visualization of separated compound spots easily.
- The method helps to identify the individual compounds.
- It helps in isolating of most of the compounds.
- The separation process is faster and the selectivity for compounds is higher (even small differences in chemistry is enough for clear separation).
- The purity standards of the given sample can be assessed easily.
- It is a cheaper chromatographic technique.

Disadvantages of Thin Layer Chromatography:

- Thin Layer Chromatography plates do not have longer stationary phase.
- When compared to other chromatographic techniques the length of separation is limited.
- The results generated from TLC are difficult to reproduce.

- Since TLC operates as an open system, some factors such as humidity and temperature can be consequences to the final outcome of the chromatogram.
- The detection limit is high and therefore if you want a lower detection limit, you cannot use TLC.
- It is only a qualitative analysis technique and not quantitative.

Thin Layer Chromatography Applications

- The qualitative testing of various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.
- TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.
- Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc
- It is widely used in separating multi component pharmaceutical formulations.
- It is used to purify of any sample and direct comparison is done between the sample and the authentic sample
- It is used in the food industry, to separate and identify colours, sweetening agent, and preservatives
- It is used in the cosmetic industry.
- It is used to study if a reaction is complete.

Gas chromatography

A gas chromatograph (GC) is an analytical instrument that measures the content of various components in a sample.

Principle of gas chromatography: The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column. To measure a sample with an unknown

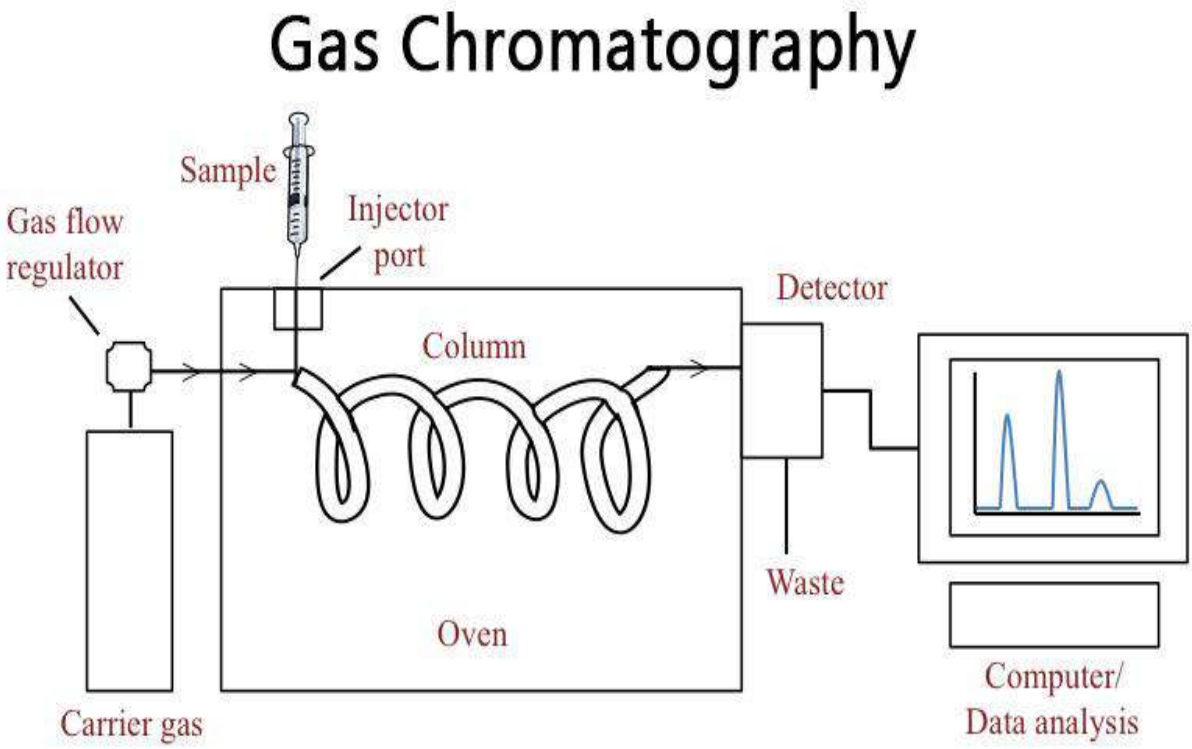
concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention time (appearance time) and area are compared to the test sample to calculate the concentration.

Gas chromatography differs from other forms of chromatography in that the mobile phase is a gas and the components are separated as vapours.

It is thus used to separate and detect small molecular weight compounds in the gas phase.

The sample is either a gas or a liquid that is vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, typically helium because of its low molecular weight and being chemically inert.

The pressure is applied and the mobile phase moves the analyte through the column. The separation is accomplished using a column coated with a stationary phase.



The Principle of separation for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.

Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer **retention time (Rt)** than samples that have a higher affinity for the mobile phase.

Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

Ideal peaks are Gaussian distributions and symmetrical, because of the random nature of the analyte interactions with the column.

The separation is hence accomplished by partitioning the sample between the gas and a thin layer of a non-volatile liquid held on a solid support.

Procedure:

- A sample containing the solutes is injected into a heated block where it is immediately vaporized and swept as a plug of vapour by the carrier gas stream into the column inlet.
- The solutes are absorbed by the stationary phase and then desorbed by a fresh carrier gas.
- The process is repeated in each plate as the sample is moved toward the outlet.
- Each solute will travel at its own rate through the column.
- Their bands will separate into distinct zones depending on the partition coefficients, and band spreading.
- The solutes are eluted one after another in the increasing order of their Rt, and enter into a detector attached to the exit end of the column.
- Here they register a series of signals resulting from concentration changes and rates of elution on the recorder as a plot of time versus the composition of carrier gas stream.
- The appearance time, height, width, and area of these peaks can be measured to yield quantitative data.

Parts of Gas chromatography

Gas chromatography is mainly composed of the following parts:

1. Carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters

- Helium, N_2, H, Argon are used as carrier gases.
- Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors.
- N_2 is preferable when a large consumption of carrier gas is employed.
- Carrier gas from the tank passes through a toggle valve, a flow meter, (1-1000 ml/min), capillary restrictors, and a pressure gauge (1-4 atm).
- Flow rate is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors.
- The operating efficiency of the gas chromatograph is directly dependant on the maintenance of constant gas flow.

2. Sample injection system

- Liquid samples are injected by a micro syringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater.
- Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves.
- Typical sample volumes range from 0.1 to 0.2 ml.

3. The separation column

- The heart of the gas chromatography is the column which is made of metals bent in U shape or coiled into an open spiral or a flat pancake shape.
- Copper is useful up to 250^0
- Swege lock fittings make column insertion easy.
- Several sizes of columns are used depending upon the requirements.

4. Liquid phases

- An infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support.

- No single phase will serve for all separation problems at all temperatures.
- **Non-Polar –** Parafin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.
- **Intermediate Polarity –** These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and non-polar solutes. For example. diethyl hexyl phthalate is used for the separation of high boiling alcohols.
- **Polar –** Carbowaxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.
- **Hydrogen bonding –** Polar liquid phases with high hydrogen bonding e.g. Glycol.
- **Specific purpose phases –** Relying on a chemical reaction with solute to achieve separations. e.g AgNO3 in glycol separates unsaturated hydrocarbons.

5. Supports

- The structure and surface characteristics of the support materials are important parameters, which determine the efficiency of the support and the degree of separation respectively.
- The support should be inert but capable of immobilizing a large volume of liquid phase as a thin film over its surface.
- The surface area should be large to ensure the rapid attainment of equilibrium between stationary and mobile phases.
- Support should be strong enough to resist breakdown in handling and be capable of packed into a uniform bed.
- Diatomaceous earth, kieselguhr treated with Na 2CO 3 for 900^0 C causes the particle fusion into coarser aggregates.
- Glass beads with a low surface area and low porosity can be used to coat up to 3% stationary phases.
- Porous polymer beads differing in the degree of cross-linking of styrene with alkyl-vinyl benzene are also used which are stable up to 250^0

6. Detector

- Detectors sense the arrival of the separated components and provide a signal.
- These are either concentration-dependent or mass dependant.
- The detector should be close to the column exit and the correct temperature to prevent decomposition.

7. Recorder

- The recorder should be generally 10 mv (full scale) fitted with a fast response pen (1 sec or less). The recorder should be connected with a series of good quality resistances connected across the input to attenuate the large signals.
- An integrator may be a good addition.

The procedure of Gas Chromatography

Step 1: Sample Injection and Vaporization

- A small amount of liquid sample to be analyzed is drawn up into a syringe.
- The syringe needle is positioned in the hot injection port of the gas chromatograph and the sample is injected quickly.
- The injection of the sample is considered to be a "point" in time, that is, it is assumed that the entire sample enters the gas chromatograph at the same time, so the sample must be injected quickly.
- The temperature is set to be higher than the boiling points of the components of the mixture so that the components will vaporize.
- The vaporized components then mix with the inert gas mobile phase to be carried to the gas chromatography column to be separated.

Step 2: Separation in the Column

- Components in the mixture are separated based on their abilities to adsorb on or bind to, the stationary phase.
- A component that adsorbs most strongly to the stationary phase will spend the most time in the column (will be retained in the column for the longest time) and

will, therefore, have the longest retention time (Rt). It will emerge from the gas chromatograph last.

- A component that adsorbs the least strongly to the stationary phase will spend the least time in the column (will be retained in the column for the shortest time) and will, therefore, have the shortest retention time (Rt). It will emerge from the gas chromatograph first.
- If we consider a 2 component mixture in which component A is more polar than component B then:
- component A will have a **longer retention time** in a polar column than component B
- component A will have a **shorter retention time** in a non-polar column than component B

Step 3: Detecting and Recording Results

- The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.
- The component that is retained the shortest time in the column is detected first. The component that is retained the longest time in the column is detected last.
- The detector sends a signal to the chart recorder which results in a peak on the chart paper. The component that is detected first is recorded first. The component that is detected last is recorded last.

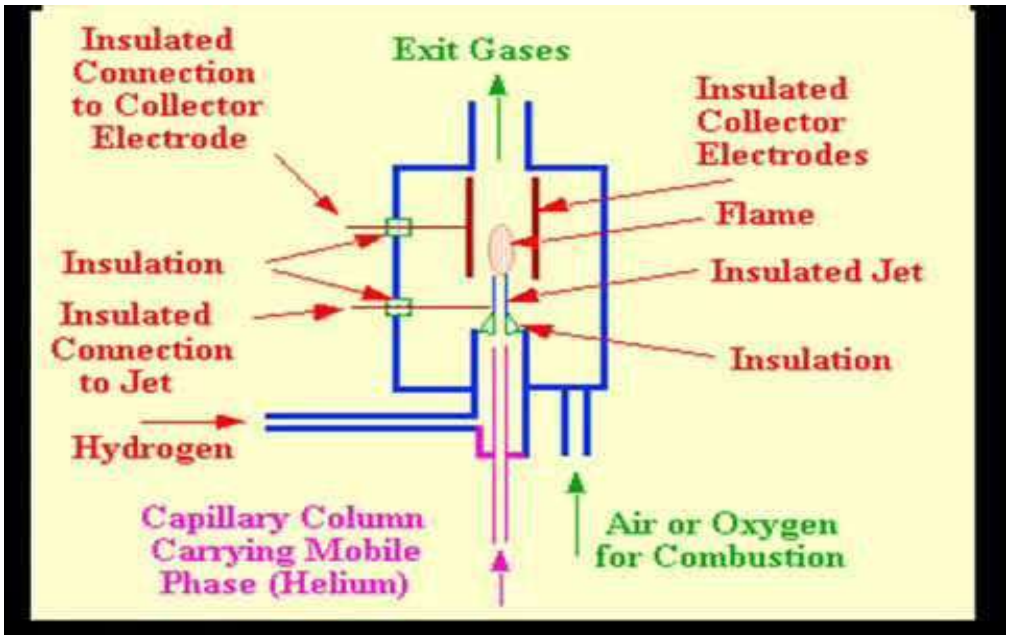
Gas chromatography Detectors

- FLAME IONIZATION DETECTOR (FID)
- THERMAL CONDUCTIVITY DETECTOR (TCD)
- ELECTRON CAPTURE DETECTOR (ECD)
- FLAME PHOTOMETRIC DETECTOR (FPD)
- PHOTOIONIZATION DETECTOR (PID)
- ELECTROLYTIC CONDUCTIVITY DETECTOR (ELCD)

- NITROGEN PHOSPHORUS DETECTOR (NPD)

Flame Ionization Detector (FID)

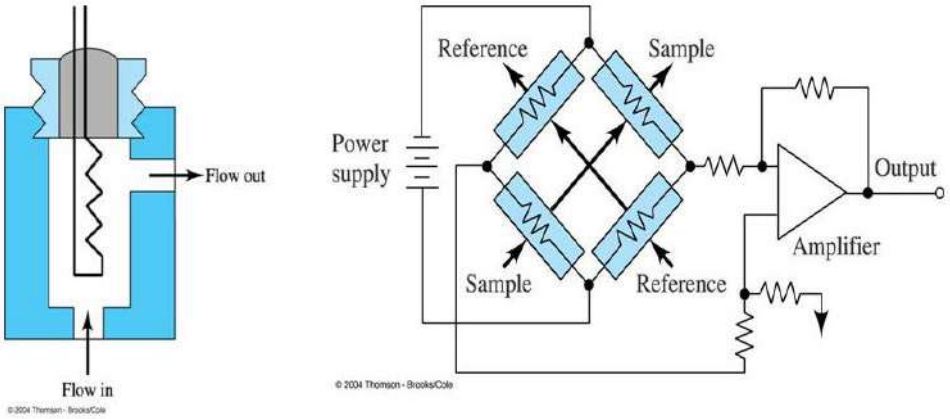
- FID is the most widely used and generally applicable detector in GC.
- Effluent from the column is directed into a small air/ H2 flame and most organic compounds produce ions and electrons when pyrolyzed at the temperature of an air /H2 flame.
- Compound is detected by monitoring the current produced by collecting the ions and electrons.
- A few hundred volts applied between the burner tip and a collector electrode located above the flame serve to collect the ions and electrons.
- The current is then measure with a sensitive picoammeter.



Thermal Conductivity Detector

- It works on the principle of Wheatstone bridge.
- Out of four resistances in the circuit, the magnitude of three resistances remains constant.
- But that of fourth resistance varies as per changes in the temperature.

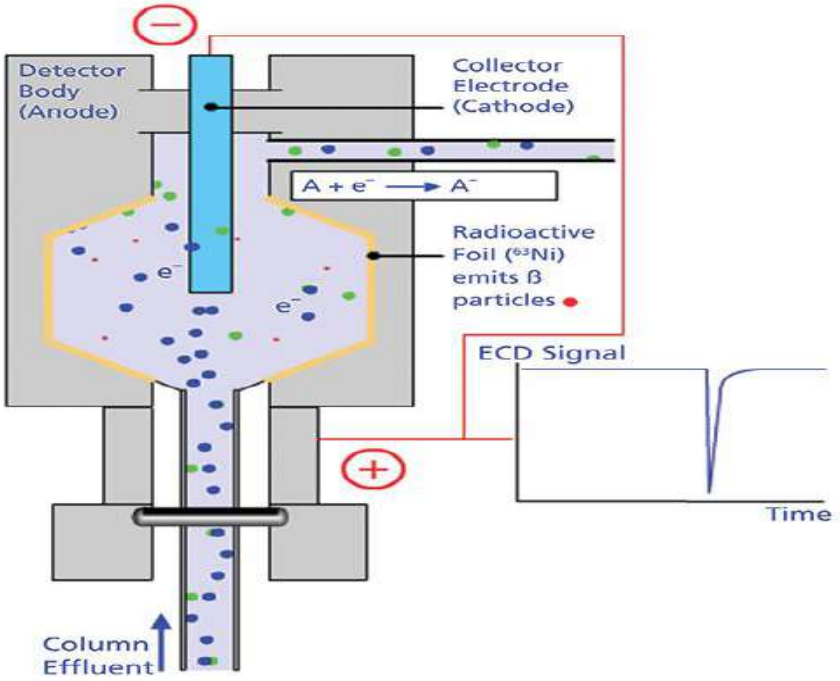
- This change is because of the difference in the capacity of solute and the carrier gas to absorb heat (thermal conductivity differences).
- The change in the temperature changes the resistance and hence the current in circuit.



Schematic of a bridge circuit for TCD detection
Two filament in one cell (reference side) --- carrier gas only
The other cell (sample side) --- carrier plus sample flowing

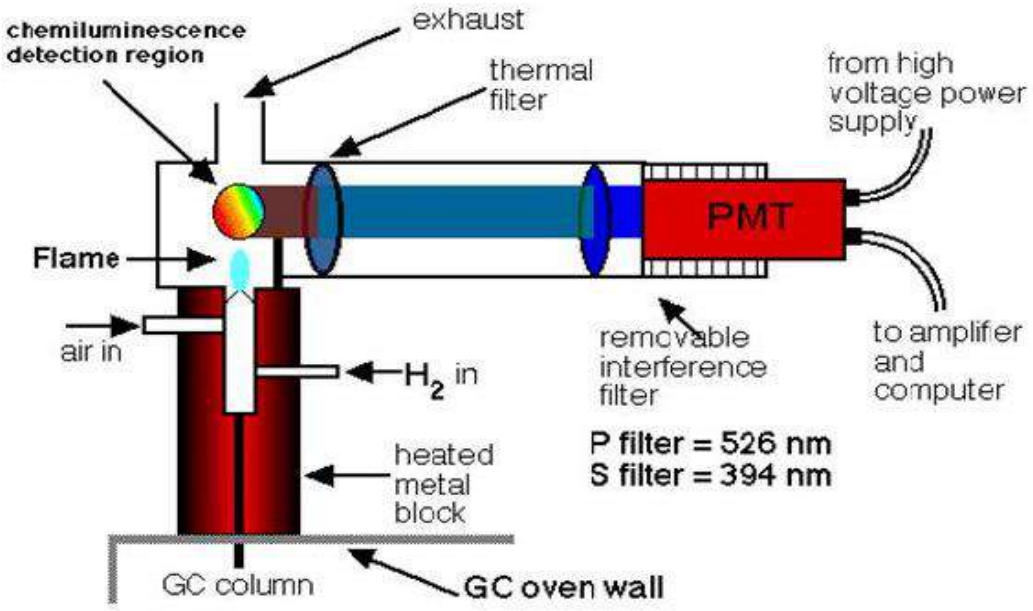
Electron Capture Detector (ECD)

- The electron capture detector has become one of the most widely used detector for environmental samples
- In ECD, the sample elute from a column is passed over a radioactive beta emitter.
- An electron from the emitter causes ionization of the carrier gas (often N2) and the Production of a burst of electrons.
- In the absence of organic species, a constant standing current between a pair of electrodes results from this ionization process.
- The current decreases in the presence of organic molecules containing electronegative functional groups that tend to capture electrons.



Flame Photometric Detector (FPD)

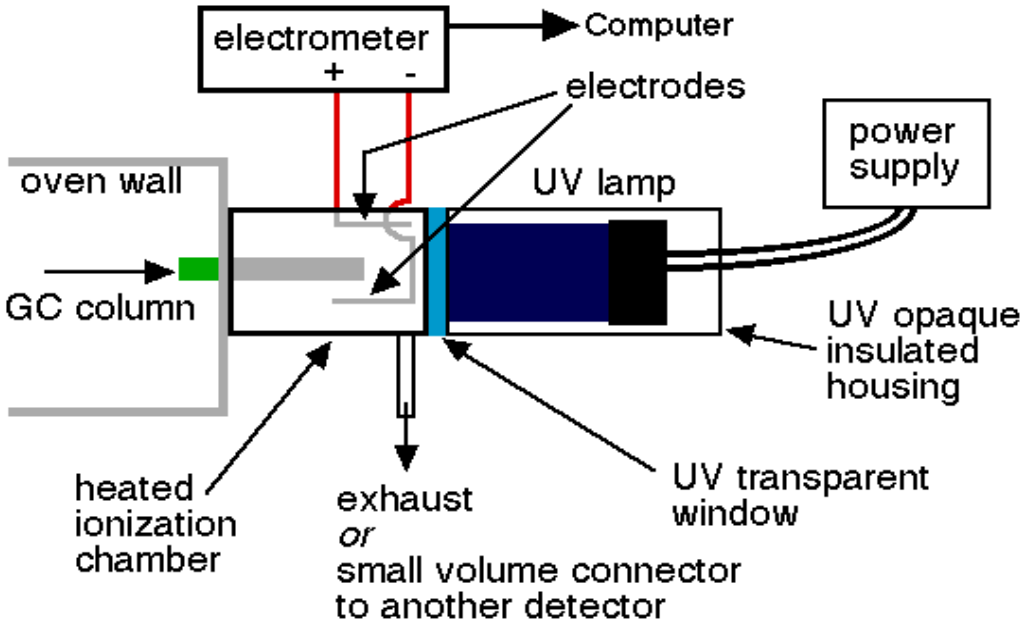
- The photoemission from the flame is monitored instead of the electric conductivity.
- The sample passes through the hydrogen/ air flame and light emission is observed due to the excitation of some of the atoms.
- The light in the UV/Visible region of the spectrum is selected by using suitable filters and is measured by a photomultiplier tube. The light emitting processes that produce the sulphur and phosphorous sensitivity occur in the upper portion of the flame that does not emit appreciably in the absence of sulphur and phosphorous.
- Selective to compounds containing sulphur and phosphorous.



Schematic of a gas chromatographic flame photometric detector

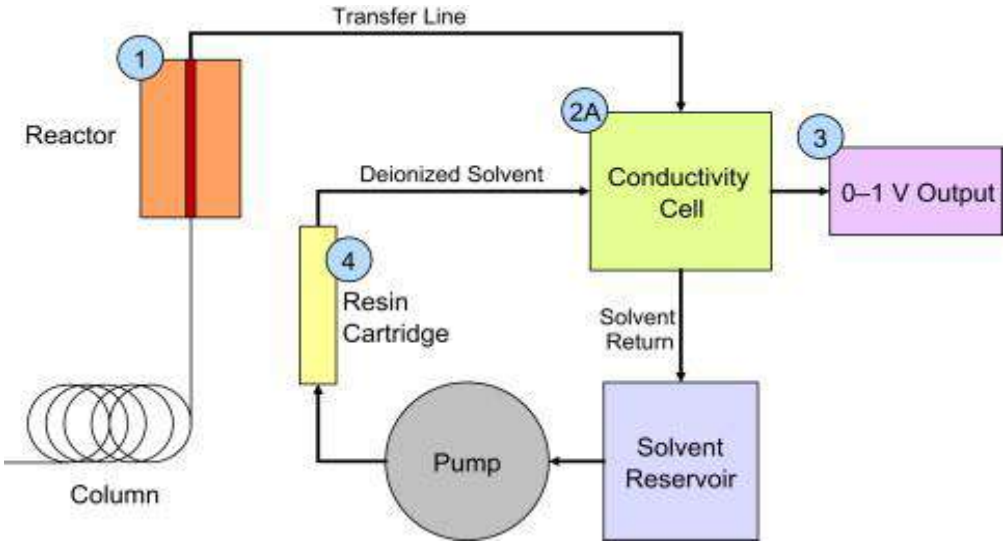
Photo Ionization Detector (PID)

- Photo Ionization Detector or GC-PID is a technique used to analyse a wide range of aromatic hydrocarbons and other organic compounds. A typical application is the analysis of hydrocarbon pollution of water. The PID uses ultraviolet light to ionize the components exiting the column.



ELECTROLYTIC CONDUCTIVITY DETECTOR (ELCD)

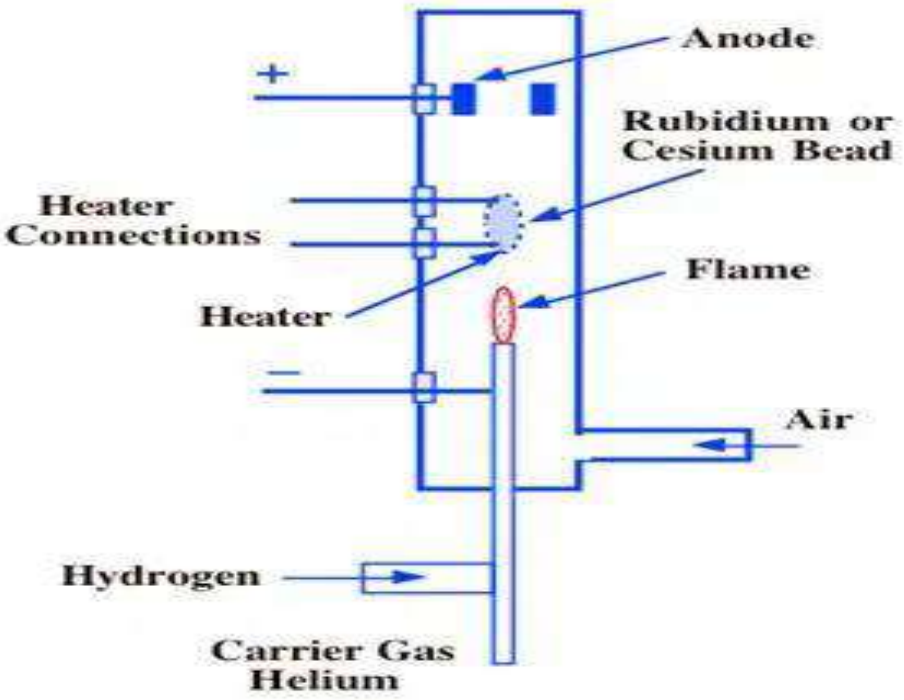
- The electrolytic conductivity detector is for compounds containing halogens, sulphur, or nitrogen.
- Compounds are mixed with a reaction gas in a small high temperature reaction tube.
- Specific reaction products are created which mix with a solvent liquid and pass through an electrolytic conductivity cell.
- The change in conductivity as a result of the presence of the active compound is then measured.
- Reaction tube temperature and solvent determine which types of compounds are detected.



NITROGEN PHOSPHORUS DETECTOR (NPD)

- The Nitrogen – Phosphorous Detector is also known as Thermionic Specified Detector (TSD) or Alkali flame ionization detector & is commonly used in gas chromatography.
- NPD uses a Hydrogen/ Air flame through which the sample is passed.
- NPD uses a rubidium/ cesium bead which is heated by a coil, over which the carrier gas mixed with Hydrogen passes.

- The hot bead emits electrons by which are collected at the anode and provides the background current.
- When a component that contains N/P exits the column, the partially combusted N/P materials are absorbed on the surface of the bead.
- This then increases the emission of electros**.**



Advantages

- The use of longer columns and higher velocity of carrier gas permits the fast separation in a matter of a few minutes.
- Higher working temperatures up to 5000C and the possibility of converting any material into a volatile component make gas chromatography one of the most versatile techniques.
- GC is popular for environmental monitoring and industrial applications because it is very reliable and can be run nearly continuously.
- GC is typically used in applications where small, volatile molecules are detected and with non-aqueous solutions.
- GC is favoured for non-polar molecules.

Limitations

- Compound to be analyzed should be stable under GC operation conditions.
- They should have a vapour pressure significantly greater than zero.
- Typically, the compounds analyzed are less than 1000 Da, because it is difficult to vaporize larger compounds.
- The samples are also required to be salt-free; they should not contain ions.
- Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

Applications

- GC analysis is used to calculate the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water.
- Gas chromatography is used in the analysis of:

  (a) air-borne pollutants

  (b) performance-enhancing drugs in athlete's urine samples

  (c) oil spills

  (d) essential oils in perfume preparation

- GC is very accurate if used properly and can measure Pico-moles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.
- Gas Chromatography is used extensively in forensic science. Disciplines as diverse as solid drug dose (pre-consumption form) identification and quantification, arson investigation, paint chip analysis, and toxicology cases, employ GC to identify and quantify various biological specimens and crime-scene evidence.

Liquid Chromatography

Liquid chromatography (LC) is a separation process used to isolate the individual components of a mixture. This process involves mass transfer of a sample through a polar mobile phase and non-polar stationary phase.

The device is a column packed with the porous medium made of a granular solid material (i.e., stationary phase), such as polymers and silica, where the sample is injected and the solvent (i.e., mobile phase) passes to transport the sample.

When a sample is injected, it is adsorbed on the stationary phase, and the solvent passes through the column to separate the compounds one by one, based on their relative affinity to the packing materials and the solvent. The component with the most affinity to the stationary phase is the last to separate. This is because high affinity corresponds to more time to travel to the end of the column.

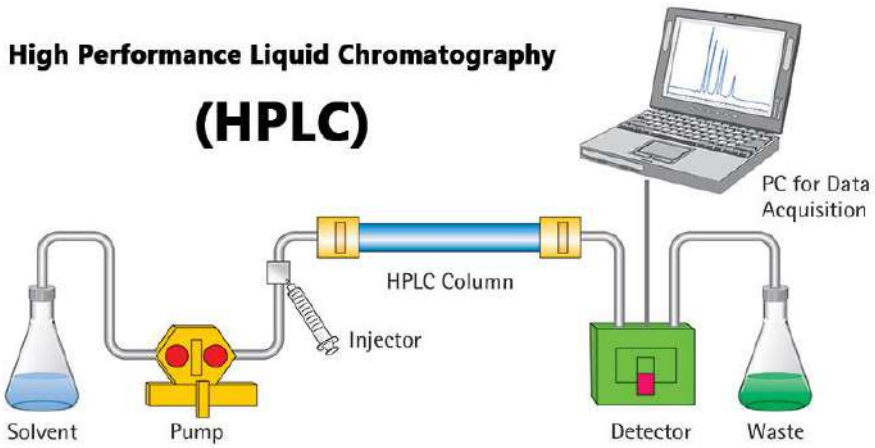
Differences between LC and HPLC

- High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography, is an advanced type of LC. HPLC is amenable to a wide range of applications, such as pharmaceuticals and food analysis. It is especially useful for low or non-volatile organic compounds, which cannot be handled with gas chromatography.
- The difference between traditional LC and HPLC is that the solvent in LC travels by the force of gravity. In the application of HPLC, the solvent travels under high pressure obtained by means of a pump to overcome the pressure drop in the packed column, which reduces the time of separation. A continuous flow syringe pump is very useful in HPLC.

High performance liquid chromatography – HPLC

- High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture.
- The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.
- In the 1960s the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.

- HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.



Principle of HPLC

- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.

- The chromatogram allows the identification and quantification of the different substances.

Instrumentation of HPLC

The Pump

- The development of HPLC led to the development of the pump system.
- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.
- High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.
- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector

- An injector is placed next to the pump.
- The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in stainless steel housing, instead of glass columns.
- The packing material generally used is silica or polymer gels compared to calcium carbonate. The eluent used for LC varies from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector

- Separation of analyte is performed inside the column, whereas a detector is used to observe the obtained separation.
- The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
- This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

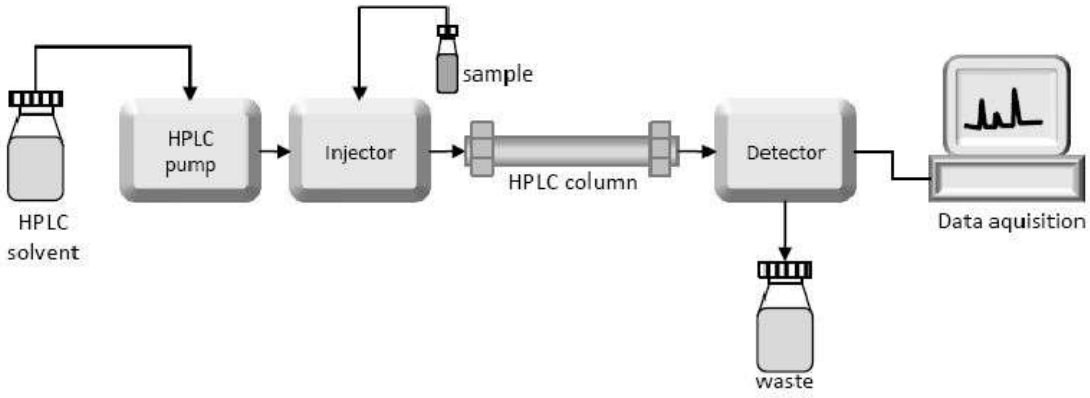
Degasser

- The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.
- When gas is present in the eluent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.
- The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.

Column Heater

- The LC separation is often largely influenced by the column temperature.

- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater).



Types of HPLC

Normal phase:

- Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds.

Reverse phase:

- The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable and ionic samples.

Ion exchange:

- Column packing contains ionic groups and the mobile phase is buffer. It is used to separate anions and cations.

Size exclusion:

- Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later.

Applications of HPLC

- The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.
- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ion-exchange chromatography of proteins

Advantages of HPLC

- Speed
- Efficiency
- Accuracy
- Versatile and extremely precise when it comes to identifying and quantifying chemical components.

Limitations

- **Cost:** Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics.

Complexity

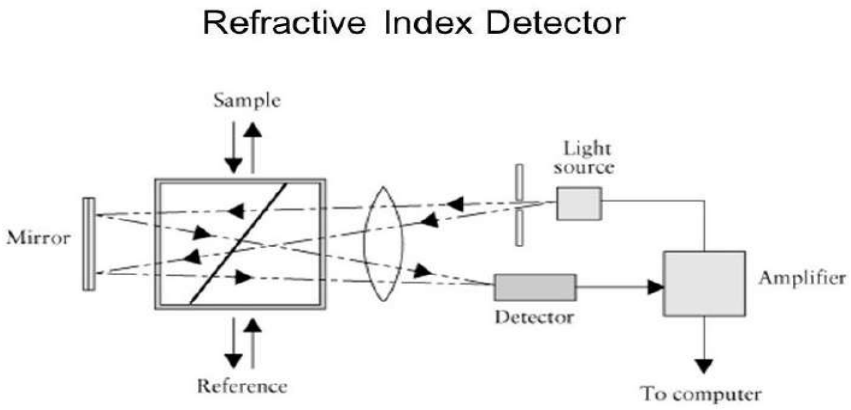
- HPLC does have **low sensitivity** for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
- Volatile substances are better separated by gas chromatography.

Detectors in HPLC

1. Refractive Index Detector
2. Evaporative Light Scattering Detector(ELSD)
3. UV/VIS Absorption Detector
4. Fluorescence Detector
5. Electrochemical Detector
6. Conductivity Detector

Refractive Index Detector

- Measures the overall ability of the mobile phase and its solutes to refract or bend light.
- When a solute is in a sample compartment, refractive index changes will shift the light beam from detector.
- Refractive index detector measures the molecule's ability to deflect light in a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference cell.
- The amount of deflection is proportional to the concentration of the solute in the mobile phase.



Evaporative Light Scattering Detector (ELSD)

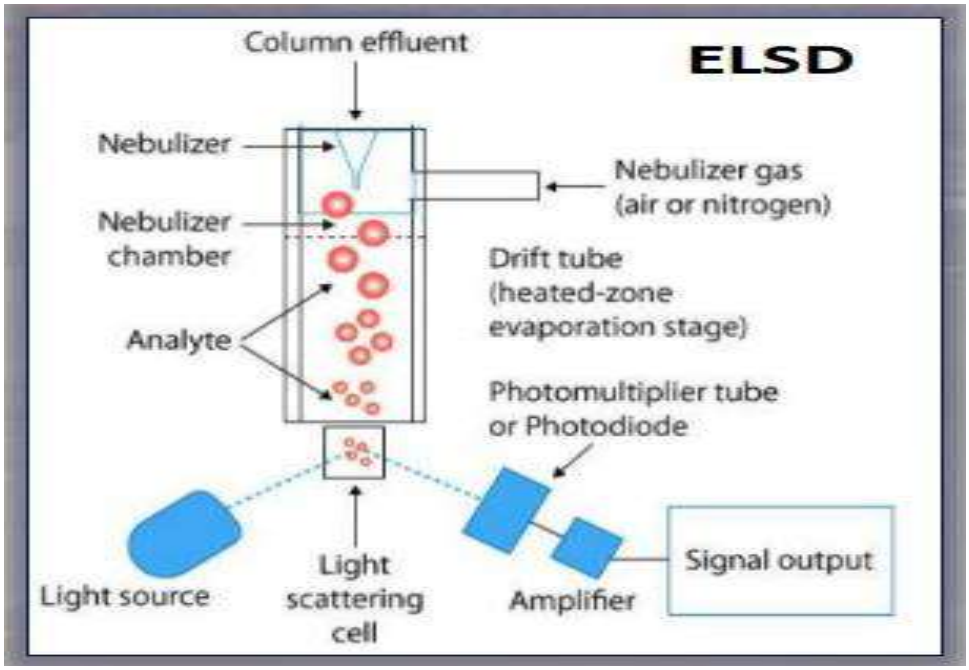
- Detection is based on the scattering of beam of light by particles of compound remaining after evaporation of the mobile phase. It is universal and destructive detector.

- There are three steps in detection:

1. Nebulization: The flow from the column is nebulized with a stream of inert gas (liquid into a fine spray or mist).

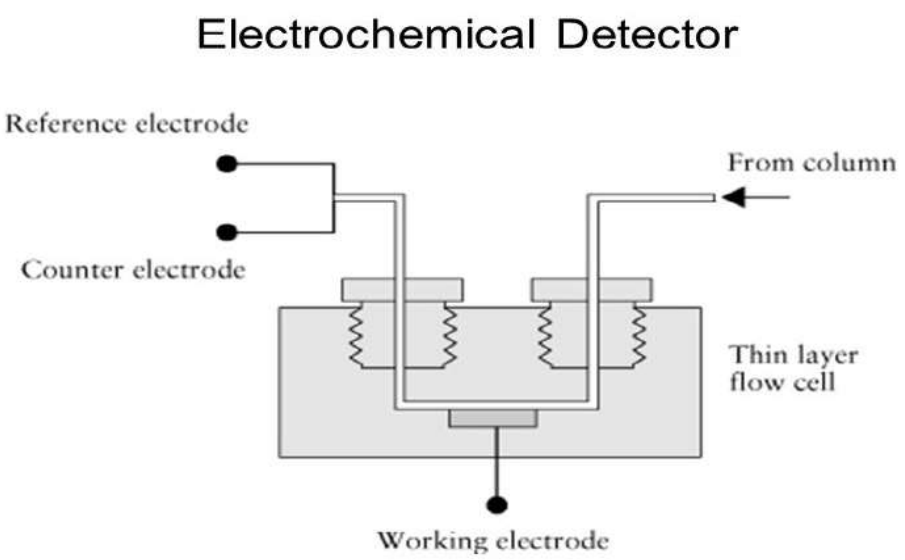
2. Mobile Phase Evaporation: The mobile phase, which must be volatile, is evaporated, leaving tiny particles of the analyte.

3. Detection: The Particles are passed through a laser beam and they scatter the laser light. The scattered light is measured at right angles to the laser beam by a photodiode detector.



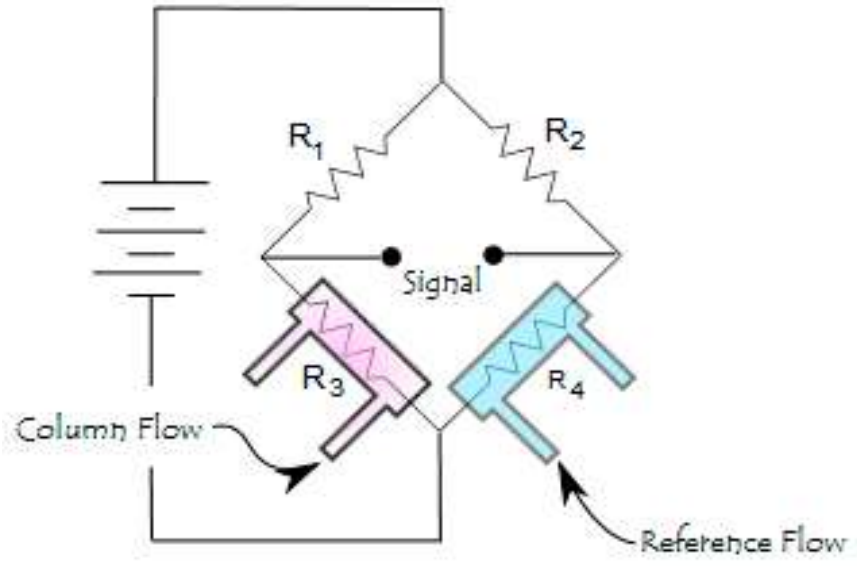
Electrochemical Detector

- It is based on the measurement of the current resulting from an oxidation/reduction reaction of the analyte at a suitable electrode.
- The level of current is directly proportional to the analyte concentration.
- Three electrodes are employed which are:
  - Working electrode: The elute is oxidized or reduced at the working electrode.
  - Auxiliary electrode
  - Reference electrode



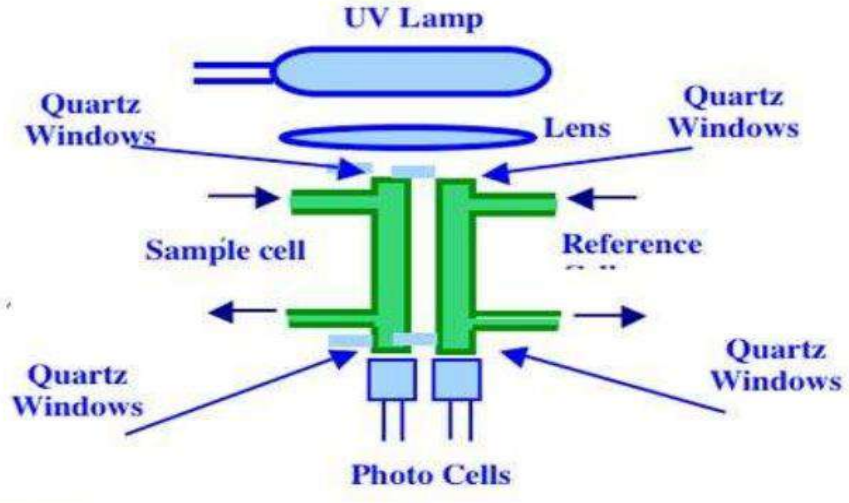
Thermal Conductivity Detector

The thermal conductivity detector (TCD), also known as a katharometer, is a bulk property detector and a chemical specific detector commonly used in gas chromatography. This detector senses changes in the thermal conductivity of the column eluent and compares it to a reference flow of carrier gas. Since most compounds have a thermal conductivity much less than that of the common carrier gases of helium or hydrogen, when an analyte elutes from the column the effluent thermal conductivity is reduced, and a detectable signal is produced.



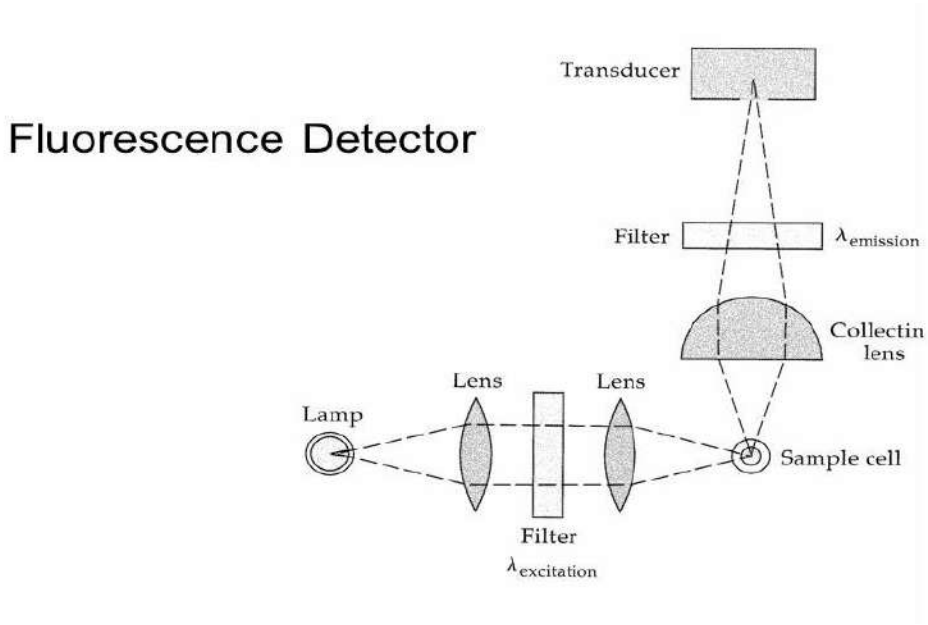
UV/VIS Absorption Detector

The UV, VIS, and PDA (Photodiode-Array Detection) detectors are categorized as absorbance detectors. They provide good sensitivity for light-absorbing compounds at ~pg level. They are easy to operate and provide good stability. UV detector is a very commonly used detector for HPLC analysis. During the analysis, sample goes through a clear color-less glass cell, called flow cell. When UV light is irradiated on the flow cell, sample absorbs a part of UV light. Thus, the intensity of UV light observed for the mobile phase (without sample) and the eluent containing sample will differ. By measuring this difference, the amount of sample can be determined. Since the UV absorbance also differs depend on what wavelength is used, it is important to choose an appropriate wavelength based on the type of analyte. A standard UV detector allows user to choose wavelength between 195 to 370 nm. Most commonly used is 254 nm. Compared to a UV detector, a VIS detector uses longer wavelength (400 to 700 nm). There are detectors that provide wider wavelength selection, covering both UV and VIS ranges (195 to 700 nm) called UV/VIS detector. PDA detects an entire spectrum simultaneously. UV and VIS detectors visualize the obtained result in two dimensions (light intensity and time), but PDA adds the third dimension (wavelength). This is convenient to determine the most suitable wavelength without repeating analyses.



Fluorescence Detector

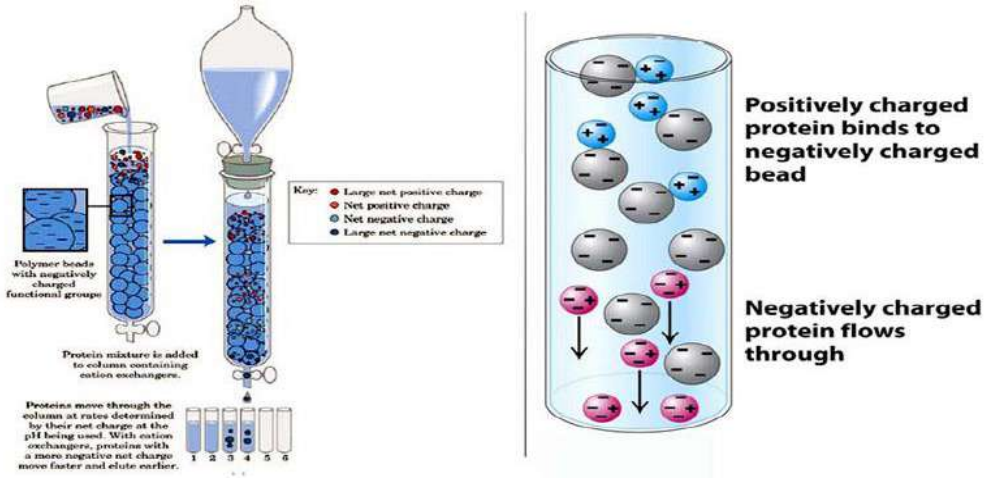
The advantage of fluorescence method is its high sensitivity for selective groups of compounds. By using a specific wavelength, analyte atoms are excited and then emit light signal (fluorescence). The intensity of this emitted light is monitored to quantify the analyte concentration. Most pharmaceuticals, natural products, clinical samples, and petroleum products have fluorescent absorbance. For some compounds which do not have fluorescence absorbance or low absorbance, they can be treated with fluorescence derivatives such as dansylchloride. The system is easy to operate and relatively stable.



Ion Exchange Chromatography

- **Chromatography** is the separation of a mixture of compounds into its individual components based on their relative interactions with an inert matrix.
- Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.
- The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.
- In this process two types of exchangers i.e., cationic and anionic exchangers can be used.
- **Cationic exchangers** possess negatively charged group, and these will attract positively charged cations. These exchangers are also called "Acidic ion exchange" materials, because their negative charges result from the ionization of acidic group.
- **Anionic exchangers** have positively charged groups that will attract negatively charged anions. These are also called "Basic ion exchange" materials.

- Ion exchange chromatography is most often performed in the form of column chromatography. However, there are also thin-layer chromatographic methods that work basically based on the principle of ion exchange.



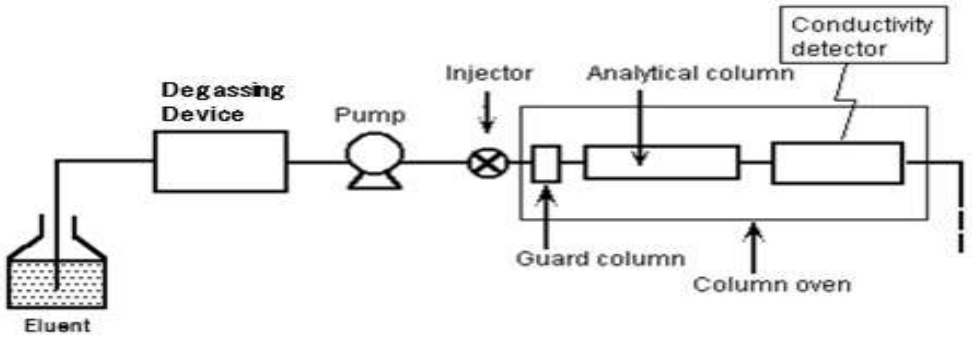
Working Principle of ion exchange chromatography

- This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
- The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
- The charged groups of the matrix can be positively or negatively charged.
- When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
- In this "ion cloud", ions can be reversibly exchanged without changing the nature and the properties of the matrix.

Instrumentation of ion exchange chromatography

Pump

- The IC pump is considered to be one of the most important components in the system which has to provide a continuous constant flow of the eluet through the IC injector, column, and detector.



Injector

- Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).

Columns

- Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass or an inert plastic such as PEEK. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyses or preparative work.

- Guard column is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column

Suppressor

- The suppressor reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. IC suppressors are membrane-based devices which are designed to convert the ionic eluet to water as a means of enhancing the sensitivity.

Detectors

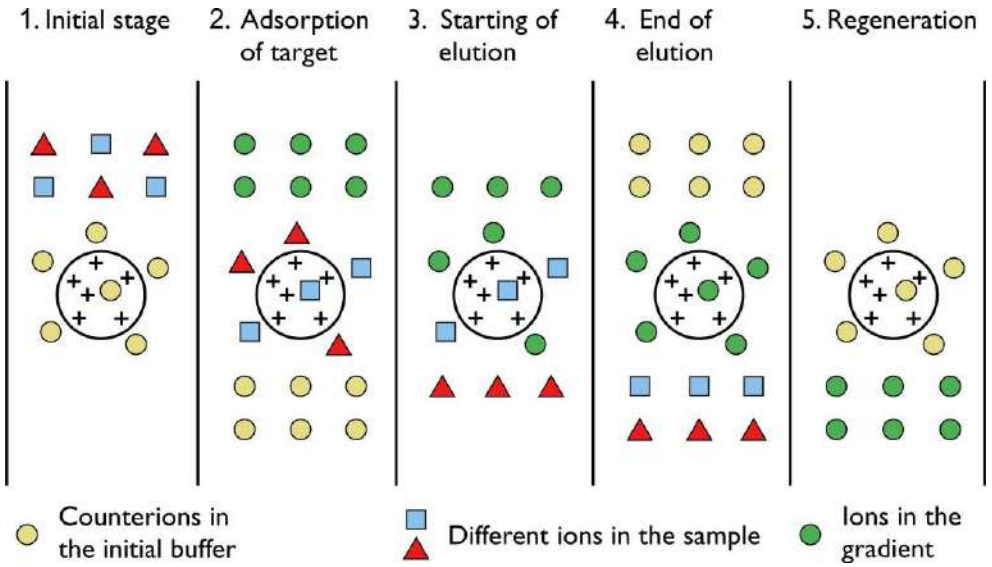
- Electrical conductivity detector is commonly use.

Data system

- In routine analysis, where no automation is needed, a pre-programmed computing integrator may be sufficient. For higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

Procedure of ion exchange chromatography

- Ion exchange separations are carried out mainly in columns packed with an ion-exchanger.
- These ionic exchangers are commercially available. They are made up of styrene and vinyl benzene. Example. DEAE-cellulose is an anionic exchanger, CM-cellulose is a cationic exchanger.
- The choice of the exchanger depends upon the charge of particle to be separated. To separate anions "Anionic exchanger" is used, to separate cations "Cationic exchanger" is used.
- First the column is filled with ion exchanger then the sample is applied followed by the buffer. The tri-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are widely used.
- The particles which have high affinity for ion exchanger will come down the column along with buffers.
- In next step using corresponding buffer separates the tightly bound particles.
- Then these particles are analyzed spectroscopic ally.



Applications of ion exchange chromatography

- An important use of ion-exchange chromatography is in the routine analysis of **amino acid** mixtures.
- The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
- This is most effective method for water purification. Complete deionization of water (or) a non-electrolyte solution is performed by exchanging solute cautions for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for softening of drinking water.
- In the analysis of products of hydrolysis of nucleic acids. In this way, information is gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
- Chelating resins are used to collect trace metals from seawater.
- To analyze lunar rocks and rare trace elements on Earth.

Advantages of ion exchange chromatography

- It is one of the most efficient methods for the separation of charged particles.
- It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.

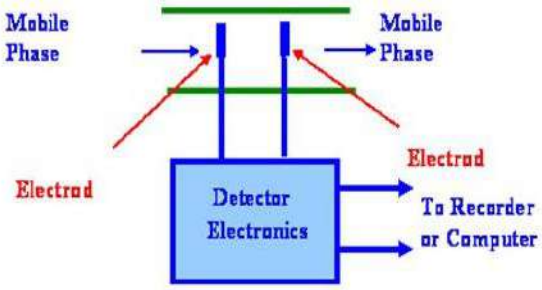
- Ion exchange is used for both analytical and preparative purposes in the laboratory, the analytical uses being the more common.
- Inorganic ions also can be separated by ion-exchange chromatography

Limitations of ion exchange chromatography

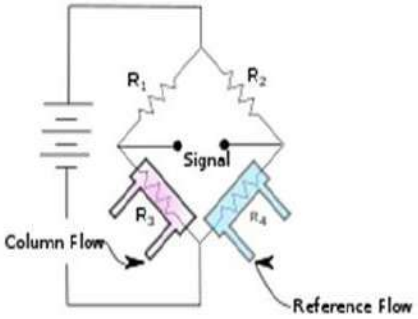
- Only charged molecules can be separated.
- Buffer Requirement

Electrical Conductivity Detector

- Inorganic ions are commonly analyzed using ion chromatographs, with the typical detection method being electric conductivity detection, which is based on detecting the electric conductivity of ions.
- When voltage is applied to a pair of parallel plate electrodes in an aqueous solution, a current flows, if ions are present, between the electrodes. The electric conductivity can be determined by measuring that current.
- If there are ions from the sample contained in the mobile phase flowing from the separation column outlet to the detector, then the electric conductivity in that area will vary according to the variation in ion concentration.
- The detector used to detect such variations is an electric conductivity detector. This detects all ions existing in aqueous solutions. Each ion has a characteristic constant called the equivalent conductivity, where the larger this value, the larger the detected peak.



Two electrodes placed in mobile phase each corresponding to one arm of a Wheatstone Bridge

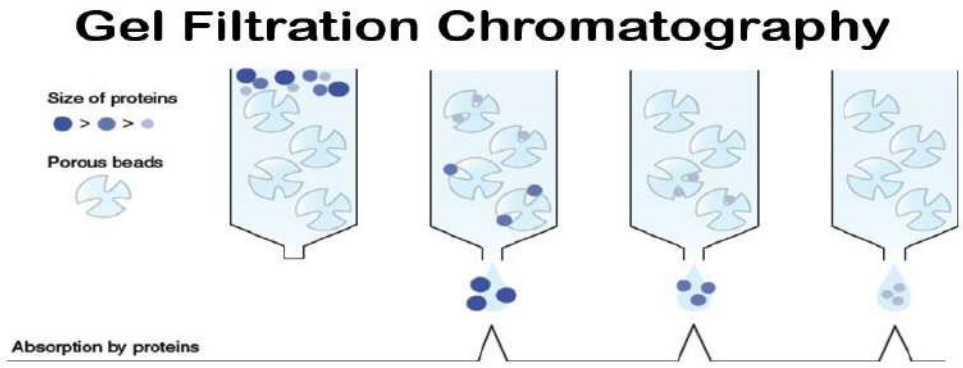


Typical Wheatstone Bridge

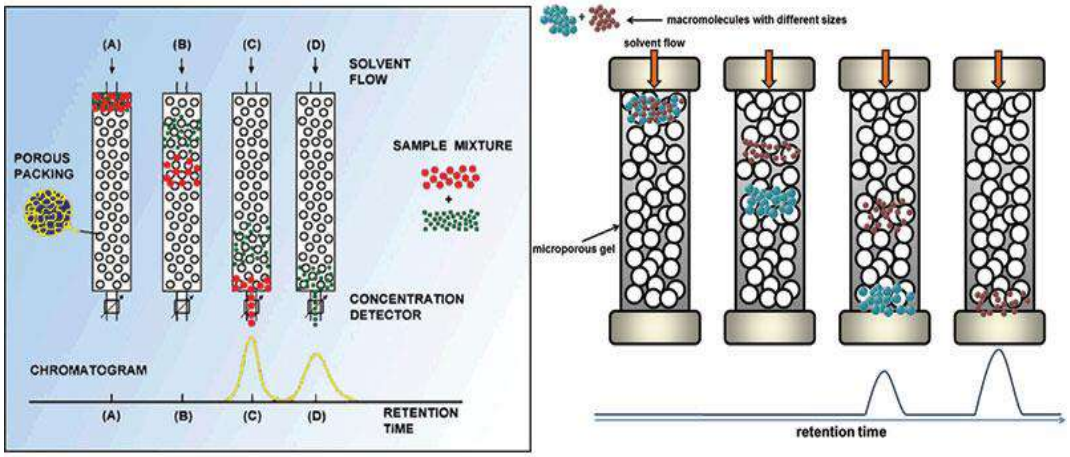
When ions flow into the sensor cell, the impedance between the electrodes changes producing an "out of balance" signal

Size Exclusion / Gel Permeation /Gel Filtration Chromatography

- Gel permeation chromatography is also called as gel filtration or size exclusion chromatography.
- In size exclusion chromatography, the stationary phase is a porous matrix made up of compounds like cross-linked polystyrene, cross-like dextrans, polyacrylamide gels, agarose gels, etc.
- The separation is based on the analyte molecular sizes since the gel behaves like a molecular sieve.
- This technique is used for the separation of proteins, polysaccharides, enzymes, and synthetic polymers.
- As a technique, size exclusion chromatography was first developed in 1955 by Lathe and Ruthven.



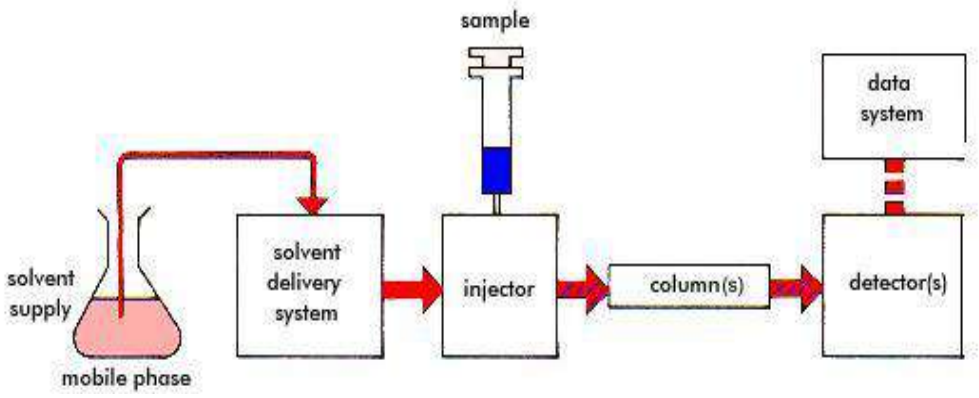
Gel Permeation Chromatography

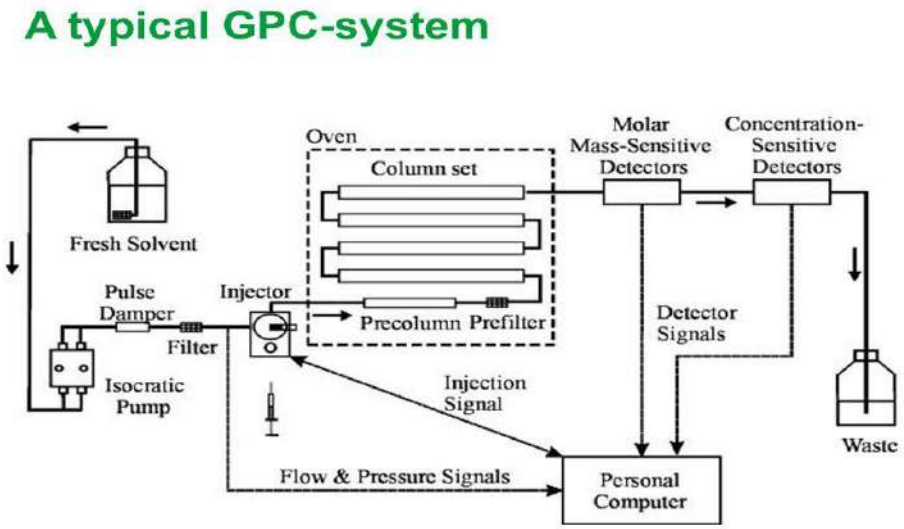


Principle of Gel Permeation Chromatography

- It is a technique in which the separation of components is based on the difference in molecular weight or size.
- The stationary phase used is a porous polymer matrix whose pores are completely filled with the solvent to be used as the mobile phase.
- The molecules in the sample are pumped through specialized columns containing such micro porous packing material (gel).
- The basis of the separation is that molecules above a certain size are totally excluded from the pores, while smaller molecules access the interior of the pores partly or wholly.
- The flow of the mobile phase hence will cause larger molecules to pass through the column unhindered, without penetrating the gel matrix, whereas smaller molecules will be retarded according to their penetration of the gel.

Components/ Instrumentation of Gel Permeation Chromatography





Components/ Instrumentation of Gel Permeation Chromatography

A. Stationary phase

- It is composed of semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.
- It has the following properties:
- Chemically inert
- Mechanically stable

- With ideal and homogeneous porous structure (wide pore size give low resolution).
- A uniform particle and pore size.
- Examples of gel:
    - **Dextran** (Sephadex) gel: An α 1-6-polymer of glucose natural gel
    - **Agarose** gel: A 1,3 linked β-D-galactose and 1,4 linked 3,6-anhydro-α, L-galactose natural gel

Acrylamide gel: A polymerized acrylamide, a synthetic gel

B. The Mobile Phase

- It is composed of a liquid used to dissolve the bio-molecules to make the mobile phase permitting high detection response and wet the packing surface.

C. Columns

- Any of the following kinds may be used:
- Analytical column- 7.5–8mm diameters.
- Preparative columns-22–25mm
- Usual column lengths-25, 30, 50, and 60 cm.
- Narrow-bore columns- 2–3mm diameter have been introduced

D. Pumps

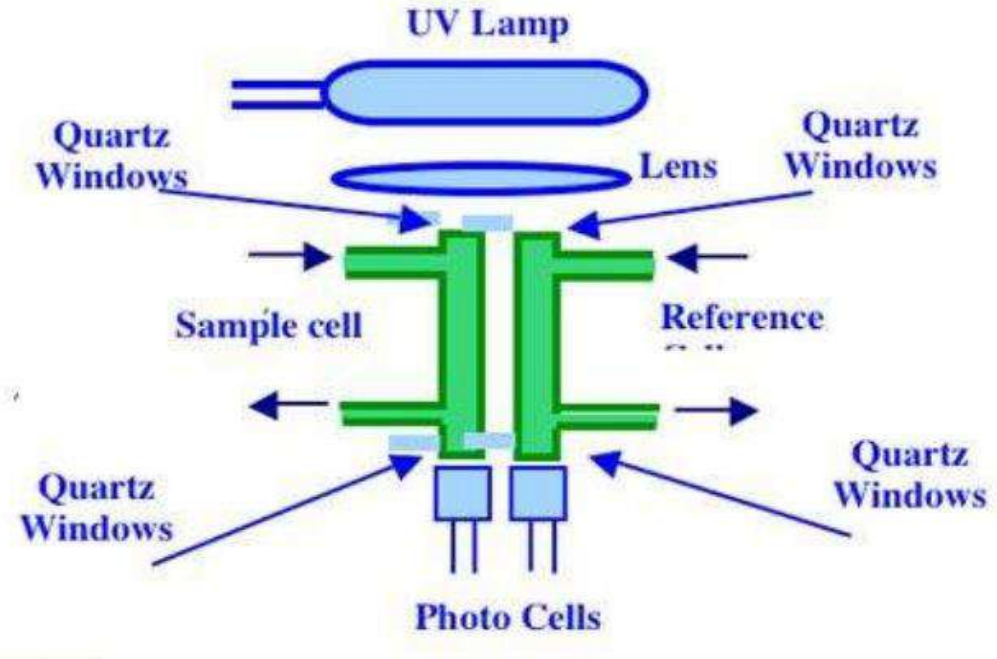
- They are either syringe pumps or reciprocating pumps with a high constant flow rate.

E. Detectors

- The detectors may be any of the following type:
- Concentration sensitive detectors
    - Bulk property detectors – Refractive Index (RI) detector
    - Solute Property Detector – Ultraviolet (UV) Absorption Detector
    - Evaporation Detector – Evaporative Light Scattering Detector(ELSD)

- Molar mass sensitive Detector
    - Light Scattering Detector

UV/VIS Absorption Detector



Refractive Index Detector

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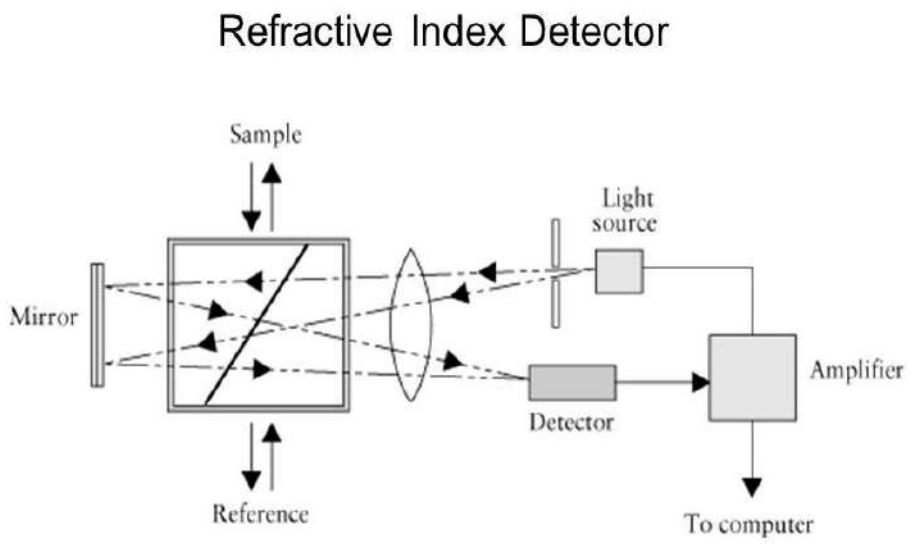

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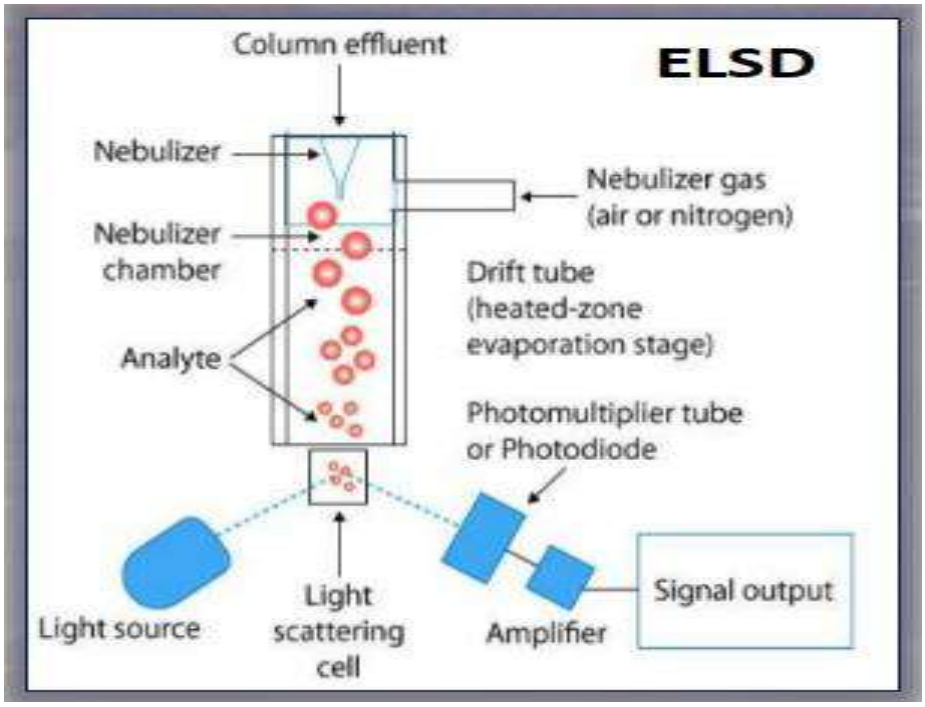
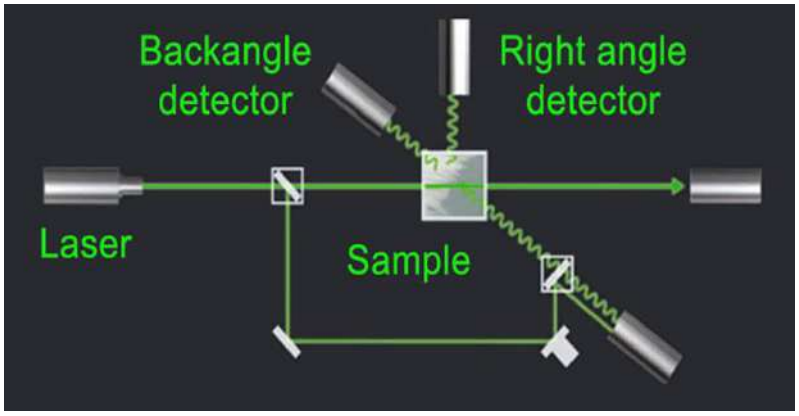


Light Scattering Detector

- Multiangle light scattering (MALS) describes a technique for measuring the light scattered by a sample into a plurality of angles. It is used for determining both the absolute molar mass and the average size of molecules in solution, by detecting how they scatter light. Collimated light from a laser source is most often used, in which case the technique can be referred to as multiangle laser light scattering (MALLS).
- With the advent of size exclusion chromatography (SEC), MALS measurements began to be used in conjunction with an on-line concentration detector to determine absolute molar mass and size of sample fractions eluting from the column, rather than depending on calibration techniques. These flow mode MALS measurements have been extended to other separation techniques such as field flow fractionation, ion exchange chromatography, and reversed-phase chromatography.

Multiangle LASER Light Scattering



Steps in Gel Permeation Chromatography

- It involves three major steps:
- **A. Preparation of column for gel filtration**
- It involves:
- Swelling of the gel
- Packing the column semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.
- Washing: After packing, several column volumes of buffer solution is passed through the column to remove any air bubbles and to test the column homogeneity.
- **B. Loading the sample onto the column using a syringe**

  C. Eluting the sample and detection of components

Applications of Gel Permeation Chromatography

- Proteins fractionation
- Purification
- Molecular weight determination.

- Separation of sugar, proteins, peptides, rubbers, and others on the basis of their size.
- Can be used to determine the quaternary structure of purified proteins.

Advantages of Gel Permeation Chromatography

- Short analysis time.
- Well defined separation.
- Narrow bands and good sensitivity.
- There is no sample loss.
- The small amount of mobile phase required.
- The flow rate can be set.

Limitations of Gel Permeation Chromatography

- The limited numbers of peaks that can be resolved within the short time scale of the GPC run.
- Filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors.
- The molecular masses of most of the chains will be too close for the GPC separation to show anything more than broad peaks.

Unit-3

Industrial Gas Analyzers and Pollution Monitoring Instruments

Process analyzers:

- Process analyzers are tools for industrial process analytics, used to determine the chemical composition or physical properties of substances involved in industrial processes. They enable process optimization, asset protection, and compliance with environmental regulations.
- Inline: For inline analysis, a sensor can be placed in a process vessel or stream of flowing material to conduct the analysis.

- Online: Analysers which are connected to a process, and conduct automatic sampling, can be called online.
- Offline: Analyser used to analyse a sample out of process is called offline.
- There are two main types of emissions monitoring systems: in-situ and extractive. In-situ measures the sample at the point source, whereas extractive extracts a sample for measurement away from the point source.

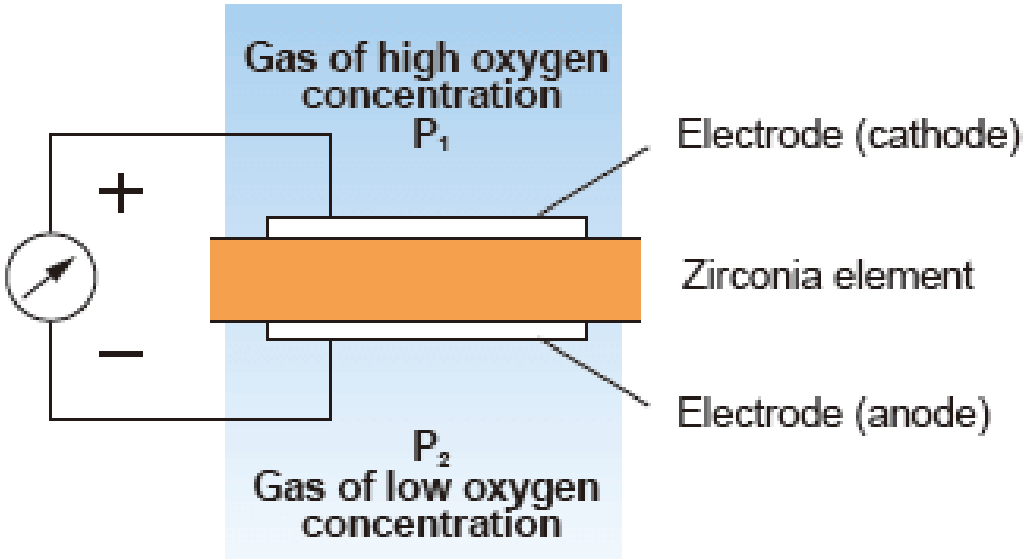
Oxygen Analyzer Working Principle

- Oxygen concentrations are measured for a variety of purposes, such as energy conservation, air pollution prevention, safety management, and quality control.
- The measurement methods of the oxygen analyzers currently available in the industry can be classified into the following categories.
- **ZIRCONIA TYPE MEASUREMENT SYSTEM**
- **PARAMAGNETIC TYPE**
- **OPTICAL TYPE**
- **ELECTROCHEMICAL TYPE**
- Since each of the measurement methods has its advantages and disadvantages, it is important to select an oxygen analyzer of an appropriate method for particular application and usage.
- The following describes an overview of each of the measurement methods and their advantages and disadvantages.

Zirconia type measurement system: Concentration cell system

- A solid electrolyte like zirconia exhibits only conductivity of oxygen.
- Zirconia cell only allow oxygen ions to pass through at high temperatures.
- As shown in the figure, when porous platinum electrodes are attached to both sides of the zirconia element to be heated up and gases of different partial oxygen concentrations are brought into contact with the respective surfaces of the zirconia, the device acts as an oxygen concentration cell.

- Oxygen ions move from the side with the highest concentration of oxygen to that of the lowest concentration.
- This phenomenon causes an electromotive force to be generated between both electrodes according to Nernst's equation. And it is proportional oxygen concentration ions at high temperature.



Advantages: Can be directly installed in a combustion process such as a boiler's flue and requires no sampling system, and response is faster.

Disadvantages: If the sample gas contains a flammable gas, a measurement error occurs (combustion exhaust gas causes almost no problem because it is completely burned).

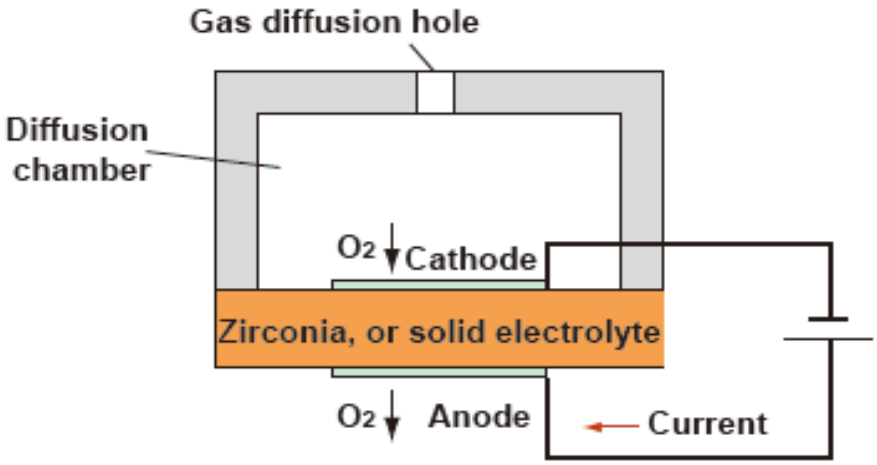
Application:

- Package boiler combustion control, gas fired
- Combustion control of power generation boilers, gas fired
- Combustion control of pulverized coal boilers
- Combustion control of hot stoves for steelmaking
- Heating and combustion exhaust gas control of coke ovens for steelmaking
- Low-oxygen concentration control of reheating and soaking furnaces for steelmaking

- Air leakage detection of sintering furnaces for steelmaking
- Combustion control of heating furnaces for oil refinery & petrochemical industry
- Oxygen concentration measurement in oxygen enrichment facilities.
- Oxygen concentration measurement of exhaust gas from activated sludge process equipment.

Zirconia type measurement system: Limiting Current type

As shown in the figure below, if the flow of oxygen into the cathode of a zirconia element heated to high temperature is limited, there appears a region where the current becomes constant even when the applied voltage is increased. This limited current is proportional to the oxygen concentration.



Advantages:

- Capable of measuring trace oxygen concentration.
- Calibration is required only on the span side (air).

Disadvantages:

- If the sample gas contains a flammable gas, a measurement error occurs.
- The presence of dust causes clogging of the gas diffusion holes on the cathode side; a filter must be installed in a preceding stage.

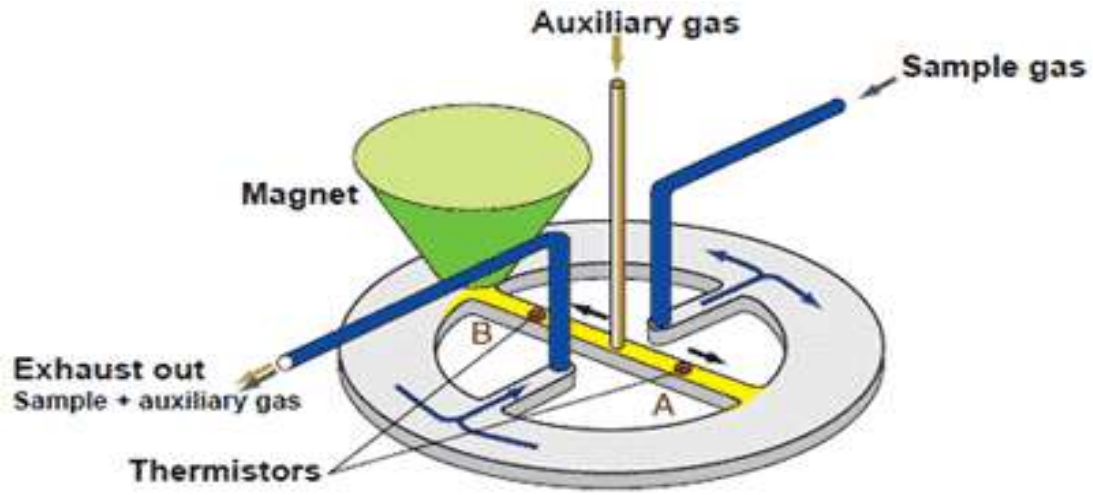
Application

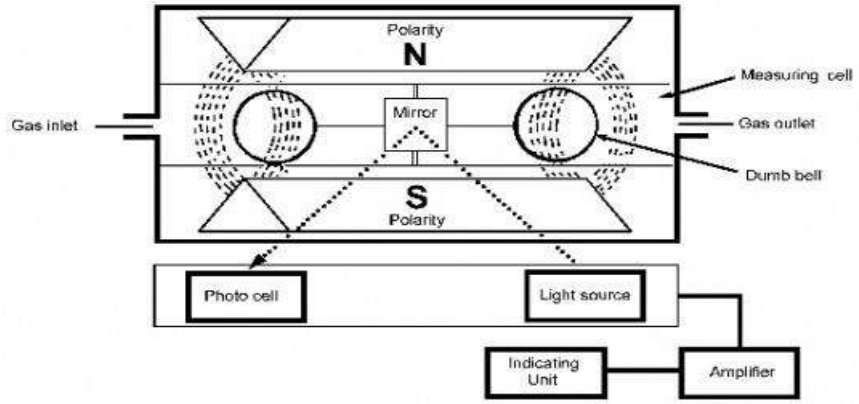
- Oxygen concentration control of N_2 reflow furnaces

- Atmospheric control of semiconductor manufacturing equipment
- N_2 and air purity control for air separators
- Oxygen deficiency prevention
- Oxygen concentration control of glove boxes for research and development and parts machining
- Oxygen concentration control of experimental clean rooms for environment, fermentation, biochemistry, etc.
- Continuous measurement of flow gases during food packaging

Magnetic type measurement system: Paramagnetic system

This is one of the methods utilizing the paramagnetic property of oxygen. When a sample gas contains oxygen, the oxygen is drawn into the magnetic field, thereby decreasing the flow rate of auxiliary gas in stream B. The difference in flow rates of the two streams, A and B, which is caused by the effect of flow restriction in stream B, is proportional to the oxygen concentration of the sample gas. The flow rates are determined by the thermistors and converted into electrical signals, the difference of which is computed as an oxygen signal.





Advantages:

- Capable of measuring flammable gas mixtures that cannot be measured by a zirconia oxygen analyzer.
- Because there is no sensor in the detecting section in contact with the sample gas, the paramagnetic system can also measure corrosive gases.
- Among the magnetic types, the paramagnetic system offers a faster response time than other systems.
- Among the magnetic types, the paramagnetic system is more resistant to vibration or shock than other systems.

Disadvantages: Requires a sampling unit corresponding to the sample gas properties or applications.

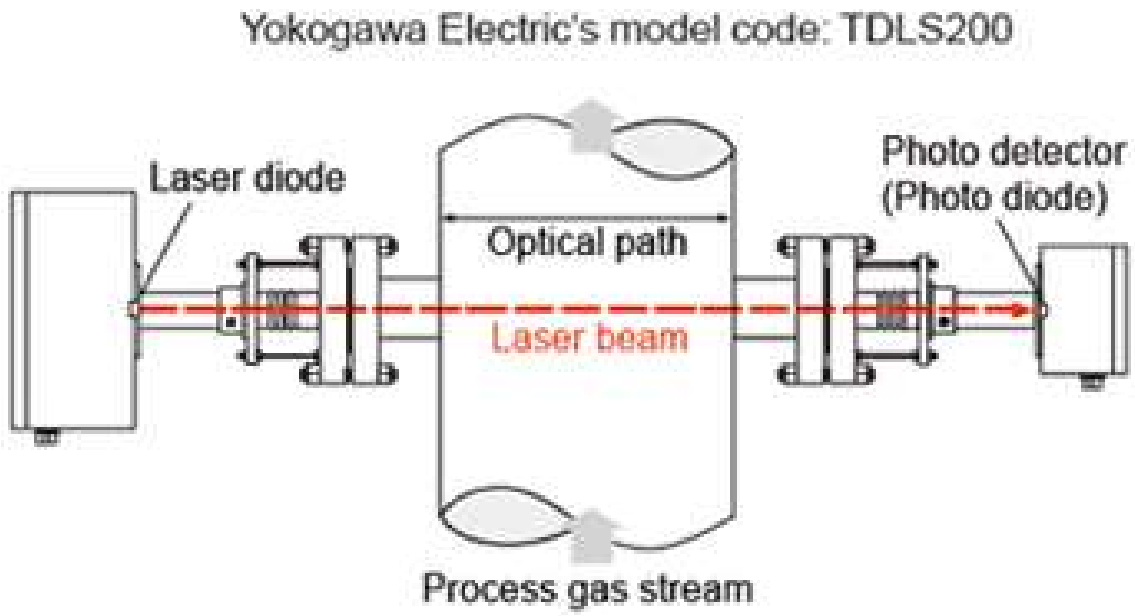
Application

- Low-oxygen concentration control of CDQ (Coke Dry Quenching) plants for steelmaking.
- Oxygen concentration control of gas containing a flammable gas
- Safety control (explosion prevention) at various plants
- Measurement of trace oxygen concentration in various manufacturing processes

City gas quality control

Optical type: Tunable Diode Laser measurement system

Tunable Diode Laser (or TDL) measurements are based on absorption spectroscopy. The True Peak Analyzer is a TDL system and operates by measuring the amount of laser light that is absorbed (lost) as it travels through the gas being measured. In the simplest form a TDL analyzer consists of a laser that produces infrared light, optical lenses to focus the laser light through the gas to be measured and then on to a detector, the detector, and electronics that control the laser and translate the detector signal into a signal representing the gas concentration.



Gas molecules absorb light at specific colors, called absorption lines. This absorption follows Beers law.

TDL Analyzers are effectively infrared analyzers which obey the Beer-Lambert Law.

$$I = I_o \cdot e^{-E \cdot G \cdot L}$$

where I is the radiation intensity after absorption,

I_0 is the initial radiation intensity,

E is the extinction coefficient,

G is the gas concentration,

and L is the path length of the measurement area.

Advantages:

- Capable of measuring a number of near infrared absorbing gases in difficult process applications.

- Capability of measuring at very high temperature, high pressures and under difficult conditions (corrosive, aggressive, high particulate service).
- Most applications are measured in-situ, reducing installation and maintenance costs.

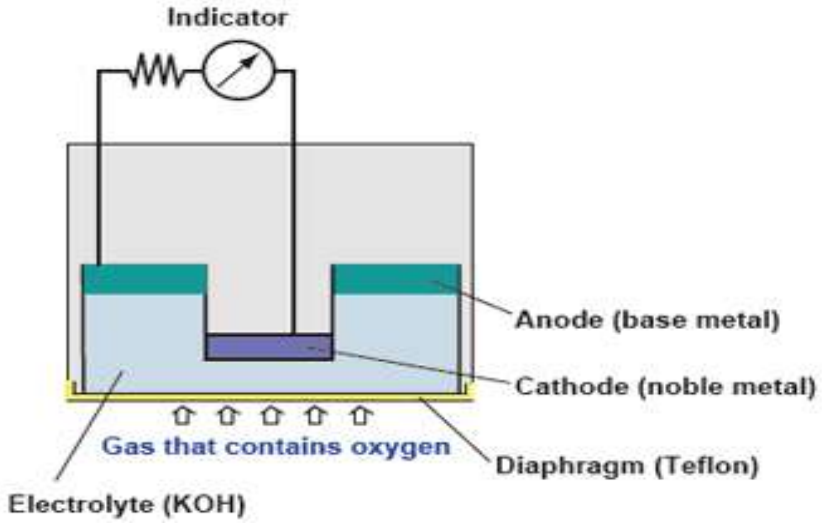
Disadvantages: The installation of the flange is necessary for both sides of the process.

Application:

- Combustion control of heating furnaces
- Incinerator combustion control
- Process control

Electrochemical type: Galvanic cell type

If oxygen is dissolved via the diaphragm in an electrolytic solution in which an anode (base metal) and cathode (noble metal) are adjacent to each other, a current proportional to the quantity of dissolved oxygen is generated. The amount of oxygen passing through the diaphragm is proportional to the partial oxygen pressure of the sample gas; therefore, the oxygen concentration can be determined by measuring the current.



Advantages:

- The detecting system can be made compact; this measurement system is available in portable or transportable form.

- Relatively inexpensive in comparison with oxygen analyzers of other measurement systems.

Disadvantages:

The cell life is limited. As it is a kind of oxygen cell, the galvanic cell deteriorates even if not used. In general, it should be replaced approximately every year.

Application:

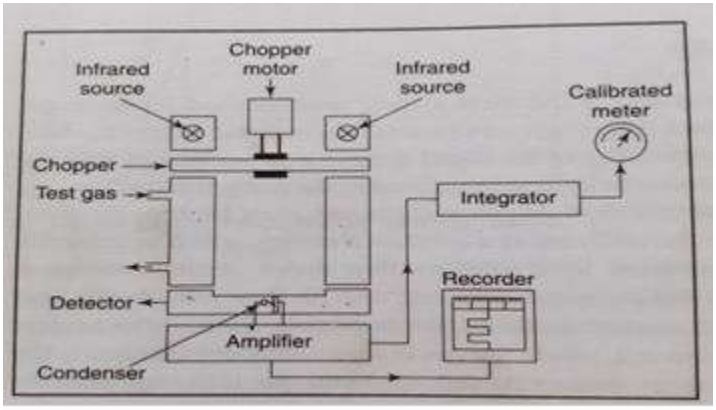
- Oxygen deficiency prevention
- Package boiler combustion control, gas fired

Types of Gas Analyzers:

- Non Dispersive Infrared Gas Analyzer
- Thermal Conductivity Analyzer
- Analyzer based on Gas Density
- Analyzer based on Ionization of Gases

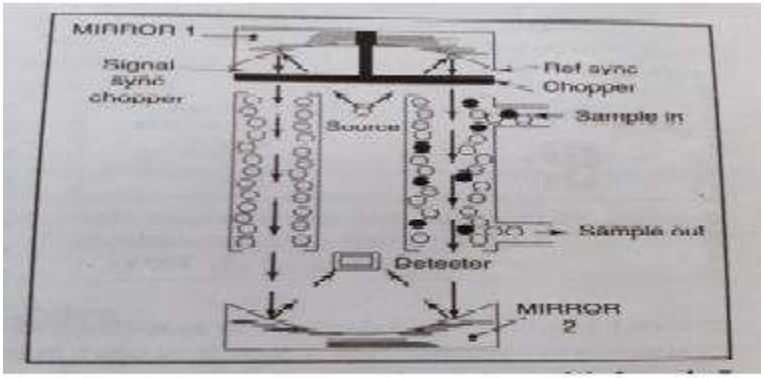
Non Dispersive Infrared Gas Analyzer (NDIR)

- Non Dispersive Infrared Gas Analyzers depend on fact that some gases absorb specific wavelength of infrared radiation. A non-dispersive infrared sensor is used in gas analysis to determine the gas concentration. One of the most commonly measured gases using infrared radiation absorption method is the carbon monoxide, carbon dioxide or hydrocarbons in a gas.



Infrared Gas Analyzer

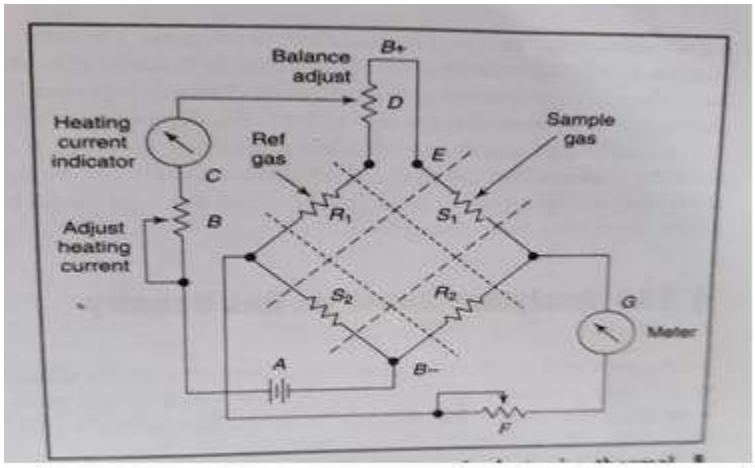
- The technique used for this purpose is the conventional double beam infrared spectrometer system having a pair of matched gas cells in the two beams. One cell is filled with a reference gas, which
- The infrared absorption principle is not applicable for analysis of two identical atoms like oxygen, hydrogen and nitrogen.



Improved version of Infrared Gas Analyzer

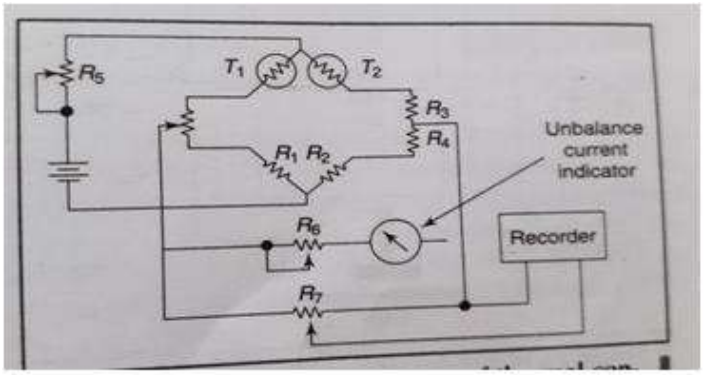
Thermal Conductivity Analyzer

- A universal detector and can detect air, hydrogen, carbon monoxide, nitrogen, sulphur oxide, inorganic gases and many other compounds.



Hot Wire Thermal Conductivity Analyzer

- Thermal conductivity (TCD) is a commonly used detector in gas chromatography. TCD works by having two parallel tubes both containing gas and heating coils. The gases are examined by comparing the heat loss rate from the heating coils into the gas. Normally one tube holds a reference gas and the sample to be tested is passed through the other. Using this principle, a TCD senses the changes in the thermal conductivity of the column effluent and compares it to a reference flow of carrier gas. Most compounds have a thermal conductivity much less than that of the common carrier gases of hydrogen or helium. Therefore, when an analyte elutes from the column, the thermal conductivity of the effluent is reduced and a detectable signal is produced.



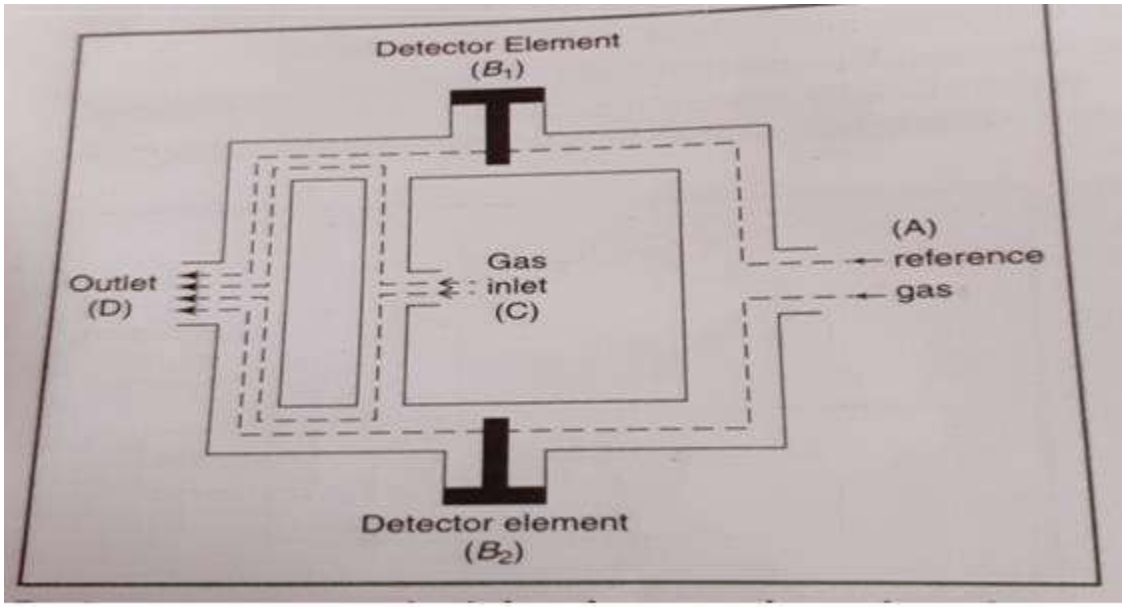
Thermal Conductivity Analyzer using Thermistor

Analyzer Based on Gas Density

The density of an ideal gas has a direct linear relation with the molecular weight of that gas. All real gases behave as ideal gases at room temperature and normal atmospheric pressure. Instruments based on the principle of gas density balance are commercially available.

The reference gas enters at point A and split into two half and passes through the detectors B1 and B2, then the reference gas leave the unit at point D. The detectors B1 & B2 are a hot wire or thermistor which is connected as two arms of a whetstone bridge. When reference gas is passing through the unit the detectors are cooled down equally so the bridge is balanced and give zero output.

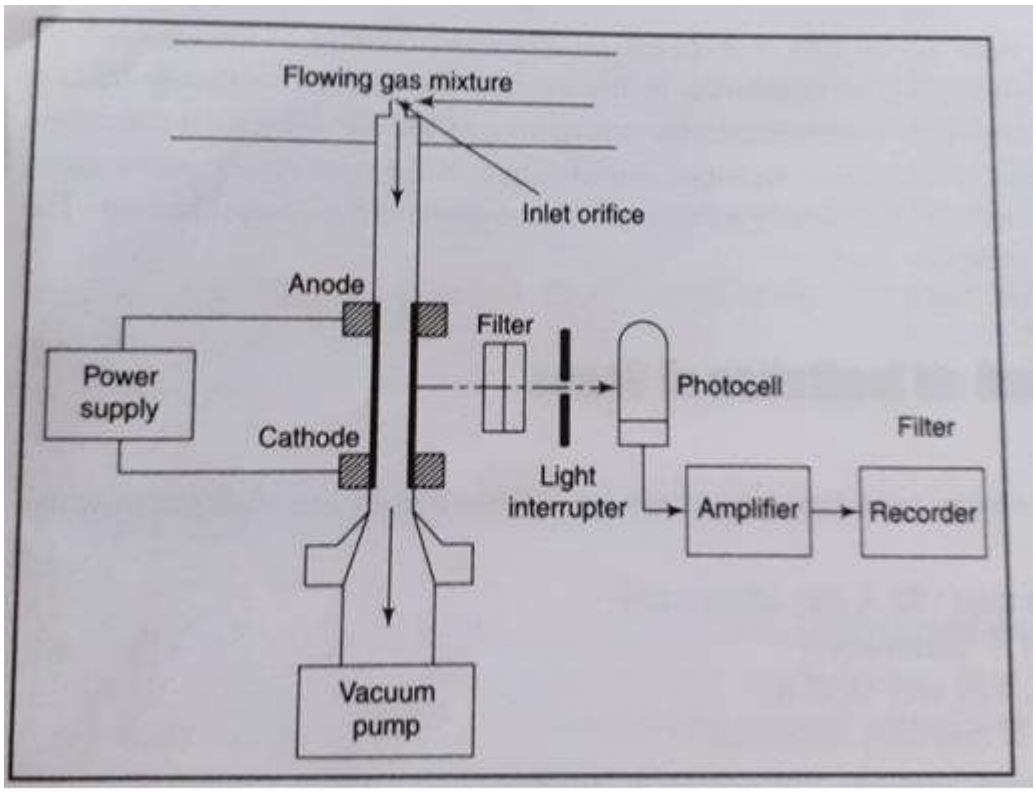
The test gas or the gas whose density is to measure is entered at point C in the measuring unit. The test gas also split into two half and leave the unit at point D. If the Density of the test gas is higher than the reference gas then the test gas is drawn mostly to downward stream that is more towards B2, so it partially obstructing the flow path A-B2-D. This would result in raising the temperature of the detector B2. This in turn increases the flow in the path A-B1-D and cause more cooling of the element B1. Since the temperature of the detectors are differed the bridge is under unbalanced condition which results in output voltage proportional to difference in density of the test gas and reference gas.



Gas Density Meter

Analyzer Based on Ionization of Gases

The spectral regions for maximum radiation absorption for different gases are of different wavelength. Nitrogen and oxygen analyses are routinely done by using these absorption bands. However, with sufficient electrical excitation and at suitable pressure, gases emit radiation in different ways like spark, arc, and glow discharge in different part of the radiation spectrum. The measurement of the emitted radiation can help in determination of the unknown concentration of a gas in a mixture. This technique has been utilized for the measurement of nitrogen gas, particularly in respiratory gases. The presence of nitrogen is detected by the emission of a characteristic purple color, when discharge takes place in a low pressure chamber containing the sample gas.



Ionization Type Gas Analyzer

Construction: the instrument generally operates in two parts. The sampling head contains the ionizing chamber, filter and the detector. The other part contains the power supply, amplifier and the display system. The ionizing chamber or the discharge tube is maintained at absolute pressure of a few Torr. A rotary oil vacuum pump draws a sample and feeds it to the discharge. The voltage required for striking the discharge in

the presence of nitrogen is of the order of 1500 V dc. This voltage is generated by using a dc-dc convener, or by rectifying the output of a high voltage transformer.

The light output from the discharger tube is interrupted by means of a rotating slotted disc, so that a chopped output is obtained. This light is then passed through optical filters to the wavelength corresponding to the purple color. The intensity of the light is measured with a photocell and an amplifier specially tuned to the chopping frequency. The light intensity is proportional to nitrogen concentration.

Pollution Monitoring Instruments

- Monitoring methodologies can be divided into three categories according to cost and the level of accuracy and precision.
- **Continuous monitoring methods**

  These are high-resolution methods that provide continuous records of contaminant levels. They can operate over extended periods (weeks or months) with minimal operator intervention. Remote communication is possible by telemetry. They have a high degree of measurement precision. As might be expected, these are the most expensive monitoring methods. A high standard of maintenance, calibration, and operational and quality control procedures are required for good data quality.

- **Gravimetric particulate methods**

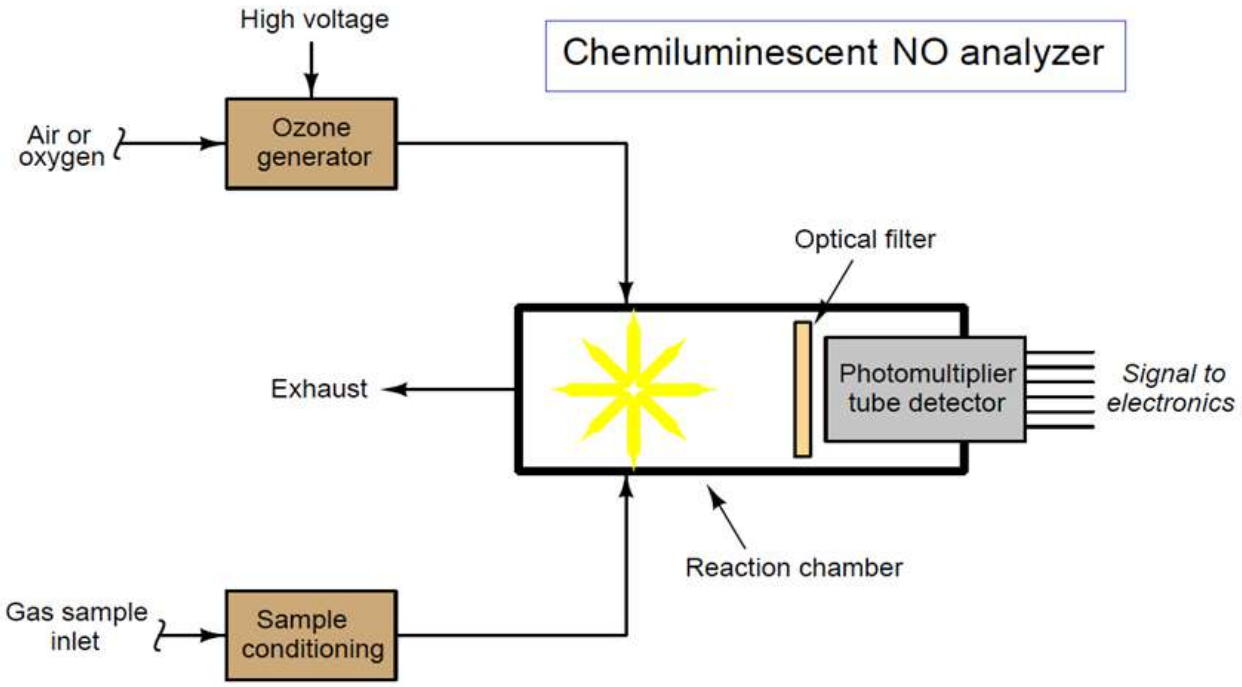
  Monitoring starts when a known volume of air is pumped through a pre-weighed filter for a known length of time (typically 24 hours). The filter is reweighed after exposure and a concentration determined. This can be done on consecutive days
- **Passive monitoring methods (diffusion tubes and badges)** Diffusion tubes work when a contaminant is diffused into a tube containing either an adsorbent or reactive material. Analysis of the tubes following a known exposure time (typically two to four weeks) will provide a time-averaged contaminant concentration. Badges work in a similar way, the difference being the sampler configuration. Because these methods are simple and cheap, they can provide a good picture of spatial variation over a large area. They are particularly useful in screening surveys and during the initial stages of an air quality monitoring programme. Though a cheap screening tool, there are a

number of limitations to this method, such as lower accuracy and no indication of peak levels.

Nitrogen oxides:

NO Monitoring – Chemiluminescence

- An exothermic chemical reaction is one that releases a net sum of energy, as opposed to an endothermic reaction which requires a greater input of energy than it releases. Combustion is a common class of exothermic reactions, with the released energy being very obviously in the forms of heat and light, with heat being the predominant form.
- Some exothermic reactions release energy primarily in the form of light rather than heat. The general term for this effect is chemiluminescence. A natural example is the "cold" light emitted by North American species of firefly. In this small insect, a chemical reaction intermittently takes place emitting significant amounts of light but insignificant amounts of heat. An artificial example is the light emitted by a "glow stick" when activated.
- Certain industrial compounds engage in chemiluminescent reactions, and this phenomenon may be used to measure the concentration of those compounds. One such compound is nitric oxide (NO), an atmospheric pollutant formed by high-temperature combustion with air as the oxidizer.
- A spontaneous chemical reaction between nitric oxide and ozone (an unstable molecule formed of three oxygen atoms: O3) is known to produce chemiluminescence:
- $NO + O3 \rightarrow NO2 + O2 + light$
- Although this process of generating light is quite inefficient (only a small fraction of the NO2 molecules formed by this reaction will emit light), it is predictable enough to be used as a quantitative measurement method for nitric oxide gas. Ozone gas is very easy to produce on demand, by exposing air or oxygen to a high-voltage electric discharge.
- A simplified diagram for a chemiluminescent nitric oxide gas analyzer appears here:



- As with many optical analyzers, a photomultiplier tube serves as the light-detecting sensor, generating an electrical signal in proportion to the amount of light observed inside the reaction chamber. The higher the concentration of NO molecules in the sample gas stream, the more light will be emitted inside the reaction chamber, resulting in a stronger electrical signal produced by the photomultiplier tube.

- Although this instrument readily measures the concentration of nitric oxide (NO), it is insensitive to other oxides of nitrogen (NO2, NO3, etc., collectively referred to as NOx, pronounced "nocks"). Normally, we would consider this selectivity to be a good thing, because it would eliminate interference problems from these other gases. However, as it so happens, these other oxides of nitrogen are every bit as polluting as nitric oxide, and therefore when we measure nitric oxide for pollution monitoring purposes, we usually also wish to measure these other oxides in combination.

- In order to use chemiluminescence to measure all oxides of nitrogen, we must chemically convert the other oxides into nitric oxide (NO) before the sample enters the reaction chamber. This is done in a special module of the analyzer called a converter. A three-way solenoid valve is shown in this diagram, providing a means to bypass the converter so the analyzer only measures nitric oxide content in the sample gas. With the solenoid valve passing all the sample

through the converter, the analyzer responds to all oxides of nitrogen (NOx) and not just nitric oxide (NO).

- One simple way to achieve the NOx → NO chemical conversion is to simply heat the sample gas to a high temperature, around 1300° F. At this temperature, the molecular structure of NO is favored over more complex oxides such as NO2, the result being a release of oxygen from the NO2 and NO3 molecules to become NO molecules. A disadvantage of this technique is that those same high temperatures also have a tendency to convert other compounds of nitrogen such as ammonia (NH3) into nitric oxide, thereby creating an unintended interference species.

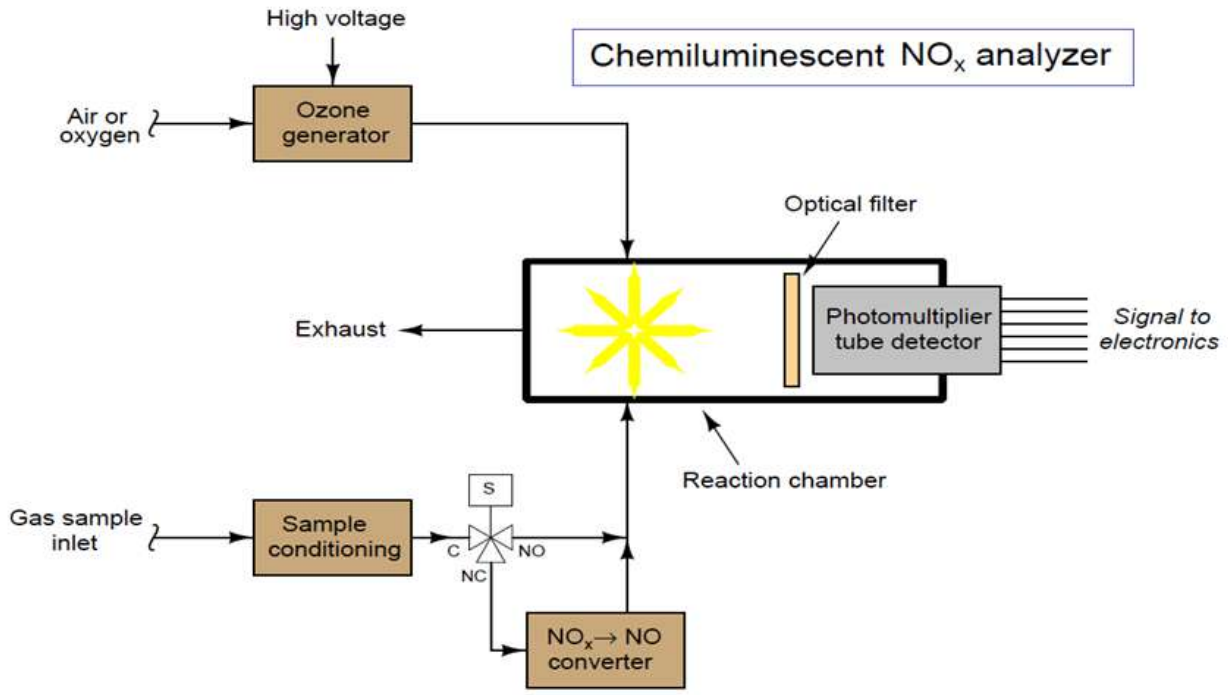
NOx Monitoring – Chemiluminescence

- An alternative NOx → NO conversion technique is to use a metallic reactant in the converter to remove the extra oxygen atoms from the NO2 molecules. One such metal that works well for this purpose is molybdenum (Mo) heated to the comparatively low temperature of 750 °F, which is too low to convert ammonia into nitric oxide. The reaction of NO2 converting to NO is as follows:

- $3NO_2 + Mo \rightarrow MoO_3 + 3NO$

- Other oxides (such as NO3) convert in similar fashion, leaving their excess oxygen atoms bound to molybdenum atoms and becoming nitric oxide (NO).

- As with other optical gas analyzers, pressure control of the gas sample is critically important for good measurement accuracy. If the pressure of the sampled gas inside the chemiluminescence reaction chamber happens to vary, it will affect the amount of light emitted even if the relative concentration of NOx gas remains stable. This is because higher pressures will pack gas molecules closer together, resulting in more reactive molecules inside the chamber for any given percentage or ppm concentration. Ensure the gas pressure inside the measurement chamber remains constant.

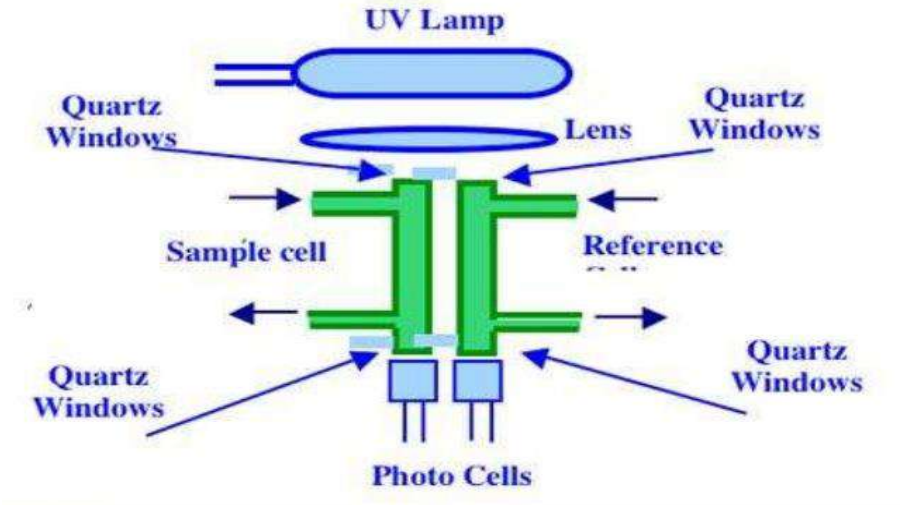


Carbon Dioxide:

1. UV/VIS Absorption Detector

The UV, VIS, and PDA (Photodiode-Array Detection) detectors are categorized as absorbance detectors. They provide good sensitivity for light-absorbing compounds at ~pg level. They are easy to operate and provide good stability. UV detector is a very commonly used detector for HPLC analysis. During the analysis, sample goes through a clear color-less glass cell, called flow cell. When UV light is irradiated on the flow cell, sample absorbs a part of UV light. Thus, the intensity of UV light observed for the mobile phase (without sample) and the eluent containing sample will differ. By measuring this difference, the amount of sample can be determined. Since the UV absorbance also differs depend on what wavelength is used, it is important to choose an appropriate wavelength based on the type of analyte. A standard UV detector allows user to choose wavelength between 195 to 370 nm. Most commonly used is 254 nm. Compared to a UV detector, a VIS detector uses longer wavelength (400 to 700 nm). There are detectors that provide wider wavelength selection, covering both UV and VIS ranges (195 to 700 nm) called UV/VIS detector. PDA detects an entire spectrum simultaneously. UV and VIS detectors visualize the obtained result in two

dimensions (light intensity and time), but PDA adds the third dimension (wavelength). This is convenient to determine the most suitable wavelength without repeating analyses.



- Gas Chromatography

  Explanation is in Unit 2.

Sulphur Dioxide:

1. **Colorimetric method**

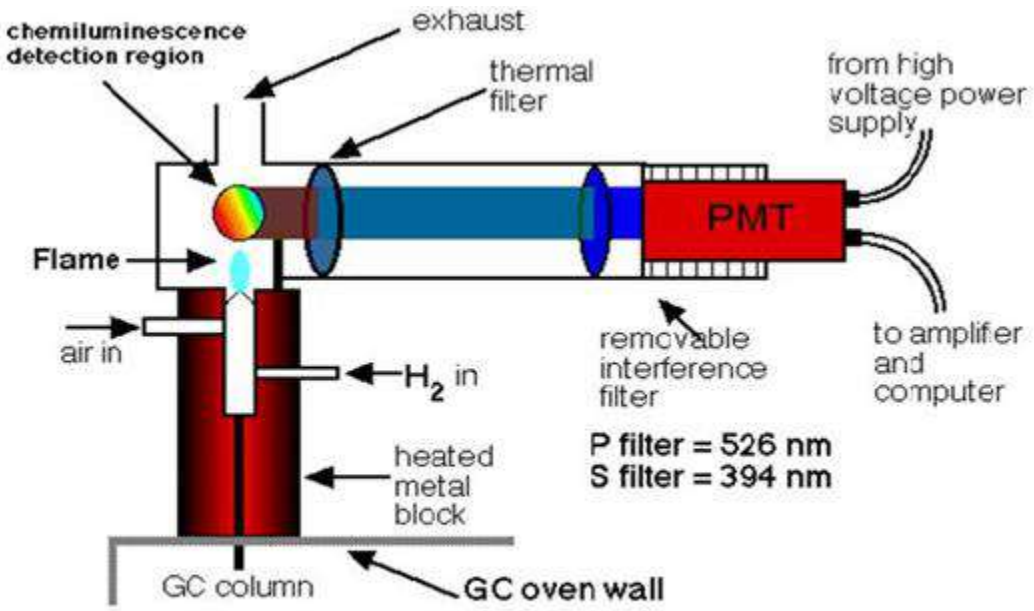
   In this method a known volume of air is passed through an aqueous solution, which contains reagent that absorb sulphur dioxide and produce a coloured substance. The amount of coloured substance is proportional to the component of interest (SO_2). This determine by measuring a solution's optical absorbance spectro-photometrically. Within limits, the absorbance is linearly proportional to the concentration of the coloured species, in accordance with the Beer's Law.

2. **Gas Chromatography**

   Explanation is in Unit 2.

3. **Flame photometric method**
   - The photoemission from the flame is monitored instead of the electric conductivity.

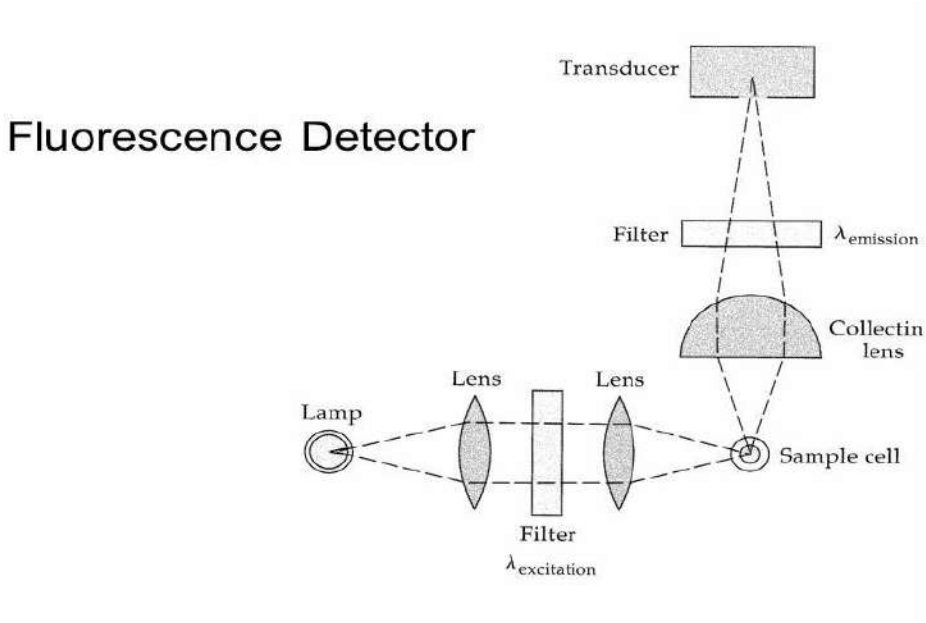
- The sample passes through the hydrogen/ air flame and light emission is observed due to the excitation of some of the atoms.
- The light in the UV/Visible region of the spectrum is selected by using suitable filters and is measured by a photomultiplier tube. The light emitting processes that produce the sulphur and phosphorous sensitivity occur in the upper portion of the flame that does not emit appreciably in the absence of sulphur and phosphorous.
- Selective to compounds containing sulphur and phosphorous.



flame photometric detector

4. Fluorescence method

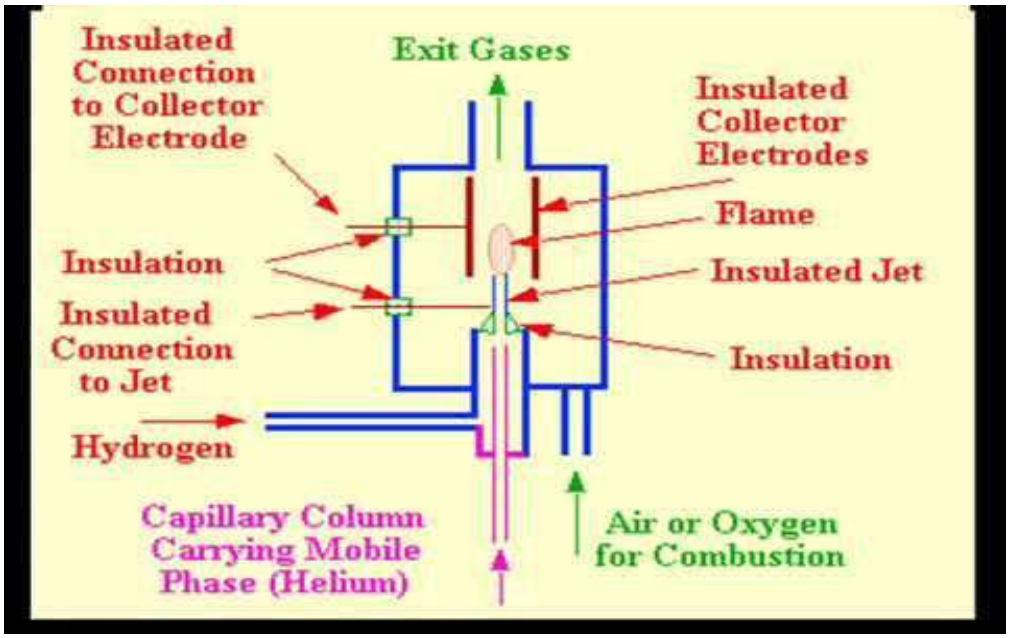
The advantage of fluorescence method is its high sensitivity for selective groups of compounds. By using a specific wavelength, analyte atoms are excited and then emit light signal (fluorescence). The intensity of this emitted light is monitored to quantify the analyte concentration. Most pharmaceuticals, natural products, clinical samples, and petroleum products have fluorescent absorbance. For some compounds which do not have fluorescence absorbance or low absorbance, they can be treated with fluorescence derivatives such as dansylchloride. The system is easy to operate and relatively stable.



Hydrocarbons:

1. **Flame ionization detector**

   - FID is the most widely used and generally applicable detector in GC and gas analyzer.
   - Effluent from the column is directed into a small air/ H2 flame and most organic compounds produce ions and electrons when pyrolyzed at the temperature of an air /H2 flame.
   - Compound is detected by monitoring the current produced by collecting the ions and electrons.
   - A few hundred volts applied between the burner tip and a collector electrode located above the flame serve to collect the ions and electrons.
   - The current is then measure with a sensitive picoammeter.



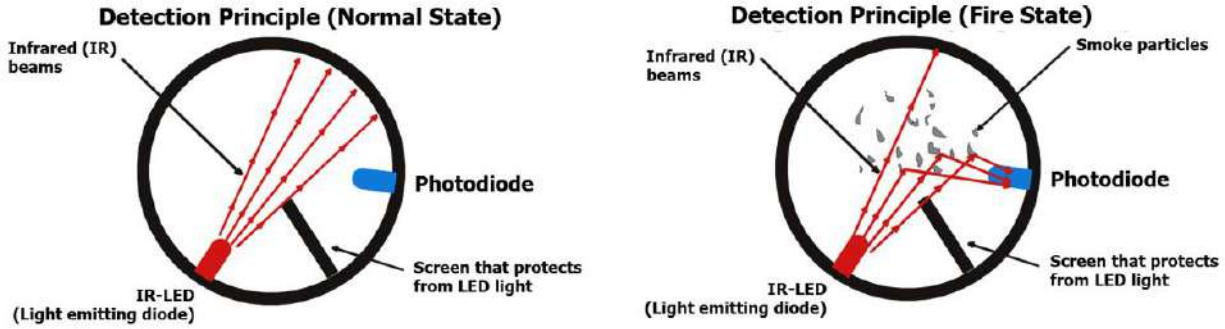
2. **Gas Chromatography**

Explanation is in Unit 2.

Dust and Smoke Detectors

1. **Optical Smoke Detector**

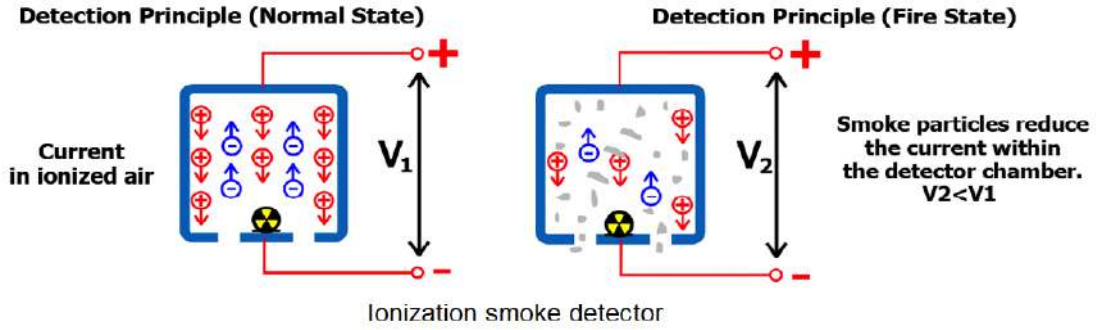
Optical smoke detector utilizes the light scatter sensing principle. It has an infrared light source, which emits signals and a photo-diode, which senses the scattering light with the effect of smoke entering the chamber. Optical smoke detector is used for the normally without dust and dirty rooms, hall, saloons etc. which have wooden and textile derivative materials. Optical smoke detector is mostly preferred at the present times.



Optical Smoke Detector

2. **Ionization smoke detector**

Ionization smoke detector consists the cell which allows to smoke entry. In this cell, there is continuously radioactive radiation. Smoke that is entered to cell cause to reference current and voltage values and smoke detector realized that changing.

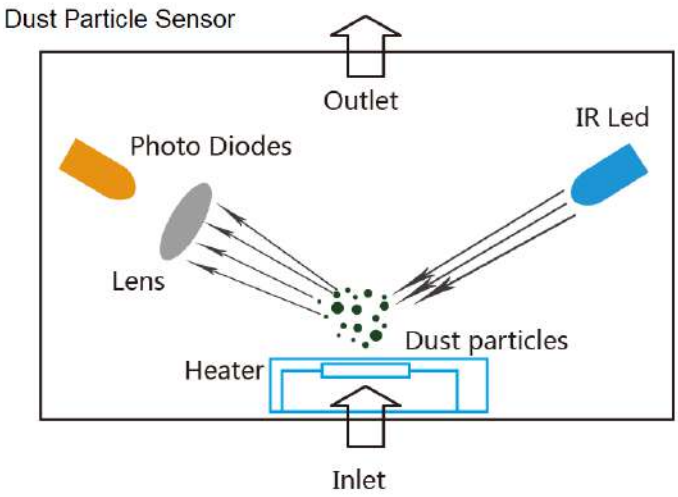


Ionization smoke detector

Ionization smoke detector can detect deodorant, cooking smells. It can be cause to fault detection. Also, when a smoke detector being contaminated, detector cannot be cleaned completely and in this process calibration is changed. Because of this, ionization smoke detectors are not preferred.

3. Dust particle sensor

With the popularization of smart home and the demand of improving air quality, dust particle sensor has been widely used in air purifier, vacuum cleaner, fresh air system and other equipment. The following is a brief introduction of the working principle of dust particle sensor. Based on the scattering principle of light, the working principle of dust particle sensor was developed. Particles and molecules scatter light when exposed to light. At the same time, they absorb some of the energy of the light that hits them.



When a parallel beam of monochromatic light enters the field of the particle being measured, it will be affected by the scattering and absorption around the particle and

the light intensity will be weakened. In this way, the relative attenuation rate of the incident light passing through the concentration field to be measured can be obtained. The relative attenuation rate can reflect the relative concentration of dust in the area to be measured almost linearly. According to the algorithm and calibration method, the dust concentration can be obtained by counting the real-time particle number concentration.

Unit - 4

pH METERS AND DISSOLVED COMPONENT ANALYZERS

Dissolved oxygen (DO) Analyzer

Dissolved oxygen is a key measure of water quality relied upon in various applications. In industrial water treatment, dissolved oxygen levels can be an indicator of water quality issues that lead to corrosion of equipment. In aquaculture, fish transport, and aquarium applications, dissolved oxygen is monitored to ensure that aquatic species have enough oxygen in their habitat to survive, grow, and reproduce. In municipal water treatment facilities, dissolved oxygen in wastewater is monitored during aeration water treatment processes.

Measuring dissolved oxygen concentration

The concentration of dissolved oxygen in water can be sampled or monitored continuously using a dissolved oxygen sensor. Commercially available dissolved oxygen sensors typically fall into 3 categories:

- Galvanic cell dissolved oxygen sensors
- Polarographic dissolved oxygen sensors
- Optical dissolved oxygen sensors

Each type of dissolved oxygen sensor has a slightly different working principle. Therefore, each dissolved oxygen sensor type has advantages and disadvantages depending on the water measurement application where it will be used.

Galvanic cell method

The membrane has high permeability to oxygen and is constructed so that the electrodes and electrolyte are isolated from the water being measured. The counter electrode (anode) is a base metal and the working electrode is a noble metal (cathode) and potassium hydroxide is used as the electrolyte. Oxygen passes through the

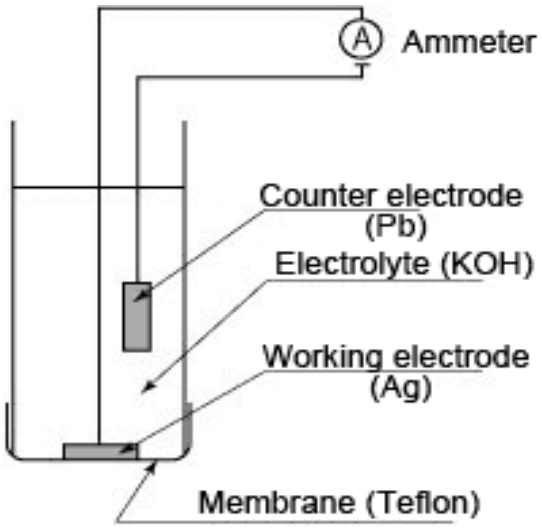
membrane and is reduced on the working electrode, and so the method measures the reduction current flowing between both electrodes, which is proportional to the concentration of dissolved oxygen.

Advantages

- No warm up time, it can be set immediately after turn-off
- The electrolyte is never used up; in theory it can be used forever.
- Fast response time.

Limitations

- The sensor continuously consumes the anode, even when turned off. Therefore the lifetime of the sensor is much shorter than of the Polarographic sensor.
- Since the electrode consumes oxygen, readings are affected by flow across the sensor tip. Thus enough flow rate at the membrane (or sample renewal rate) must be ensured for accurate results.



Polarographic method

The sensor construction is almost the same as that of the galvanic cell method. The counter electrode is silver-silver chloride and the working electrode is gold or platinum. When a voltage of 0.5–0.8 V is applied between both electrodes, oxygen that has permeated through the membrane initiates a reduction reaction on the working

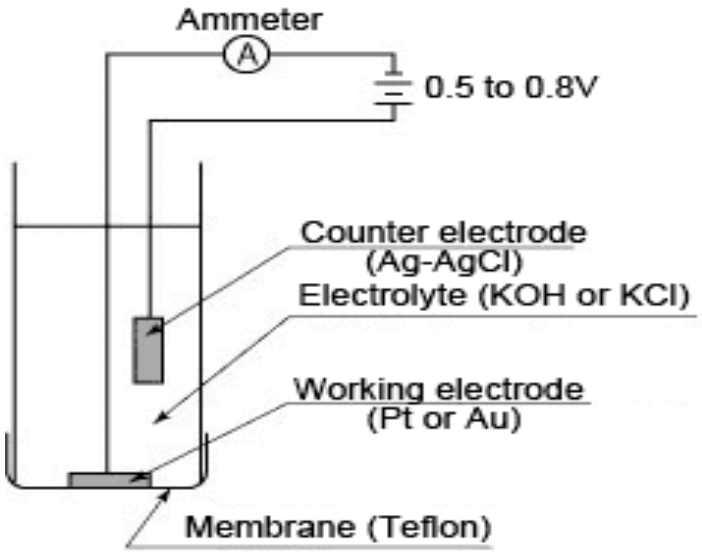
electrode, causing a Polarographic limiting current to flow which is proportional to the oxygen concentration. This method measures the concentration of dissolved oxygen based on this current value.

Advantages

- Fast response time therefore best suited where fastest response time is necessary or for huge amount of measurements.

Limitations

- Warm-up time for this type is approximately 10 minutes. Wrong readings will occur if measurements are made when the required amount of time has not been attained.
- Chloride ions in the electrolyte will be eventually consumed resulting in gradual drift in the electrode signal. The electrolyte must be replaced.
- Since the electrode consumes oxygen, readings are affected by flow across the sensor tip. Thus enough flow rate at the membrane (or sample renewal rate) must be ensured for accurate results.



Optical method

An optical dissolved oxygen sensor does not have an anode or cathode, and oxygen is not reduced during measurement. Instead, the sensor cap contains a luminescent dye, which glows red when exposed to blue light. Oxygen interferes with the luminescent

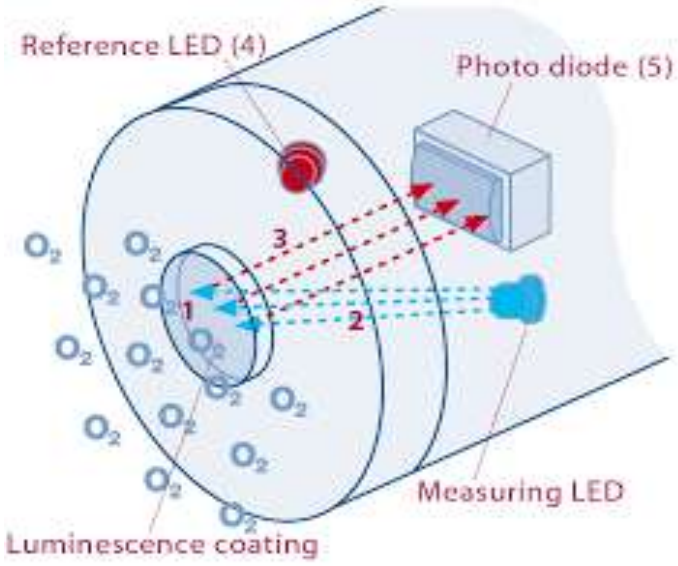
properties of the dye, an effect called "quenching." A photodiode compares the "quenched" luminescence to a reference reading, allowing the calculation of dissolved oxygen concentration in water.

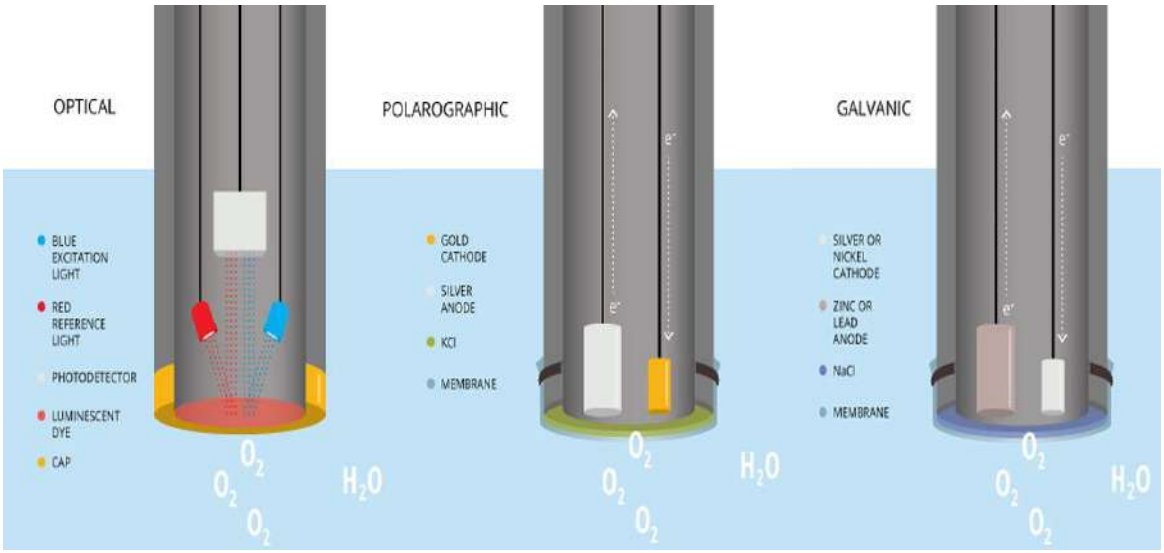
Advantages:

- **Reliable measurements:** Optical sensor technology delivers accurate DO monitoring with no drift and no minimum flow
- **Long operating life with minimal maintenance:** No membranes, electrolyte solutions, or anodes – just replace the cap once in every 1 or 2 years.

Disadvantages:

- Higher initial Cost.
- Slower measurement response time.
- Higher power consumption than other sensors.





Dissolved oxygen (DO) Analyzer

Applications

A. Foods and Beverages

Many foodstuffs are packed in conditions where require a low or controlled oxygen level. Dissolved oxygen levels in drinks, such as beer, should be kept in specific range. Practice of adding oxygen under pressure to bottled water to make oxygenated water has become more common. These dissolved oxygen measurements required dissolved oxygen probes that can be cleaned at elevated temperatures without being removed from the application.

B. Aquaculture (Fish Farming)

Dissolved oxygen sensors, such as multi-channel dissolved oxygen meters, are needed for fish farmers. It is essential to have such instrument to measure and control the dissolved oxygen level in the water body. Dissolved oxygen monitoring and logger are encompassing alert units with both high dissolved oxygen alarm and low dissolved oxygen alarm.

C. Sea cages

Since it is difficult to control the dissolved oxygen content of the sea, dissolved oxygen measurement is very important because the feed uptake and dissolved oxygen levels are interconnected. Intensive feeding after fish have experienced low dissolved oxygen levels can not only be a waste of food, but can actually harm the fish. The measurement of dissolved oxygen levels enables feed to be dosed optimally and, if relayed to the shore can warn that the cage should be moved if extremely low dissolved oxygen levels should occur.

D. Waste Water Treatment

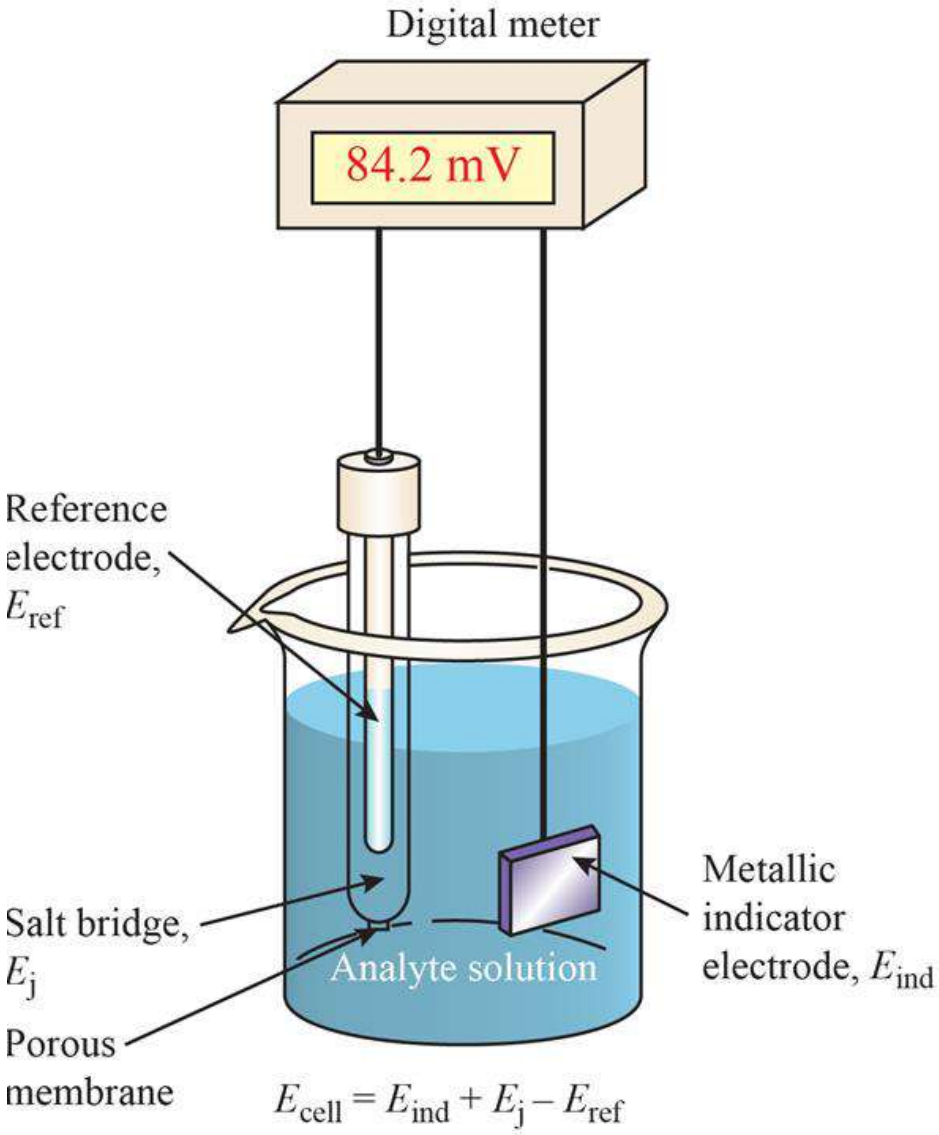
Waste water treatment is critical in these years. It is no longer enough just to filter the water and dump it into the sea directly. The larger part of the waste is mainly organic, and this must treated in sludge tanks to break it down for further filtration. Sludge tank dissolved oxygen measurement and control is kept. Flow measurement, such as suspended solids measurement, sludge blanket detection, conductivity measurement, nitrate measurement and phosphate measurement utilizing the DO sensors are also all used to enable the efficient and effective cleaning of waste water.

E. Safety Monitoring

DO sensors can be utilized for safety monitoring such as oxygen detection in flammable gas and oxygen monitoring in ambient air. Blanket gas is often used where flammable substances occur. Blanket gas is gas that cannot burn or sustain fire, i.e. it does not contain oxygen. Volumetric oxygen measurement is carried out both on the blanket gas and the surrounding air, the latter for worker safety. Special versions of the dissolved oxygen electrodes are approved for use in potentially dangerous atmospheres, i.e. in classified areas.

Electrodes:

Potentiometric Methods: Based on measurement of the potential of electrochemical cells in the absence of appreciable currents.



Instrumention of potentiometric technique

Basics components required:

- Reference electrode: gives reference for potential measurement
- Indicator electrode: where species of interest is measured
- Potential measuring device

The main theory involved in the potentiometry is, when the known potential electrode immersed in the sample solution then the potential is given by Nernst equation:

$$E = E^0 + \left(\frac{0.592}{n}\right) \log C$$

Where E is the potential of the solution; E^0 is the standard electrode potential; n is the valency of the ions; c is the concentration of sample solution; 0.592 is the value obtained from the RT/F; where R is the gas constant, T is the temperature in Kelvin, F is the faradays constant.

Electrodes: These are mainly used to measure the voltages. Mainly two electrodes are used in the potentiometry. They are as follows

- Reference electrode
- Indicator electrode

Reference Electrode:

The Reference Electrode: An important part of the measurement is the use of a stable reference electrode.

Example:

- Standard hydrogen electrode
- Silver- Silver Chloride electrode
- Saturated calomel electrode

The **reference electrodes** are classified into two main classes they are as follows:

- Primary standard electrodes
  - Standard hydrogen electrode
- Secondary standard electrodes
  - Silver- Silver Chloride electrode
  - Saturated calomel electrode

Indicator electrode:

It is used to measure the potential of the analyte solution comparing with that of reference electrode. Its potential is directly proportional to ion concentration.

Example:

- Hydrogen electrode.
- Glass electrode.
- Antimony –Antimony oxide electrode.

The Indicator electrodes are classified into two main classes they are as follows:

- Metal indicator electrodes
- Ion-selective electrodes

Reference Electrode

It is clear that at least two electrodes are necessary to make a potential measurement. As Kissinger and Bott have expressed, "electrochemistry with a single electrode is like the sound of one hand clapping". In potentiometry, those two electrodes are generally called the indicator electrode and the reference electrode. The indicator electrode possesses some characteristic that allows it to selectively respond to changes in the activity of the analyte being measured. For the measured potential to have meaning in this context, the reference electrode must be constructed so that its composition is fixed and its response is stable over time, with observed changes in measured potential due solely to changes in analyte concentration. The standard reduction potential, or E^0, allows you to predict the ease with which a half-cell reaction occurs relative to other half-reactions. Values of E^0 are most often reported as the potential measured in an electrochemical cell for which the standard hydrogen electrode is used as a reference.

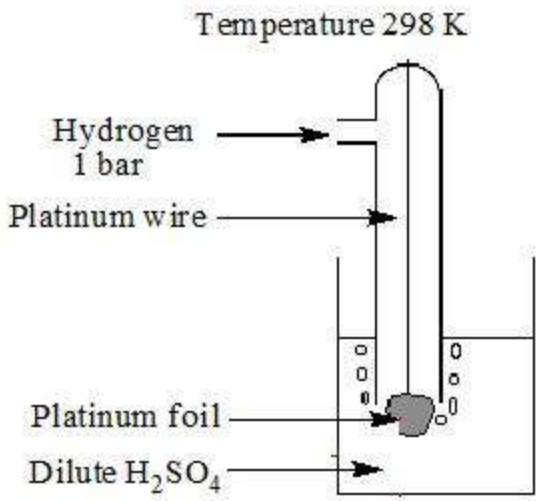
Standard Hydrogen Electrode

The standard hydrogen electrode, or SHE, is composed of an inert solid like platinum on which hydrogen gas is adsorbed, immersed in a solution containing hydrogen ions at unit activity. The half-cell reaction for the SHE is given by

$2H^+ (aq) + 2e^- = H_2 (g)$

and the half-cell potential arbitrarily assigned a value of zero ($E^0 = 0.000$ V).

Practical application of the SHE is limited by the difficulty in preparing and maintaining the electrode, primarily due to the requirement for H_2 (g) in the half-cell.



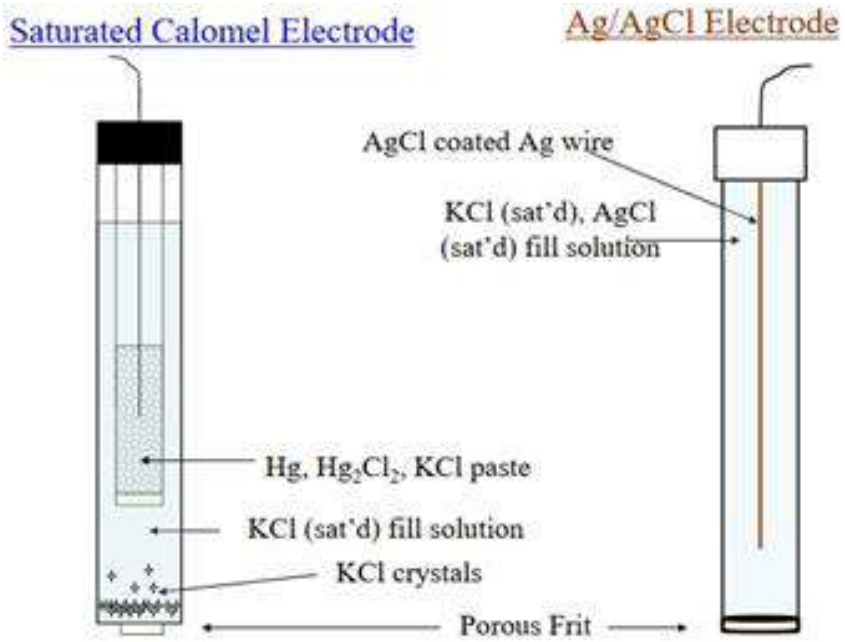
Most Potentiometric methods employ one of two other common reference half-cells – the saturated calomel electrode (SCE) or the silver-silver chloride electrode (Ag/AgCl).

1. Saturated Calomel Electrode (SCE)

The SCE is a half cell composed of mercurous chloride (Hg_2Cl_2, calomel) in contact with a mercury pool. These components are either layered under a saturated solution of potassium chloride (KCl) or within a fritted compartment surrounded by the saturated KCl solution (called a double-junction arrangement). A platinum wire is generally used to allow contact to the external circuit. The half reaction is described by

$$Hg_2Cl_2(s) + 2e^- = 2Hg(l) + 2Cl^-(sat'd)$$

with an E^0 value of +0.244 V. A common arrangement for the SCE is shown below, left side. In this arrangement, a paste is prepared of the calomel and solution that is saturated with KCl. The solution over the past is also saturated with KCl, with some solid KCl crystals present. Contact to the measurement cell is made through a porous glass frit or fiber which allows the movement of ions, but not the bulk solution. In many electrodes designed for potentiometry, the reference half cell is contained within the body of the sensing electrode. This arrangement is referred to as a "combination" electrode.



2. Silver/Silver Chloride (Ag/AgCl)

The silver/silver chloride reference electrode is composed of a silver wire, sometimes coated with a layer of solid silver chloride, immersed in a solution that is saturated with potassium chloride and silver chloride. The pertinent half reaction is

$$AgCl(s) + e^- \Leftrightarrow Ag(s) + Cl^-(sat'd)$$

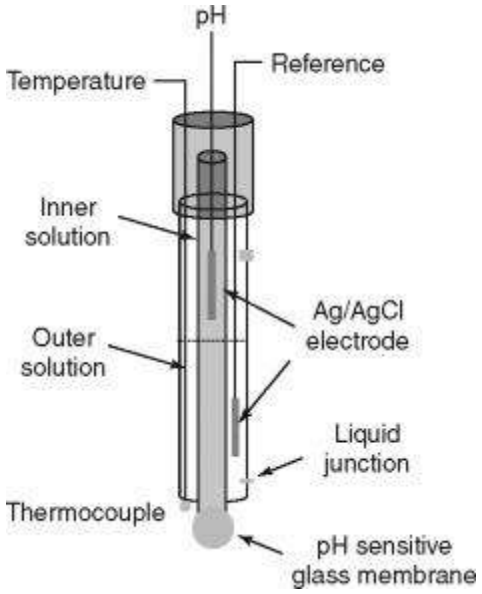
with a value for E^0 of +0.222 V. The actual potential of the half-cell prepared in this way is +0.197 V vs SHE, which arises because in addition to KCl, AgCl also contributes to the chloride activity, which is not exactly unity. A schematic of the Ag/AgCl reference electrode is shown at right in the figure.

Both the SCE and the Ag/AgCl reference electrodes offer stable half-cell potentials that do not change over time or with temperature. In addition, the loss of electrolyte to evaporation does not change the saturated nature of the solution, nor the potential. One must be aware that the contact junctions of the half cells by nature slowly leak fill solution into the external solution in which they are found. As such, there are instances where measurements of certain ions, like chloride, might be affected by the ions introduced to the measurement solution by leakage.

Glass Electrode:

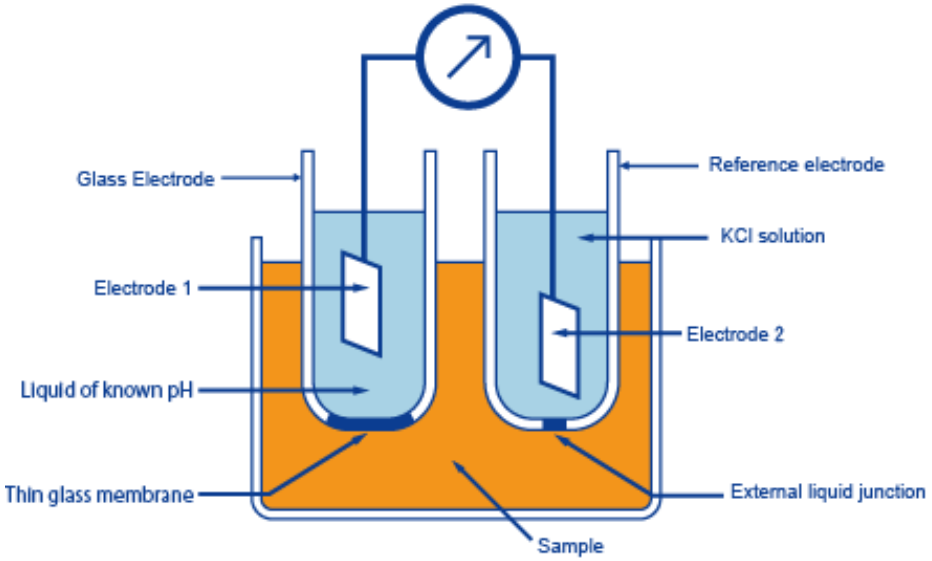
In the glass-electrode method, the known pH of a reference solution is determined by using two electrodes, a glass electrode and a reference electrode, and measuring the voltage (difference in potential) generated between the two electrodes. The difference in

pH between solutions inside and outside the thin glass membrane creates electromotive force in proportion to this difference in pH. This thin membrane is called the electrode membrane. Normally, when the temperature of the solution is 30 ℃, if the pH inside is different from that of outside by 1, it will create approximately 60 mV of electromotive force.



The liquid inside the glass electrode usually has a pH of 7. Thus, if one measures the electromotive force generated at the electrode membrane, the pH of the sample can be found by calculation.

A second electrode is necessary when measuring the electromotive force generated at the electrode membrane of a glass electrode. This other electrode, paired with the glass electrode, is called the reference electrode. The reference electrode must have extremely stable potential. Therefore, it is provided with a pinhole or a ceramic material at the liquid junction.
In other words, a glass electrode is devised to generate accurate electromotive force due to the difference in pH. And a reference electrode is devised not to cause electromotive force due to a difference in pH.



Glass Electrode

A glass-electrode pH meter consists of a detector, indicator and reference solution. A brief description of each part follows:

Detector (Glass electrode, Reference electrode, Temperature-compensation electrode, Combination electrode)

There is also a composite electrode, in which the glass electrode and reference electrode are integrated into one unit, and the combination electrode, into which all three of the above-mentioned electrodes are integrated into a single unit.

Indicator

The combination of a glass electrode and reference electrode can be thought of as a battery with high internal resistance. Thus, you cannot measure the difference in potential accurately if you connect it to an ordinary potentiometer (voltmeter) as-is. Need an amplifier with high input impedance. The indicator of the pH meter has such an amplifier built in, and allows adjustment.

Reference Solution

A reference solution must always be calibrated for the pH meter before measuring pH.

Ion Selective Electrode

An electrode that responds to a particular ion's activity is called ion-selective (or) ion – sensitive electrode (ISE). An Ion selective electrode is a sensor which converts the activity of a specific ion (dissolved in a solution) into a voltage (potential), which can be

measured by a mV or Ion meter. The voltage is theoretically dependent on the logarithm of the ion activity, as described by the Nernst Equation.

$E = E^0 + (2.303*RT/nF) \log(A)$

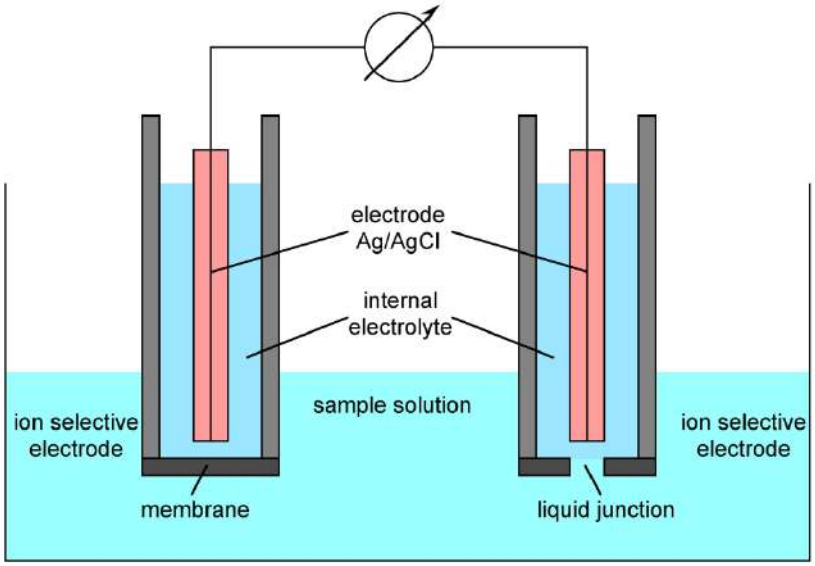
The sensing part of the electrode is usually made from an ion specific membrane, coupled together with a reference electrode (either separate or as a combination).

Working Principle: When ever two solutions of different concentrations are separated by a membrane, a potential difference is set up across the membrane due to the unequal distribution of ions in the solutions. This potential difference is known as membrane potentials. This membrane potential difference will be measured by ion-selectometers with the help of ion selective electrodes.

- Potentiometric methods of analysis involve the direct measurement of electrical potential due to the activity of free ions.
- ISEs are designed to be selectively sensitive toward individual ions.
- The potential produced at the membrane sample solution interface is proportional to the ionic activity or concentration.
- Ion-selective membranes typically consist of glass, crystalline, or polymeric materials.
- The chemical composition of the membrane is designed to achieve optimal perm selectivity toward the ion of interest.
- Other ions exhibit slight interaction with membrane sites and display some degree of interferences.
- Clinically, a correction is required when the interference exceed an acceptable value.
- Measurements with ISE are simple, often rapid, nondestructive, and applicable to a wide range of concentrations.

Components of ISE:

1. Ion selective electrode with membrane at the end allows ions of interest to pass, but excludes the passage of the other ions.
2. Internal reference electrode- present within the ion selective electrode which is made of silver wire coated with solid silver chloride, embedded in concentrated potassium chloride solution (filling solution) saturated with silver chloride. This solution also contains the same ions as that to be measured.



Ion Selective Electrode (ISE)

Types of Ion Selective Electrodes

There are six types of ISEs based on the membrane used in it:

1. Glass membrane electrode
2. Liquid membrane Electrode
3. Solid membrane Electrode
4. Gas sensing Electrode
5. Bio catalytic membrane Electrode (Enzyme – based Electrode).

Glass Membrane Electrode

Glass electrode consists of a stem of non-cation responsive, high resistance glass on which is fused a thin walled bulb of cation responsive glass. Selectivity of a glass electrode is a function of the components of the glass. Glass membrane electrodes are formed from special ionically conducting glass. By altering the composition and constituents of the glass, changes can be made to selectivity, chemical resistance, response time, and impedance. The most common glass membrane ion selective electrode is the pH electrode. Another common one is the sodium selective electrode.
There are three types of glass electrodes

i. Type : pH
ii. Type : Cation sensitive
iii. Type : Sodium sensitive

Liquid Membrane Electrode

Liquid membrane or ion exchanger electrode are prepared using an organic liquid ion-exchanger which is immiscible with water, or with ion sensing material is dissolved in an organic solvent which is immiscible with water. The solvent is placed in a tube sealed at the lower end by a thin hydrophobic membrane such as cellulose acetate paper; aqueous solution will not penetrate this film. Example for liquid membrane electrode is calcium responsive electrode.

Solid State Electrode

Solid state electrodes are selective primarily to anions. It may be a homogenous membrane electrode or heterogeneous membrane electrode. Homogenous membrane electrode: ions selective electrodes in which the membrane is a crystalline material (Agl/Ag2S). The membrane consisting of the sparingly soluble salt in the inert binding material is a heterogeneous membrane electrode.

Crystalline or solid state membrane electrodes are made from relatively insoluble ionically conducting inorganic salts. These are available in homogeneous and heterogeneous forms. They have good selectivity since only ions which can introduce themselves into the crystal lattice can interfere. Examples include the Fluoride electrode which uses a doped LaF3 crystal, and the Chloride electrode which uses silver chloride powder.

Gas Sensing Electrode

This type of electrodes consists of permeable membrane and an internal buffer solution. The pH of the buffer change as the gas reacts with it. The change is detected by a combination of pH sensor. This type of electrode does not require an external reference electrode. Used for the measurement of Ammonia, Carbon dioxide and Nitrogen oxide.

Bio catalytic membrane Electrode

The Potentiometric electrodes for the analysis of molecules of biochemical importance can be constructed in fashion similar to that used for gas sensing electrodes. The most common class of Potentiometric biosensors are so called enzyme electrodes, in which an enzyme is trapped or immobilized at the enzyme produces a product whose concentration is monitored by the ion selective electrode.

Polymer Membrane Electrodes

Polymer Membrane Electrodes are based on special organic polymer membranes which contain various ion-exchange ionophores incorporated into an inert matrix. These are used in electrodes to measure ions such as Potassium, Calcium, and Nitrate.

pH Measurement:

In chemistry, **pH** is a scale used to specify the acidity or basicity of an aqueous solution. Acidic solutions (solutions with higher concentrations of H^+ ions) are measured to have lower pH values than basic or alkaline solutions.

The pH scale is logarithmic and inversely indicates the concentration of hydrogen ions in the solution. When the hydrogen ions outnumber the hydroxide ions, the solution is acidic. If the reverse is true, then the solution is alkaline. So, pH is defined by the following formula:

$$\mathbf{pH=-\log_{10}[H^+]}$$

The concentration of hydrogen ions in any solution we are likely to encounter will range from 1 mol to 0.000001 mol per liter of solution. However, solutions with extremely low hydrogen-ion concentration could conceivably rack up a pretty long parade of zeros after the decimal point. Danish biochemist S.P.L. Sorensen was the first to use the pH system, which defines inverse numbers of hydrogen-ion concentration shown in common logarithm as pH. That is,

$$\mathbf{pH=-\log_{10}[H^+]}$$

In the case of a neutral solution,

$[H^+]=10^{-7}$, which we call a pH of 7.

The methods for measuring pH fall roughly into the following four categories:

- **Indicator methods**
- **Metal-electrode methods** (including the hydrogen-electrode method, quinhydron-electrode method and antimony-electrode method)
- **Glass-electrode methods**
- **Semiconductor sensor methods**

(1) Measuring pH using an Indicator

This category basically includes two methods: One involves comparing the standard color corresponding to a known pH with the color of an indicator immersed in the test liquid using buffer solution. The other method involves preparing pH test paper which is soaked in the indicator, then immersing the paper in the test liquid and comparing its color with the standard color. This method is simple, but prone to error. A high degree of accuracy cannot be expected. Various errors include; Error due to high salt

concentration in the test liquid, Error due to the temperature of the test liquid, Error due to organic substances in the test liquid. The indicator method cannot measure the pH of high-purity water, since the influence of the indicator itself is too large.

(2) Hydrogen-Electrode Method

A hydrogen electrode is made by adding platinum black to platinum wire or a platinum plate. It is immersed in the test solution and an electric charge is applied to the solution and the solution is saturated with hydrogen gas. The electrode potential is measured between platinum black electrode and silver chloride electrode. This potential is inversely proportional to pH of the solution. The hydrogen-electrode method is a standard among the various methods for measuring pH. The values derived using other methods become trustworthy only when they match those measured using hydrogen electrode method.

However, this method is not appropriate for daily use because of the effort and expense involved, with the inconvenience of handling hydrogen gas and great influence of highly oxidizing or reducing substances in the test solution.

(3) Quinhydron-Electrode Method

When quinhydrone is added to a solution, it separates into hydroquinone and quinone. Because quinone's solubility varies depending on the pH value of the solution, pH can be determined from the voltage between a platinum and reference electrode. Although this method is simple, it is seldom used today, because it does not work when oxidizing or reducing substances are involved, or when the test solution has a pH above 8 or 9.

(4) Antimony-Electrode Method

This method involves immersing the tip of a polished antimony rod into a test solution, also immersing a reference electrode, and measuring pH from the difference in potential between them. This method was once widely used because the apparatus is sturdy and easy to handle. However, its application is now quite limited because results vary depending on the degree of polish of the electrode, and reproducibility is low.

(5) Glass-Electrode Method

The glass electrode method uses two electrodes, a glass electrode and reference electrode, to determine the pH of a solution by measuring the voltage (potential) between them. This method is the one most commonly used for pH measurement, since the potential quickly reaches equilibrium and shows good reproducibility, and because the method can be used on various types of solutions, with oxidizing or reducing substances having very little impact on the result. The glass electrode method is widely used, not only in industry but also in many other fields.

（6）Semiconductor sensor methods

The semiconductor pH sensor, whose development started around 1970, replaces a glass electrode with a semiconductor chip. This sensor, known as an ion sensitive field effect transistor (ISFET), is not only resistant to damage but also easily miniaturized. Miniaturization allows the use of smaller amounts of sample for measurement, and makes it possible to perform measurements in very small spaces and on solid state surfaces. This sensor promises useful applications in measurement in the fields of biology and medicine.

Unit - 5

NUCLEAR MAGNETIC RESONANCE AND RADIATION TECHNIQUES

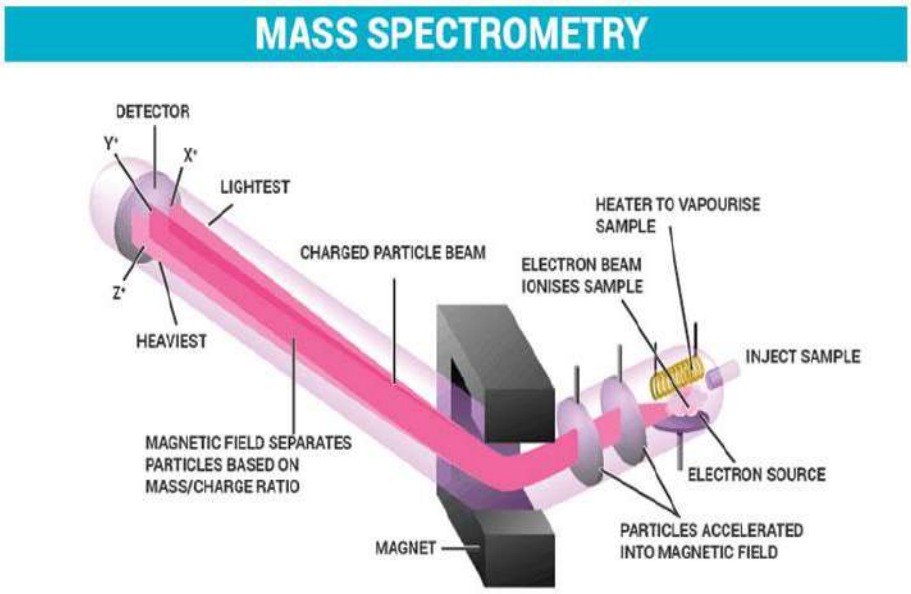
Mass Spectrometry

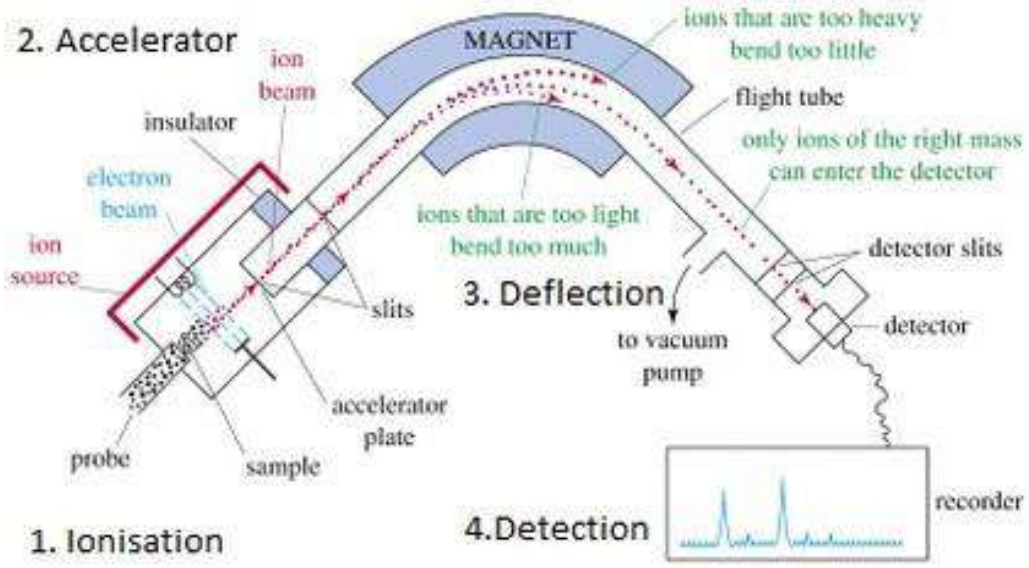
- Mass Spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions.
- In this instrumental technique, sample is converted to rapidly moving ions by electron bombardment and charged particles are separated according to their masses.
- Mass spectrum is a plot of relative abundance against the ratio of mass/charge (m/e).
- These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules and other chemical compounds.

Principle of Mass Spectrometry (MS)

- There are four stages in a mass spectrometer which we need to consider, these are – ionisation, acceleration, deflection, and detection.
- In this technique, molecules are bombarded with a beam of energetic electrons.
- The molecules are ionized and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge, i.e. m/e ratio (value).
- For most ions, the charge is one and thus, m/e ratio is simply the molecular mass of the ion.

- The ions pass through magnetic and electric fields to reach detector where they are detected and signals are recorded to give a mass spectra.



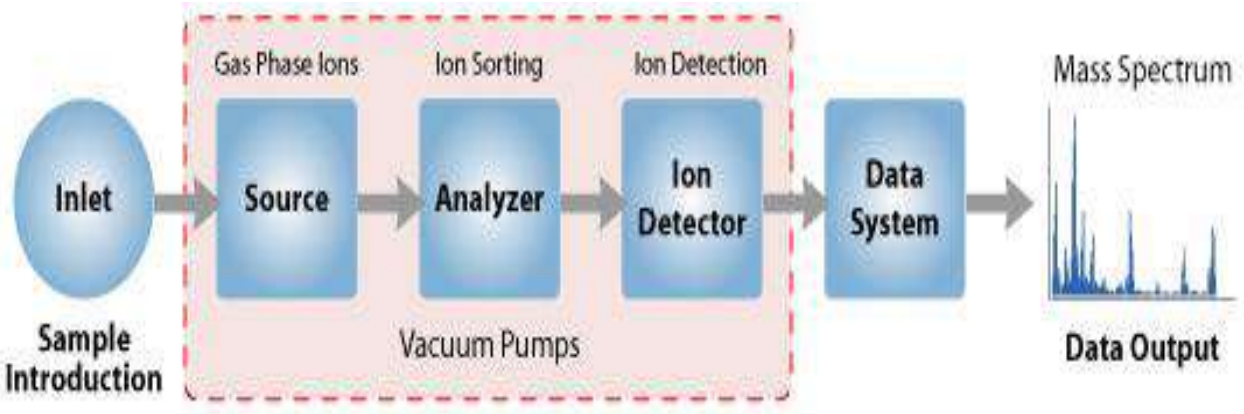


Working of Mass Spectrometry (MS)

- In a typical procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons.

- This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field.
- Ions of the same mass-to-charge ratio will undergo the same amount of deflection.
- The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio.
- The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

Instrumentation and Steps of Mass Spectrometry (MS)



Instrumentation and Steps of Mass Spectrometry (MS)

A. Sample Inlet

- Sample stored in large reservoir from which molecules reaches ionization chamber at low pressure in steady stream by a pinhole called "Molecular leak".

B. Ionization

- Atoms are ionized by knocking one or more electrons off to give positive ions by bombardment with a stream of electrons. Most of the positive ions formed will carry charge of +1.
- Ionization can be achieved by :

The classic methods are

- **Electron Impact (EI)**
- **Fast Atom Bombardment (FAB).**

More modern techniques are

- **Atmospheric Pressure Chemical Ionization (APCI)**
- **Electrospray Ionization (ESI)**
- **Matrix Assisted Laser Desorption Ionization (MALDI)**

C. Acceleration

- Ions are accelerated so that they all have same kinetic energy.
- Positive ions pass through 3 slits with voltage in decreasing order.
- Middle slit carries intermediate and finals at zero volts.

D. Deflection

- Ions are deflected by a magnetic field due to difference in their masses.
- The lighter the mass, more they are deflected.
- It also depends upon the no. of +ve charge an ion is carrying; the more +ve charge, more it will be deflected.
- There are six general types of mass analyzers that can be used for the separation of ions in a mass spectrometry.
    - Quadrupole Mass Analyzer.
    - Time of Flight Mass Analyzer.
    - Magnetic Sector Mass Analyzer.
    - Electrostatic Sector Mass Analyzer.
    - Quadrupole Ion Trap Mass Analyzers.
    - Ion Cyclotron Resonance.

E. Detection

- The beam of ions passing through the mass analyzer is detected by detector on the basis of m/e ratio. The most common types of ion detector used in modern instruments are :
- **Faraday Cup detector (or Cylinder electrode)**
- **Electron Multiplier**
- **Photomultiplier (or Scintillation Counter)**

Applications of Mass Spectrometry (MS)

- Environmental monitoring and analysis (soil, water and air pollutants, water quality, etc.)
- Geochemistry – age determination, soil and rock composition, oil and gas surveying
- Chemical and Petrochemical industry – Quality control
- Identify structures of biomolecules, such as carbohydrates, nucleic acids
- Determination of molecular mass of peptides, proteins.
- Monitoring gases in patients breathe during surgery.
- Identification of drugs abuse and metabolites of drugs of abuse in blood, urine, and saliva.
- Analyses of aerosol particles.
- Determination of pesticides residues in food

Mass Spectrometry Ionization Methods

- There are many types of ionization methods are used in mass spectrometry methods.

The classic methods are

- **Electron Impact (EI)**
- **Fast Atom Bombardment (FAB).**

More modern techniques are

- **Atmospheric Pressure Chemical Ionization (APCI)**
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- **Matrix Assisted Laser Desorption Ionization (MALDI)**

Electron Impact ionization (EI)

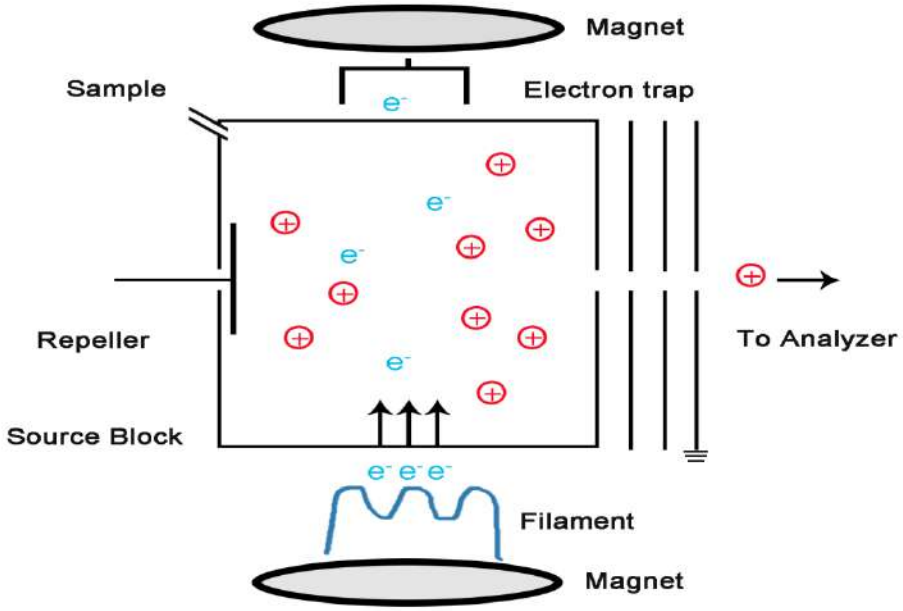
EI is done by volatilizing a sample directly in the source that is contained in a vacuum system directly attached to the analyzer. The gas phase molecules are bombarded by a beam of electrons formed by heating a filament bias at a negative voltage compared to the source. The bias voltage is most commonly at -70 volts. The electron beam ejects an ion from the gas phase molecule producing a radical ion. This technique is considered a hard ionization technique, because it causes the ion to fragment. EI is also the method that is most commonly used for GC-MS.

Advantages

- Reproducible Method
- High Ionization Efficiency
- Ionization is non-selective
- Interface to GC

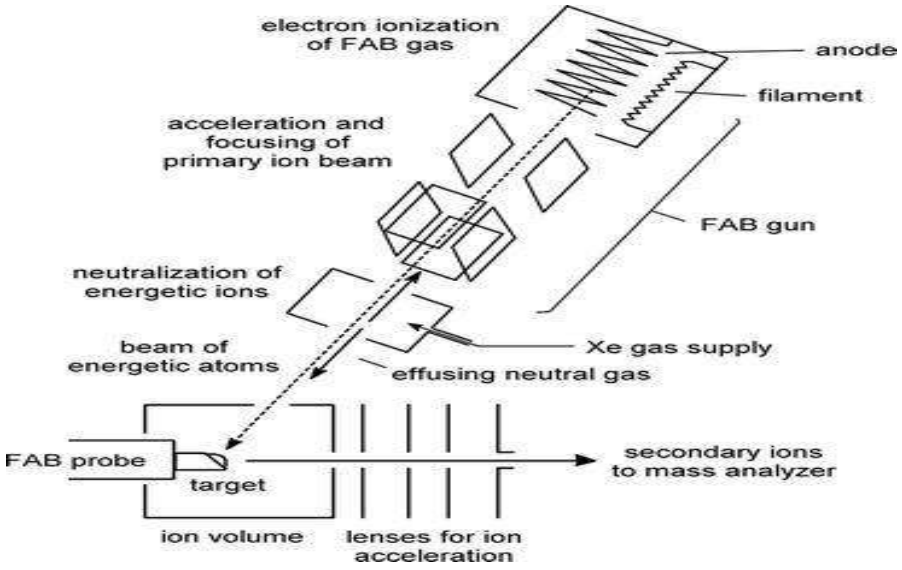
Disadvantages

- Only positive ions are formed
- Sample has to be volatile
- Large internal energy method
- No interface to LC



Fast Atom Bombardment (FAB)

FAB is a technique that was popular in the 80's to early 90's because it was the first technique that allowed ionization of non-volatile compounds that could be done simply. It was done by bombarding a sample in a vacuum with a beam of atoms, typically argon or xenon accelerated to Kilovolt energies. The sample was typically mixed in a matrix(the material to be analyzed is mixed with a non-volatile chemical protection environment, called a matrix). The two most common matrixes were glycerol and 3 Nitro-benzoic acid. The matrix allowed the sample to refresh itself. A variation of FAB was replacement of the atom beam with a beam of ions, typically cesium ions, which was called secondary ion mass spectrometry (SIMS). SIMS spectra were typically identical to FAB spectra and the terms became interchangeable.



Electrospray ionization (ESI)

Electrospray ionization (ESI) is a technique to generate ions for mass spectrometry by applying a high voltage to a liquid to produce an aerosol. Due to relatively fragile bio macro molecules, their structures are easily destroyed during the process of dissociation and ionization. ESI overcomes the tendency of these molecules to fragment upon ionization. ESI applies a high voltage at the outlet of the capillary, and the high electric field generated atomizes the liquid flowing out of the capillary into tiny charged droplets. Desolvation: The droplets entering the spray chamber are evaporated by the counter current of the heated dry gas (like nitrogen gas), and the diameter of the droplets becomes smaller, and the surface charge density increases. When reaching the Rayleigh limit, the Coulomb repulsive force between the charges is sufficient to counteract the surface tension of the droplet, and the droplets fission, resulting in smaller charged droplets. As the solvent evaporates, the charge intensity on the surface of the droplet gradually increases, and finally the droplet splits into one or a plurality of charged ions, allowing the analyte to enter the gas phase in the form of a single charge or multiple charges and become a gas phase ion.

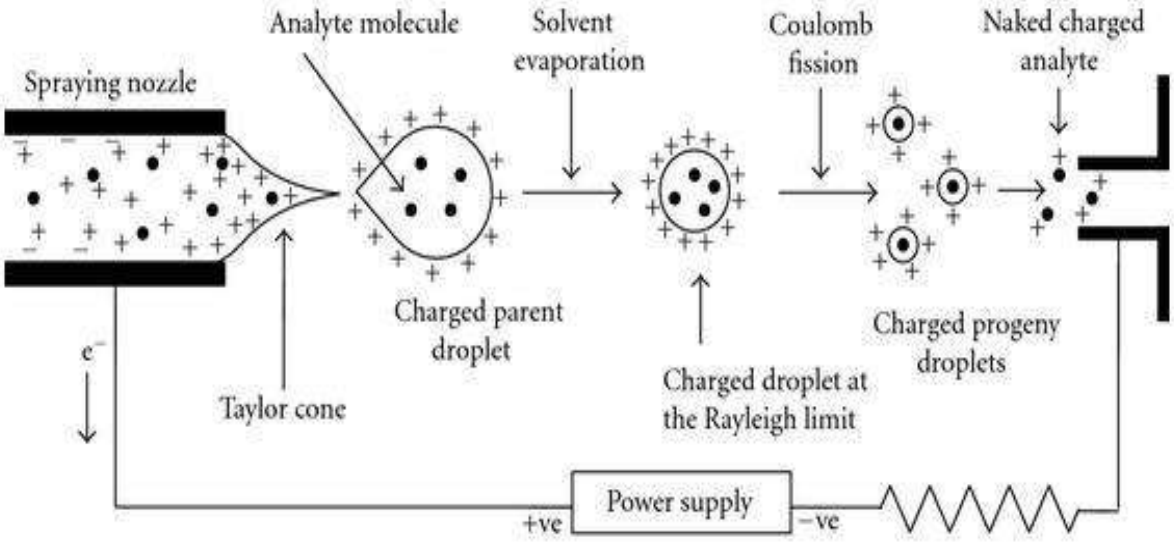
Advantages

- Simple method to ionize non-volatile solutions.
- In Electrospray mass spectrometry, high molecular weight molecules typically carry multiple charges, and the distribution of charge states accurately quantifies molecular weight, providing both accurate molecular mass and structural information.

- Multiple ionization modes to choose from: positive ion mode and negative ion mode.
- Effectively combined with a variety of chromatographs for complex system analysis.

Disadvantages

- There is a limit to the choice of solvent and the range of solutions that can be used.
- Since the solution parameters control the spray process, there is fluctuation in the ion signal even under good conditions.



Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric Pressure Chemical Ionization (APCI)- Atmospheric pressure chemical ionization (APCI) is a new ionization method that is primarily used as an interface technology in high performance liquid chromatography and mass spectrometry. APCI has a wide ionization range, high sensitivity and high selectivity. It matches the high-efficiency separation ability of liquid chromatography, making liquid chromatography-atmospheric pressure chemical ionization mass spectrometry a standard analytical technique in the field of biological and environmental chemistry. APCI is a method that is typically done using a similar source as ESI, but instead of putting a voltage on the spray itself, the voltage is placed on a needle that creates a corona discharge at

atmospheric pressures. With gas assistance, the solvent and sample flow through the injector. The solvent and sample are vaporized by the heater in the injector and ejected from the injector outlet. At the outlet of the injector, the solvent is ionized by electro-acupuncture corona discharge. The solvent ions react with the sample molecules to ionize the sample.

$$G + e^- \rightarrow G^+ + 2e^-$$

$$G^+ + R \rightarrow R^+ + G$$

$$R^+ + M \rightarrow M^+ + R$$

$$N_2 + e^- \rightarrow N_2^+ + 2e^-$$

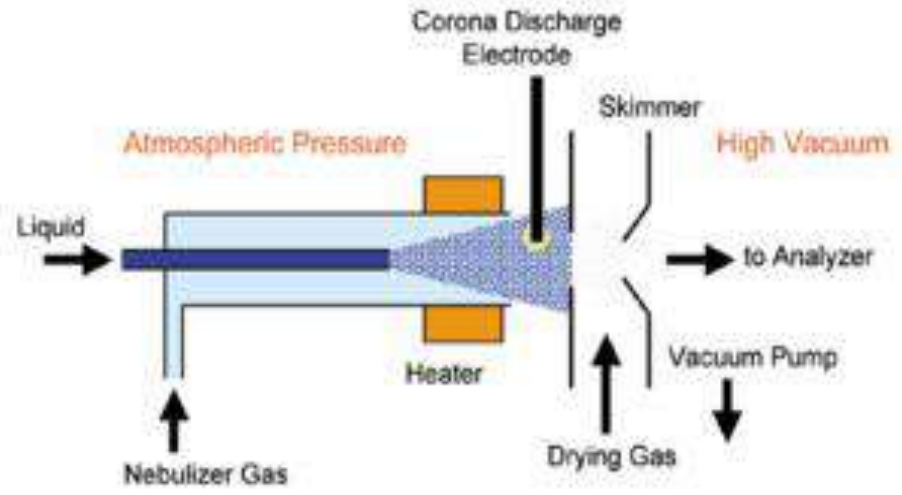
$$N_2^+ + 2N_2 \rightarrow N_4^+ + N_2$$

$$N_4^+ + H_2O \rightarrow H_2O^+ + 2N_2$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + HO\cdot$$

$$H_3O^+ + M \rightarrow [M+H]^+ + H_2O$$

In the formula, G represents a reaction gas, R represents a solvent, and M represents a sample molecule. When N_2 is used as the reaction gas and water is used as the solvent, the above reaction is as above.



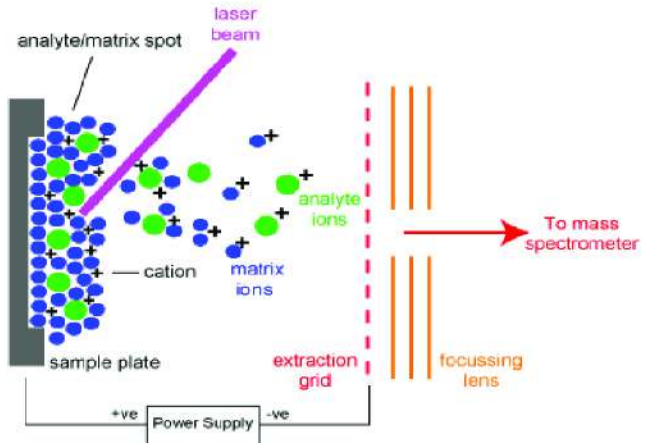
This reaction process is very similar to traditional chemical ionization. The difference is that conventional chemical ionization is the electron bombardment of a solvent under vacuum to ionize it, while atmospheric pressure chemical ionization is to ionize the sample by a discharge needle corona discharge at normal pressure. Atmospheric pressure chemical ionization is mainly used to analyze samples with good thermal stability. Compared with Electrospray ionization, APCI has the advantage of a wider range of mobile phase adaptation.

Advantages and Disadvantages of APCI

- APCI produces a singly charged product, and the product molecular mass spectrum can be directly observed. That is, APCI does not suffer from the problem of multi-charged ion products leading to signal overlap and reduced resolution of the mass spectrum. APCI must vaporize the sample and is therefore suitable for the analysis of weakly polar compounds with good thermal stability and low proton affinity. In terms of mobile phase flow rate, APCI can accommodate higher flow rates than ESI.
- In addition, APCI is not suitable for the analysis of biological macromolecules because it cannot generate a series of multi-charged ions. APCI produces very few fragment ions and therefore has limited structural information.

Matrix Assisted Laser Desorption Ionization (MALDI)

MALDI is a method of ionization in which the sample is bombarded with a laser. The sample is typically mixed with a matrix that absorbs the laser radiation and transfer a proton to the sample. Some small mass samples can be ionized without matrix, but this is typically called laser desorption. The laser is always pulsed, and typically in a vacuum. In addition, MALDI mostly forms singularly charged ions. This means MALDI is mostly performed on specially built time-of-flight instruments. One major application of MALDI besides simple analysis is imaging mass spectrometry.



Types of Mass Analyzers in Mass Spectrometry (MS)

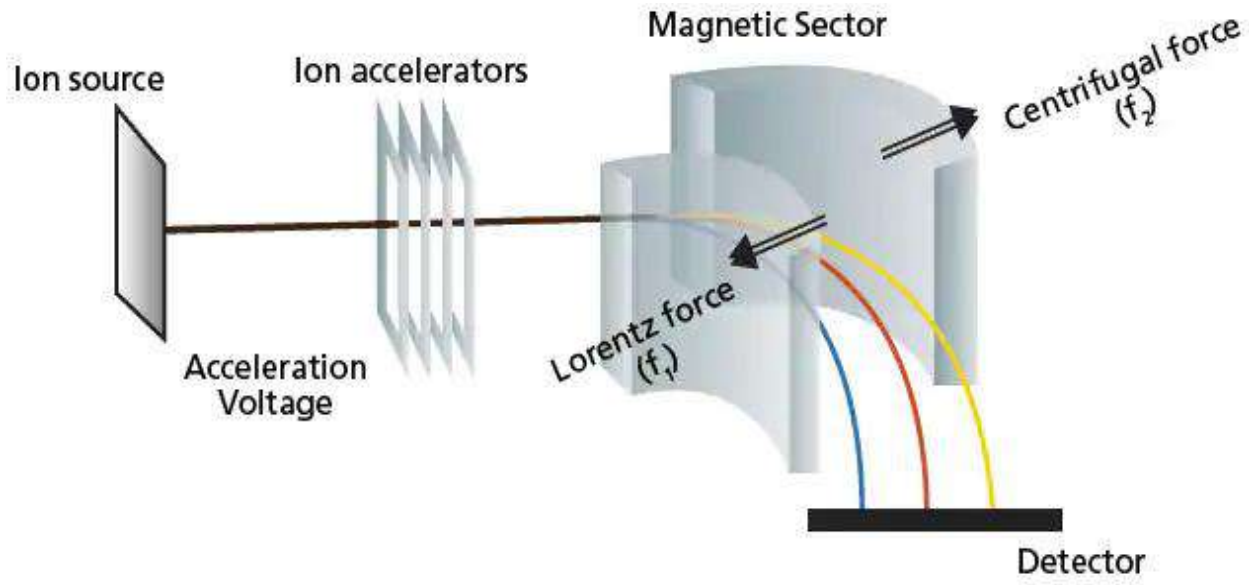
There are six general types of mass analyzers that can be used for the separation of ions in a mass spectrometry:

- Quadrupole Mass Analyzer
- Time of Flight Mass Analyzer
- Magnetic Sector Mass Analyzer
- Electrostatic Sector Mass Analyzer
- Quadrupole Ion Trap Mass Analyzers
- FT-Ion Cyclotron Resonance

Magnetic Sector Mass Analyzer

Magnetic sector, a continuous MS, has been used historically the longest. As the name implies, the mass analyzer uses magnetic field to separate ions of different *m/z* values. High voltage is first applied to the ions to accelerate them into the magnetic sector. A continuous ion source is generated and supplied from the ionization unit to the magnetic sector. Once the ions enter, are exposed to the magnetic field. As a result, ions are deflected. The deflections differ based on their *m/z* where lighter ions (of the same charge) will experience more deflection.

Ions experience a Lorentz force (f_1) from the magnetic field that can be calculated according to Equation 3. As the direction of the ion changes, a centrifugal force (f_2), expressed by Equation 4, acts on the ion. For the ions to pass through the magnetic field region and reach the detector, it must travel along a curved path of a given radius (r) where f_1 and f_2 are balanced (Equation 5). Furthermore, the kinetic energy of ions accelerated by voltage V is shown in Equation 6. By eliminating the velocity of the ion (v), Equations 5 and 6 are simplified to give Equation 7. By keeping the ion acceleration voltage V constant and varying the magnetic flux B (or keeping B constant and varying V), a detector placed on the corresponding path radius r could detect any mass m (given the same charge).



In reality, only one ion detector is used and both the acceleration voltage (V) and curve path radius (r) are kept constant while the magnetic flux density (B) is scanned. This means that ions with different masses (m) all pass along the same path through the magnetic field, one after another, and reach the detector. One mass spectrum is obtained from each scan of the magnetic field. That is to say that magnetic sector mass analyzer function by ion transmission and scanning mode. Besides the described single magnetic sector MS, there are also models of MS with both the electric sector and magnetic sector in a single MS, which is known as a dual-focusing MS. This setup is able to focus and converge ions of different energy and identical mass thereby obtaining higher mass resolution.

Some of the key features of magnetic sector MS are its high resolution and high dynamic range. However, to improve the performance of the magnetic sector MS, the strength of the magnetic field needs to be raised which means costly and larger systems are required. This limits the development of the magnetic sector MS. In addition, magnetic sector MS systems require an extremely high vacuum level of 10^{-7} Pa, causing difficulty in the LCMS interface. Furthermore, they have the disadvantage of a slower scan speed than other MS systems. Therefore, these mass analyzers are now rarely used in LCMS systems. On the other hand, they interface relatively easily to a GC unit.

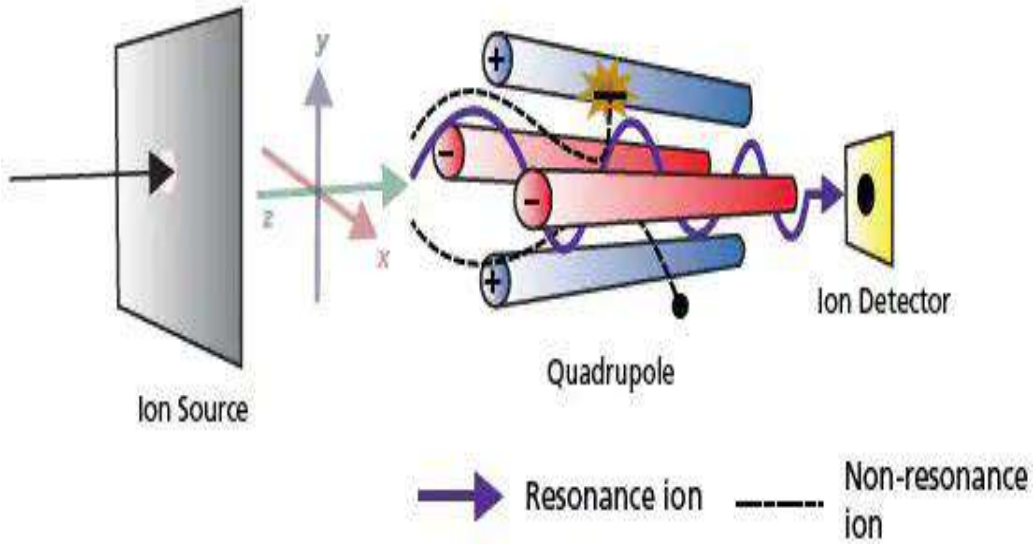
Quadrupole Mass Analyzer

The other MS which functions by scanning of ions and allowing ion transmission is the single quadrupole mass analyzer. As its name suggests, it contains four parallel

cylindrical metal rods inside a vacuum chamber, positioned equidistant from the center axis. Both a direct current (D.C.) and radiofrequency (RF) are applied to the quadrupole, so that only the ions with the target *m/z* successfully pass through the quadrupole and get to the detector. The quantity of ions that reach the detector is converted to a signal and output to a computer.

Continuous ion source generated in the ionization unit are first accelerated in the z-direction by a relatively weak voltage of only a few dozen volts. These ions pass through a tiny orifice and enter the quadrupole.

Voltage of the same polarity is applied to diagonally opposite poles and opposite voltage polarity is applied to adjacent poles as depicted by the blue and red rods in Figure. When a combination of the direct current voltage and high-frequency alternating current voltage is applied to each pole, an electric field with a rapidly varying phase is generated within the quadrupole.



Consequently, ions passing through this electric field oscillate in the x- and y directions. When a given set of parameters are applied to the poles, certain ions of a specific *m/z* range maintain a stable oscillation and pass through the quadrupole to reach the detector. On the contrary, the oscillations of ions with other *m/z* values become unstable, causing them to collide with the poles, fly out of the system, and not be detected.

The oscillation of ions within the quadrupole MS is known to occur according to the Mathieu Equation. The motion of the ion in a quadrupole follows this equation

regardless of its initial velocity or position. For a single quadrupole MS system, it can operate in two modes: **(A) Scan and (B) Selected Ion Monitoring (SIM).** In scan mode, the DC voltage to the quadruples is larger than the RF Frequency, such that the smaller *m/z* value ions will hit the detector first. For the SIM mode the RF frequency is kept higher than the DC voltage so that the heavy *m/z* value ions will hit the detector first. This SIM mode offers higher sensitivity.

Mathieu Equation

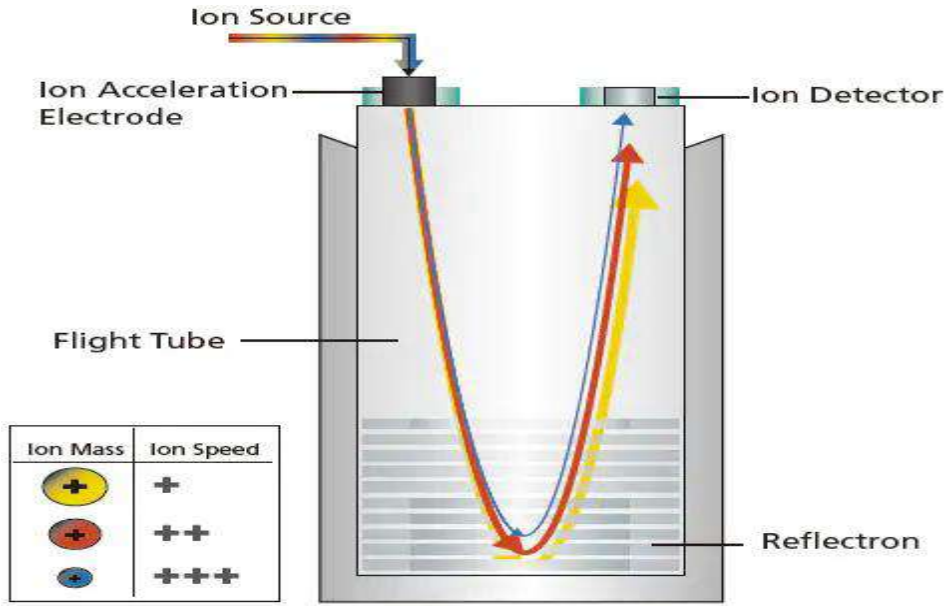
$$\frac{m}{z} = \mathrm{K}\,\frac{\mathrm{v}}{\mathrm{r}^2\omega^2}$$

m: mass of the ion
z: charge of the ion
K: constant
V: voltage applied
r: effective distance between the electrodes
ω: oscillation frequency

TOF (Time of Flight) Mass Analyzer

Unlike magnetic sector and quadrupole MS, Time-of-Flight (TOF) MS is a pulsed and non-scanning MS. It has a simple construction, consisting of an accelerator, a field-free region, a reflectron and detector inside a high vacuum chamber called a flight tube.

TOF MS separates and detects ions of different *m/z* by measuring the time taken for the ions to travel through a field-free region. First, ions generated in an ionization unit are accumulated and introduced in pulses to a flight tube. These ions are accelerated by applying a high acceleration voltage between the electrodes. The corresponding kinetic energy is obtained as described in Equation. Given a constant acceleration voltage as well as kinetic energy, each ion flies at its unique velocity inside the flight tube to reach the ion detector, which is higher for ions with smaller masses and lower for ions with larger masses.



$$T = \frac{\text{distance}}{\text{velocity}} = \sqrt{\frac{m}{z}} \times \frac{L}{\sqrt{2eV}}$$

T: Time of flight
m: mass of the ion
z: charge of the ion
e: elementary charge
V: acceleration voltage applied to ions
L: flight distance in TOF

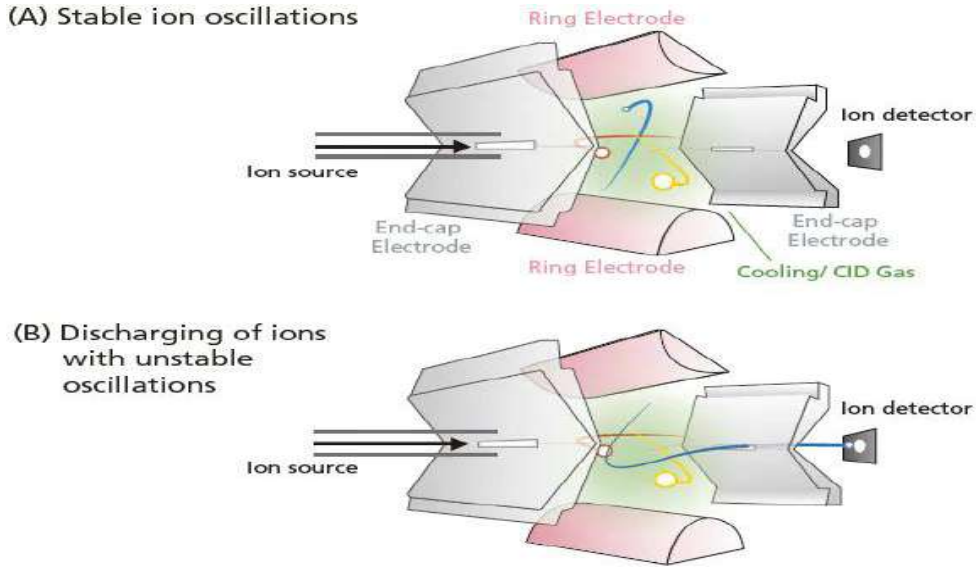
As shown in Equation , Time of flight (T) is proportional to the square root of *m/z*, i.e. for a fixed flight distance (L), ions with smaller *m/z* reach the detector sooner than those with larger *m/z*. Therefore, by keeping all other parameters constant, the time of flight (T) can be converted directly to *m/z*, which is how a mass spectrum is generated in a TOF MS. Since there is no limit to the time of flight in TOF MS, it can theoretically measure an unlimited mass range. Due to its operating principle, TOF MS systems use MALDI method of ionization.

Quadrupole Ion Trap Mass Analyzers

The IT MS employ the same principle as the quadrupole MS and the motion of ions within the mass analyzer follows the Mathieu Equation. It generally consists of a donut-shaped ring electrode sandwiched between two end-cap electrodes. An ionization unit is located at the entrance and a detector at the exit. Just like a quadrupole system, this can be thought of as the entrance and exit of a quadrupole connected in a ring shape.

Normally, the IT MS is used without applying the direct current voltage (U) to the electrodes, which corresponds to movement and operation along the horizontal axis.

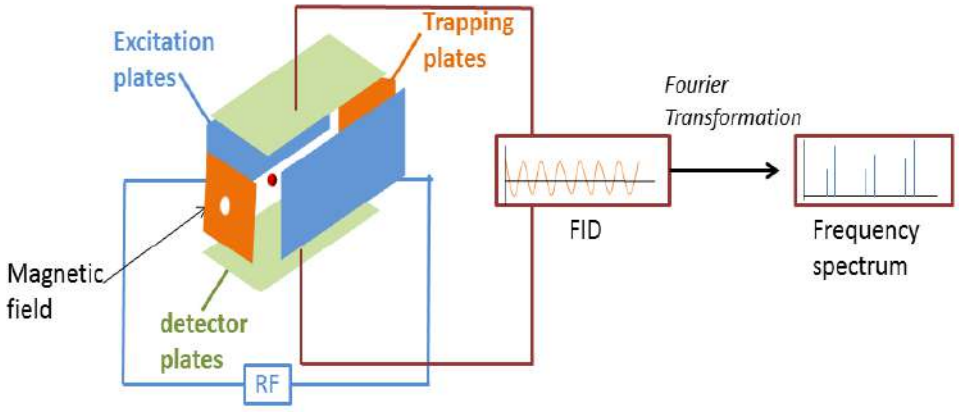
To measure a spectrum, end-cap electrodes are first grounded, and then a low high-frequency voltage is applied to the ring electrode. The ions are introduced into the IT MS in a pulse mode, where they are all temporarily trapped inside the electrode. This state, where the ions with varying mass are experiencing stable oscillations. Subsequently, to detect a specific ion, the high-frequency voltage is gradually increased, while keeping the direct current (U) to zero. As the voltage increases, the oscillation of the smaller m/z ion becomes unstable at point and then heavy m/z ion becomes unstable at point later, at which time these ions are discharged via the hole in the end-cap electrode. Quadrupole MS systems separate and detect masses by letting oscillating ions pass through the quadrupole to reach a detector, whereas ion trap MS systems separate and detect masses by discharging ions with unstable oscillations from the system.



As the name implies, ion trap MS systems trap the generated ions before separating them by mass. Furthermore, they operate by pulsed mode and only a limited quantity of ions can be trapped, resulting in a narrower dynamic range than quadrupole MS systems. However, all trapped ions are detected in the IT MS, this provides higher sensitivity in scanning analysis than quadrupole models. In addition, it enables the trapping of a specific ion, fragmenting it and then trapping a specific product ion for further fragmentation, and so forth. Therefore, IT MS is considered a mass spectrometer specialized for elucidating the fragmentation pathway for structural determination of a target molecule.

Fourier Transform Ion Cyclotron Resonance (FT-ICR)

In addition to the two kinds of IT MS, there are other similar trapping type of MS such as the Fourier Transform Ion Cyclotron Resonance (FT-ICR) and Orbitrap. They adopt similar mechanism and principles. In the FT-ICR setup, the use of both the electric and magnetic field generates the stable oscillation and motion of the ions. To detect the ions, the selected ions are accelerated such that its radius of oscillating motion increases, the oscillation becomes unstable and eventually the ion gets removed. By determining the cyclotron frequency, it can be Fourier transformed and the ion mass is deduced. For Orbitrap MS systems, it only requires the use of electric field to trap and separate the ions. These MS systems demonstrate excellent mass resolution and mass accuracy.

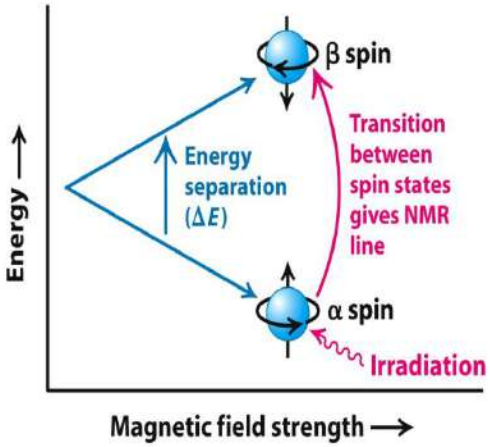


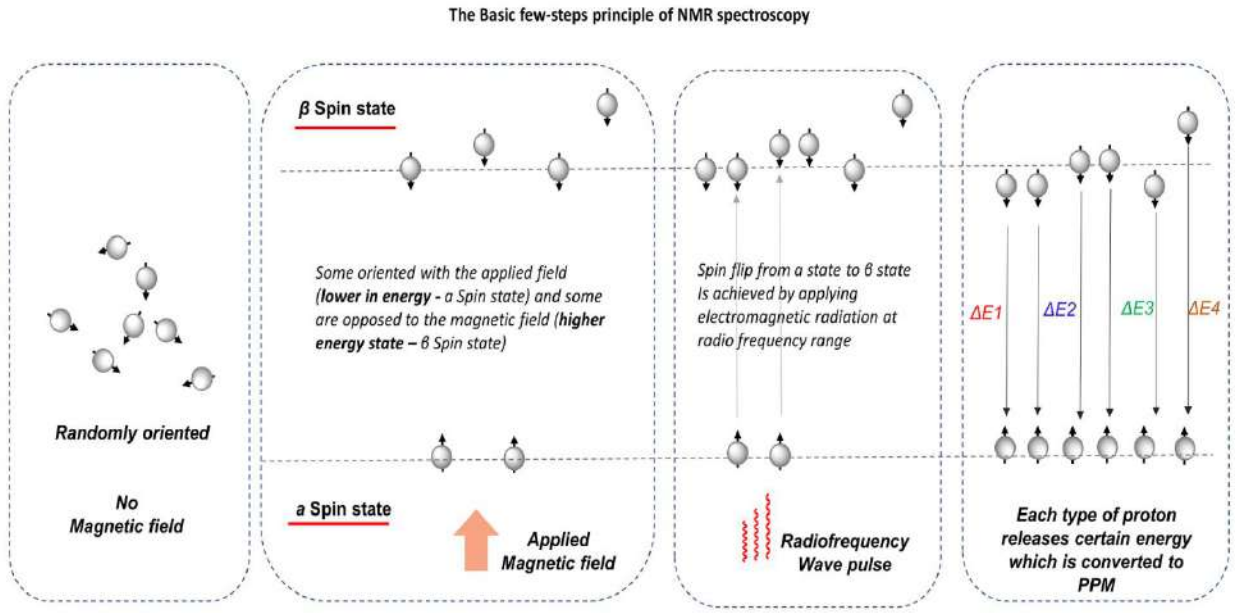
Nuclear Magnetic Resonance (NMR) Spectroscopy

- Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei.
- It is a spectroscopy technique which is based on the absorption of electromagnetic radiation in the radio frequency region 4 to 900 MHz by nuclei of the atoms.
- Over the past fifty years, NMR has become the most powerful technique for determining the structure of organic compounds.
- Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected.

Principle of Nuclear Magnetic Resonance (NMR) Spectroscopy

- The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).
- The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.
- The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.





Types of NMR

- **Continuous Wave Nuclear Magnetic Resonance (NMR) Spectroscopy(CW-NMR)**
- **Pulsed Fourier Transform Nuclear Magnetic Resonance (NMR) Spectroscopy (FT-NMR)**

Working of CW-NMR Spectroscopy

- The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers.
- The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.
- As the fields are unique or highly characteristic to individual compounds, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.
- Besides identification, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.
- The most common types of NMR are proton and carbon-13 NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.

Spectrum Scan in CW-NMR

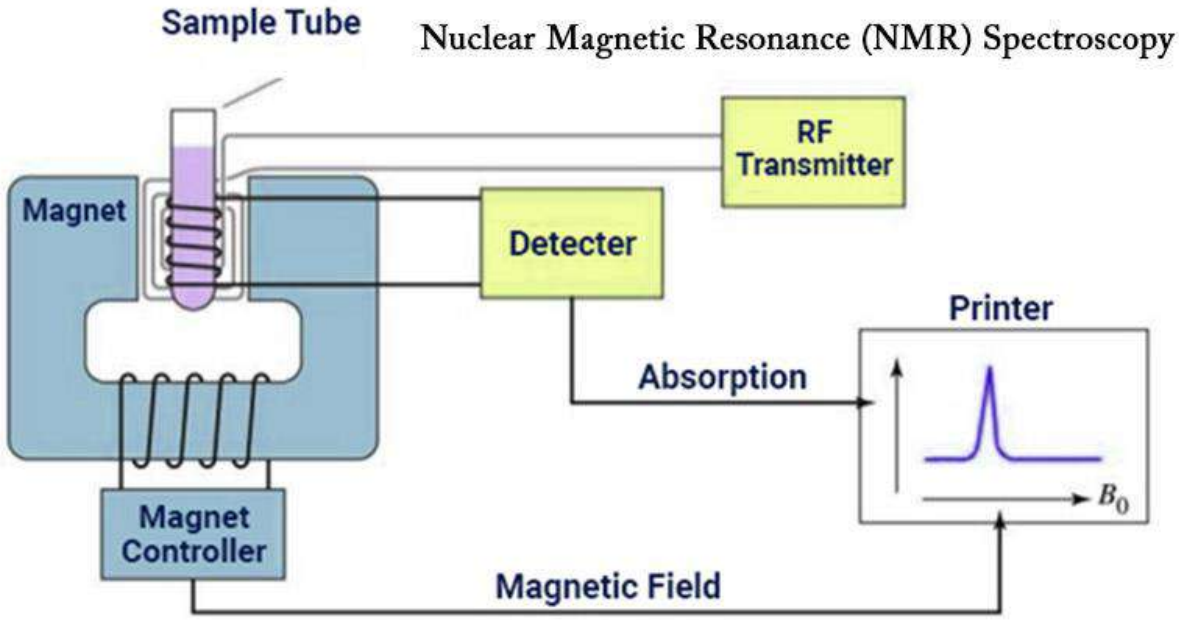
The spectrum can be scanned by the two methods.

- **Frequency Sweep method**

In the frequency sweep method the magnetic field is held constant, which keeps the nuclear spin energy level constant then the RF signal is swept(varied continuously) to determine the frequencies at which energy is absorbed.

- **Field sweep method**

In the field sweep method , the RF signal is held constant then the magnetic field is wept which varies the energy level to determine the magnetic field strength that produce resonance at a fixed resonance frequency.



Instrumentation of CW-Nuclear Magnetic Resonance (NMR) Spectroscopy

Sample holder

- Glass tube with 8.5 cm long, 0.3 cm in diameter.

Permanent magnet

- It provides homogeneous magnetic field at 60-100 MHZ

Magnetic coils

- These coils induce magnetic field when current flows through them

Sweep generator

- To produce the equal amount of magnetic field pass through the sample

Radio frequency transmitter

- A radio transmitter coil transmitter that produces a short powerful pulse of radio waves

Radio frequency receiver

- A radio receiver coil that detects radio frequencies emitted as nuclei relax to a lower energy level

Read out systems

- A computer that analyses and record the data.

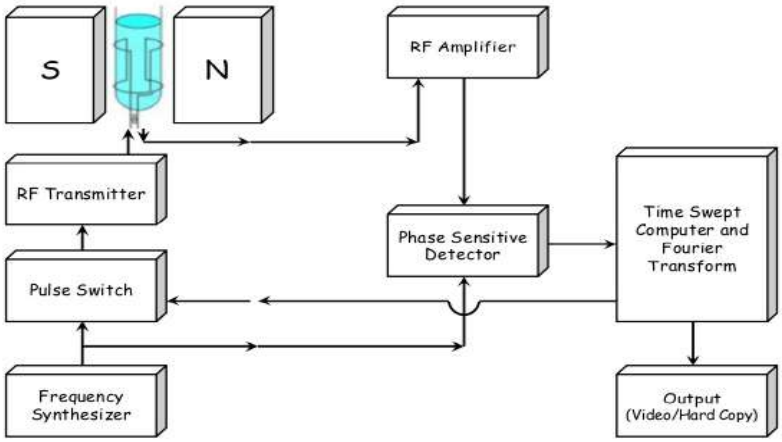
Pulsed – Fourier Transform NMR

- **Fourier Transform NMR (FT-NMR):** A method to collect an NMR spectrum in which a pulse of radio frequency energy is used to excite all nuclei of a particular in the molecule simultaneously. As the excited nuclei relax, they re-emit radio wave photons which produce an interference pattern called the **free induction decay** (**FID**). A mathematical process called a Fourier transform is used to convert the FID into the NMR spectrum.

ADVANTAGES OF FT-NMR

- FT-NMR is more sensitive and can measure weaker signals.
- The pulsed FT-NMR is much faster as compared to continuous wave NMR.
- FT-NMR can be obtained with less than 0.5 mg of compound. This is important in the biological chemistry, where only micro gram quantities of the material may be available.
- The FT method also gives improved spectra for sparingly soluble compounds.
- Pulsed FT-NMR is therefore especially suitable for examination of nuclei that are magnetic or very dilute sample.

FT-NMR INSTRUMENTATION



Applications of Nuclear Magnetic Resonance (NMR) Spectroscopy

- Spectroscopy is the study of the interaction of electromagnetic radiation with matter. NMR spectroscopy is the use of the NMR phenomenon to study physical, chemical and biological properties of matter.
- It is an analytical chemistry technique used in quality control.
- It is used in research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze mixtures containing known compounds.
- NMR spectroscopy is routinely used by chemists to study chemical structure using simple one-dimensional techniques. Two-dimensional techniques are used to determine the structure of more complicated molecules.
- These techniques are replacing x-ray crystallography for the determination of protein structure.
- Time domain NMR spectroscopy techniques are used to probe molecular dynamics in solution.
- Solid state NMR spectroscopy is used to determine the molecular structure of solids.
- Other scientists have developed NMR methods-of measuring diffusion coefficients.

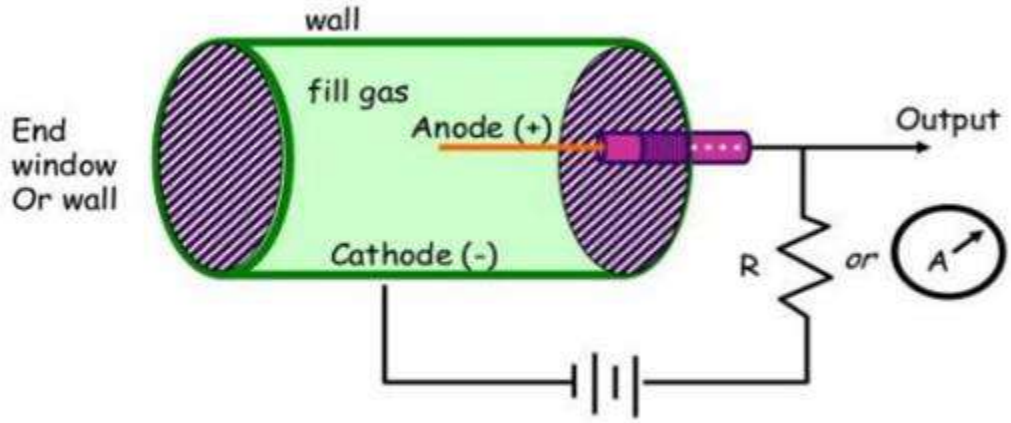
Nuclear Radiation Detector:

Nuclear Radiation Detectors are used to measure alpha and beta particles, neutrons, and gamma rays.

Types of **Nuclear Radiation Detector:**

- **Gas Filled Detectors**
    - **Ionization Chamber**
    - **Proportional Counter**
    - **Geiger Muller Counter**

- **Scintillation Detector**
- **Semiconductor Detector/ Solid State detectors**



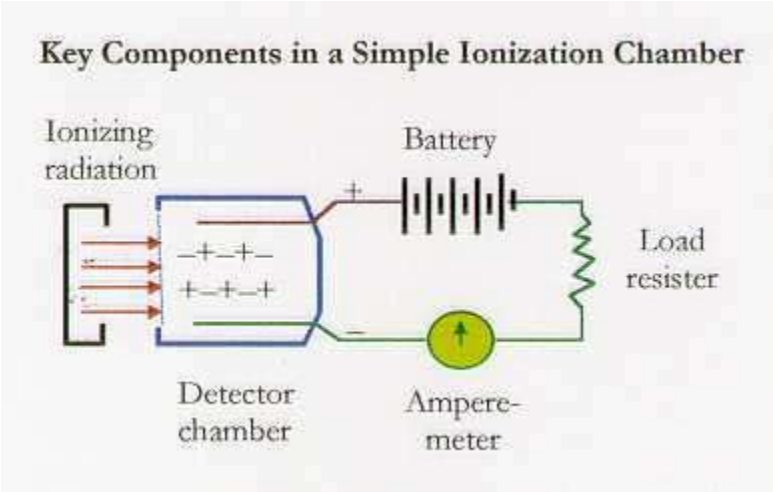
Gas Filled Detectors

Ionization Chamber:

The **ionization chamber** is the simplest of all gas-filled radiation detectors, and is widely used for the detection and measurement of certain types of ionizing radiation; X-rays, gamma rays, and beta particles. Conventionally, the term "ionization chamber" is used exclusively to describe those detectors which collect all the charges created by direct ionization within the gas through the application of an electric field. It only uses the discrete charges created by each interaction between the incident radiation and the gas, and does not involve the gas multiplication mechanisms used by other radiation instruments, such as the Geiger counter or the proportional counter.

Ion chambers have a good uniform response to radiation over a wide range of energies and are the preferred means of measuring high levels of gamma radiation. They are widely used in the nuclear power industry, research labs, radiography, radiobiology, and environmental monitoring.

Working: An ionization chamber measures the charge from the number of ion pairs created within a gas caused by incident radiation. It consists of a gas-filled chamber with two electrodes; known as anode and cathode. The electrodes may be in the form of parallel plates (Parallel Plate Ionization Chambers: PPIC), or a cylinder arrangement with a coaxially located internal anode wire.



A voltage potential is applied between the electrodes to create an electric field in the fill gas. When gas between the electrodes is ionized by incident ionizing radiation, ion-pairs are created and the resultant positive ions and dissociated electrons move to the electrodes of the opposite polarity under the influence of the electric field. This generates an ionization current which is measured by an electrometer circuit. The electrometer must be capable of measuring the very small output current which is in the region of femtoamperes to picoamperes, depending on the chamber design, radiation dose and applied voltage.

Each ion pair created deposits or removes a small electric charge to or from an electrode, such that the accumulated charge is proportional to the number of ion pairs created, and hence the radiation dose. This continual generation of charge produces an ionization current, which is a measure of the total ionizing dose entering the chamber.

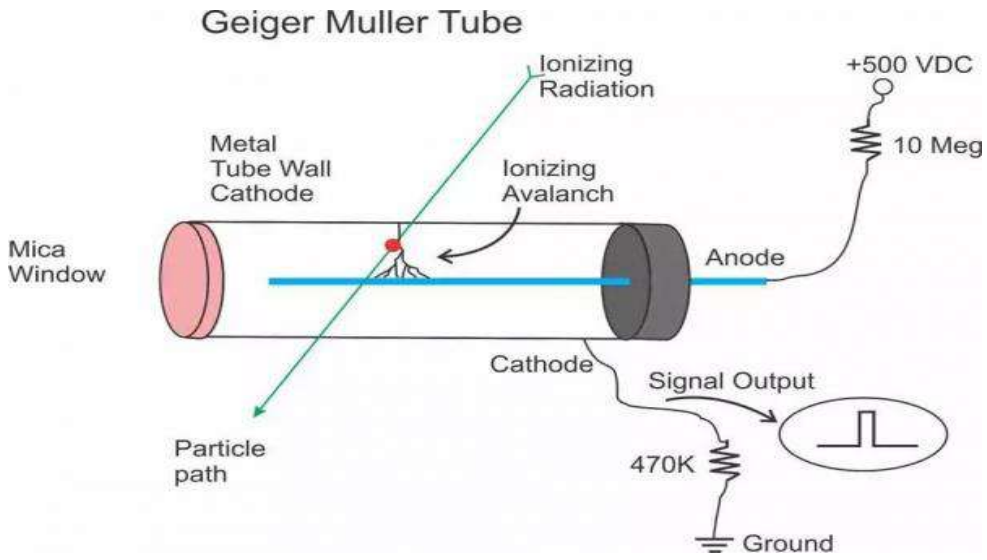
Geiger Muller Counter

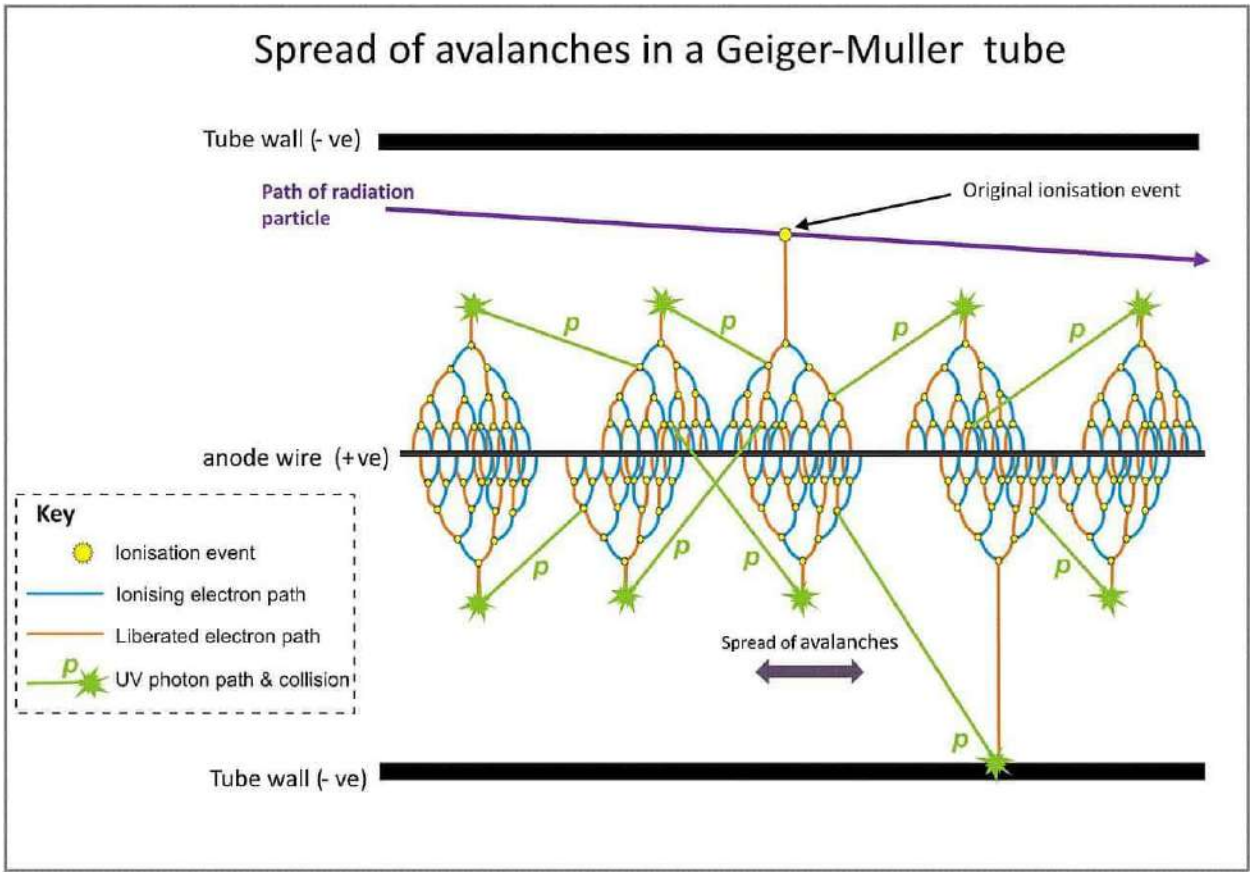
- Geiger Muller Counter is named after its developers: Geiger and Muller.
- A Geiger counter (Geiger-Muller tube) is a device used for the detection and measurement of all types of radiation: alpha, beta and gamma radiation. It is a metal cylinder filled with low-pressure gas sealed with a plastic or ceramic window at one end. Basically it consists of a pair of electrodes surrounded by a gas. The electrodes have a high voltage across them. The gas used is usually Helium or Argon.
- This counter works in Geiger region with two specialities.

- The gas multiplication factor is so large that an avalanche dies in at one point but spreads all over the entire length of the central wire.
- Large output pulses are obtained as the output pulse is independent both of the energy and nature of the particles detected.

The Principle of GM Counter

- The voltage of detector is adjusted so that the conditions correspond to the Geiger-Mueller region.
- In this region, the voltage is high enough to provide the primary electrons with sufficient acceleration and energy so that they can ionize additional atoms of the medium. These secondary ions (gas amplification) formed are also accelerated causing an effect known as **Townsend avalanches.** These avalanches can be triggered and propagated by photons emitted by atoms excited in the original avalanche. Since these photons are not affected by the electric field, they may interact far (e.g. laterally to the axis) from the primary avalanche, the entire Geiger tube is participating in the process.





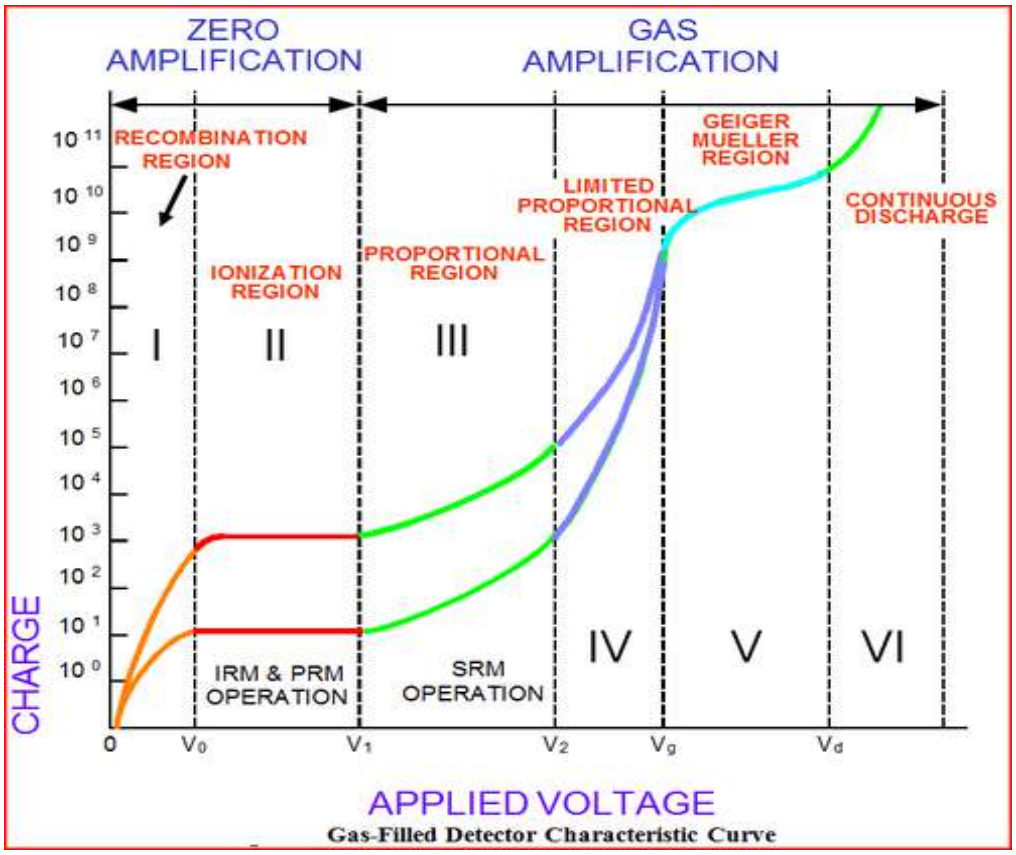
The Working of GM Counter

The Geiger counter has a cathode and an anode that are held at high voltage, and the device is characterized by a capacitance that is determined by the geometry of the electrodes. In a Geiger counter the fill gas of the chamber is an inert gas which is ionized by incident radiation, and a quench gas of 5–10% of an organic vapor or a halogen gas to prevent spurious pulsing by quenching the electron avalanches.

As ionizing radiation enters the gas between the electrodes, a finite number of ion-pairs are formed. In air, the average energy needed to produce an ion is about 34 eV, therefore a 1 MeV radiation completely absorbed in the detector produces about 3 x 10^4 pair of ions. The behavior of the resultant ion-pairs is affected by the potential gradient of the electric field within the gas and the type and pressure of the fill gas. Under the influence of the electric field, the positive ions will move toward the negatively charged electrode (outer cylinder), and the negative ions (electrons) will migrate toward the positive electrode (central wire). The electric field in this region keeps the ions from recombining with the electrons. In the immediate vicinity of the anode wire, the field strength becomes large enough to produce Townsend avalanches. These avalanches can be triggered and propagated by photons emitted by atoms excited

in the original avalanche. Since these photons are not affected by the electric field, they may interact far (e.g. laterally to the axis) from the primary avalanche, the entire Geiger tube is participating in the process. A strong signal (the amplification factor can reach about 10^{10}) is produced by these avalanches with shape and height independently of the primary ionization and the energy of the detected photon. The high amplification factor of the Geiger counter is the major advantage over the ionization chamber. Geiger counter is therefore a much more sensitive device than other chambers. It is often used in the detection of low-level gamma rays and beta particles for this reason.

- Since the positive ions do not move far from the avalanche region, a positively charged ion cloud disturbs the electric field and terminates the avalanche process. In practice the termination of the avalanche is improved by the use of "quenching" techniques.
- The collection of all these electrons will produce a charge on the electrodes and an electrical pulse across the detection circuit. Each pulse corresponds to one gamma ray or neutron interaction. The pulse height is not proportional to the number of original electrons produced. Therefore, Geiger counters are not capable of particle identification and energy measurement (spectroscopy). Since the process of charge amplification greatly improves the signal-to-noise ratio of the detector, the subsequent electronic amplification is usually not required.
- It is very useful for general measurement of nuclear radiation, but it has two important disadvantages.
- Since the pulse height is independent of the type and energy of radiation, discrimination is not possible. There is no information whatsoever on the nature of the ionization that caused the pulse.
- Because of the large avalanche induced by any ionization, a Geiger counter takes a long time (about 1 ms) to recover between successive pulses. Therefore, Geiger counters are not able to measure high radiation rates due to the "dead time" of the tube.



Gas-Filled Detector Characteristic Curve

Resolving Time or Dead Time and Quenching

After a count has been recorded, it takes the G-M tube a certain amount of time to reset itself to be ready to record the next count. The resolving time or ”dead time”, T, of a detector is the time it takes for the detector to ”reset” itself. Since the detector is ”not operating” while it is being reset, the measured activity is not the true activity of the sample. If the counting rate is high, then the effect of dead time is very important.

However, for each electron collected in the chamber, there is a positively charged gas ion left over. These gas ions are heavy compared to an electron and move much more slowly. Free electrons are much lighter than the positive ions, thus, they are drawn toward the positive central electrode much faster than the positive ions are drawn to the chamber wall. The resulting cloud of positive ions near the electrode leads to distortions in gas multiplication. Eventually the positive ions move away from the positively charged central wire to the negatively charged wall and are neutralized by gaining an electron. These atoms then return to their ground state by emitting photons which in turn produce further ionisation and thereby spurious secondary discharges. The electrons produced by this ionization move toward the central wire and are multiplied en route. This pulse of charge is unrelated to the radiation to be detected and can set off

a series of pulses. In practice the termination of the avalanche is improved by the use of "quenching" techniques.

The quenching gas molecules have a weaker affinity for electrons than the chamber gas does; therefore, the ionized atoms of the chamber gas readily take electrons from the quenching gas molecules. Thus, the ionized molecules of quenching gas reach the chamber wall instead of the chamber gas. The ionized molecules of the quenching gas are neutralized by gaining an electron, and the energy liberated does not cause further ionization, but causes dissociation of the molecule. This type of quenching is known as self-quenching or internal-quenching, since tubes stop the discharge without external assistance.

For Geiger counters, external quenching, sometimes called "active quenching" or "electronic quenching", is also a possibility. Electronic quenching uses simplistic high speed control electronics to rapidly remove and re-apply the high voltage between the electrodes for a fixed time after each discharge peak in order to increase the maximum count rate and lifetime of the tube.

Advantages of GM Counter

- It can count alpha, beta, gamma particles as well as cosmic rays.
- It has high sensitivity.
- Power supply need not be precisely regulated as the pulse height is constant over a large range.
- Because of the fact that output pulse is very high, so the Amplification needed is also very subtle.
- They are relatively inexpensive
- They are durable and easily portable

Disadvantages of GM Counter

- They cannot differentiate which type of radiation is being detected.
- They cannot be used to determine the exact energy of the detected radiation
- They have a very low efficiency.
- It cannot detect uncharged particles like Neutrons.

- It is less efficient due to the large paralysis time limits and large dead time.
- Quenching agent used in this counter often decomposes, leading to less lifetime of the GM Counter.

Proportional Counter

The proportional counter, also known as the proportional detector, is electrical device that detects various types of ionizing radiation. The voltage of detector is adjusted so that the conditions correspond to the proportional region.

A proportional counter, also known as the proportional detector, is an electrical device that detects various types of ionizing radiation. The voltage of detector is adjusted so that the conditions correspond to the proportional region. In this region, the voltage is high enough to provide the primary electrons with sufficient acceleration and energy so that they can ionize additional atoms of the medium. These secondary ions (gas amplification) formed are also accelerated causing an effect known as Townsend avalanches, which creates a single large electrical pulse. Gaseous proportional counters usually operate in high electric fields of the order of 10 kV/cm and achieve typical amplification factors of about 10^5. Since the amplification factor is strongly dependent on the applied voltage, the charge collected (output signal) is also dependent on the applied voltage and proportional counters require constant voltage.

This is a subtle, but important difference between ionization chambers and proportional counters. An ionization chamber will produce a current that is proportional to the number of electrons collected each second. This current is averaged and is used to drive a display reading in Bq, or μSv/h. Proportional counters do not work in this way. Instead, they amplify each of the individual bursts of ionisation so that each ionising event is detected separately. They therefore measure the number of ionising events (which is why they are called counters).

The process of charge amplification greatly improves the signal-to-noise ratio of the detector and reduces the subsequent electronic amplification required. When instruments are operated in the proportional region, the voltage must be kept constant. If a voltage remains constant the gas amplification factor also does not change. Proportional counter detection instruments are very sensitive to low levels of radiation. By proper functional arrangements, modifications, and biasing, the proportional counter can be used to detect alpha, beta, gamma, or neutron radiation in mixed radiation fields. Moreover, proportional counters are capable of particle identification and energy measurement (spectroscopy). The pulse height reflects the

energy deposited by the incident radiation in the detector gas. As such, it is possible to distinguish the larger pulses produced by alpha particles from the smaller pulses produced by beta particles or gamma rays.

While ionization chambers can be operated in current or pulse mode, proportional counters or Geiger counters are almost always used in pulse mode. Detectors of ionizing radiation can be used both for activity measurements as well as for dose measurement. With knowledge about the energy needed to form an pair of ions – the dose can be obtained.

Argon and helium are the most frequently used fill gases and allow for the detection of alpha, beta, and gamma radiation. For neutron detection He-3 and BF_3 (Boron Trifluoride) are the most commonly employed gases. For special purposes other mixtures of gases have been used, such as a tissue equivalent gas mixture consisting of 64.4% methane, 32.4% carbon dioxide and 3.2% nitrogen.

Basic Principle of Proportional Counters

The proportional counter has a cathode and an anode that are held at some voltage (above 1000 V), and the device is characterized by a capacitance that is determined by the geometry of the electrodes. In a proportional counter the fill gas of the chamber is an inert gas which is ionized by incident radiation, and a quench gas to ensure each pulse discharge terminates; a common mixture is 90% argon, 10% methane, known as P-10.

As ionizing radiation enters the gas between the electrodes, a finite number of ion-pairs are formed. In air, the average energy needed to produce an ion is about 34 eV, therefore a 1 MeV radiation completely absorbed in the detector produces about 3×10^4 pair of ions. The behavior of the resultant ion-pairs is affected by the potential gradient of the electric field within the gas and the type and pressure of the fill gas. Under the influence of the electric field, the positive ions will move toward the negatively charged electrode (outer cylinder), and the negative ions (electrons) will migrate toward the positive electrode (central wire). The electric field in this region keeps the ions from recombining with the electrons. In the immediate vicinity of the anode wire, the field strength becomes large enough to produce Townsend avalanches. This avalanche region occurs only fractions of a millimeter from the anode wire, which itself is of a very small diameter. The purpose of this is to use the multiplication effect of the avalanche produced by each ion pair. This is the "avalanche" region. A key design goal is that each original ionizing event due to incident radiation produces only one avalanche. Gas amplification factors can range from unity in the ionization region

to 10^3 or 10^4 in the proportional region. The high amplification factor of the proportional counter is the major advantage over the ionization chamber.

The collection of all these electrons will produce a charge on the electrodes and an electrical pulse across the detection circuit. Each pulse corresponds to one gamma ray or particle interaction. The pulse height is proportional to the number of original electrons produced. But in this case the pulse height is significantly amplified by the detector. The proportionality factor in this case is the gas amplification factor. The number of electrons produced is proportional to the energy of the incident particle. Therefore, proportional counters are capable of particle identification and energy measurement (spectroscopy). Different energies of radiation and different types of radiation can be distinguished by analyzing the pulse height, since they significantly differ in the primary ionization (low-LET vs high-LET). Since the process of charge amplification greatly improves the signal-to-noise ratio of the detector, the subsequent electronic amplification is usually not required.

Ionization chamber construction differs from the proportional counter. The flat plate design is preferred for ionization chambers, or concentric cylinders may be utilized in the construction to allow for the integration of pulses produced by the incident radiation. Proportional counters and Geiger counters usually utilize cylinder and central electrode. The proportional counter would require such exact control of the electric field between the electrodes that it would not be practical.

Quenching – Proportional Counters

In a proportional counter the fill gas of the chamber is an inert gas which is ionized by incident radiation, and a quench gas to ensure each pulse discharge terminates; a common mixture is 90% argon, 10% methane, known as P-10.

For each electron collected in the chamber, there is a positively charged gas ion left over. These gas ions are heavy compared to an electron and move much more slowly. Free electrons are much lighter than the positive ions, thus, they are drawn toward the positive central electrode much faster than the positive ions are drawn to the chamber wall. The resulting cloud of positive ions near the electrode leads to distortions in gas multiplication. Eventually the positive ions move away from the positively charged central wire to the negatively charged wall and are neutralized by gaining an electron. In the process, some energy is given off, which causes additional ionization of the gas atoms. The electrons produced by this ionization move toward the central wire and are multiplied en route. This pulse of charge is unrelated to the radiation to be detected and

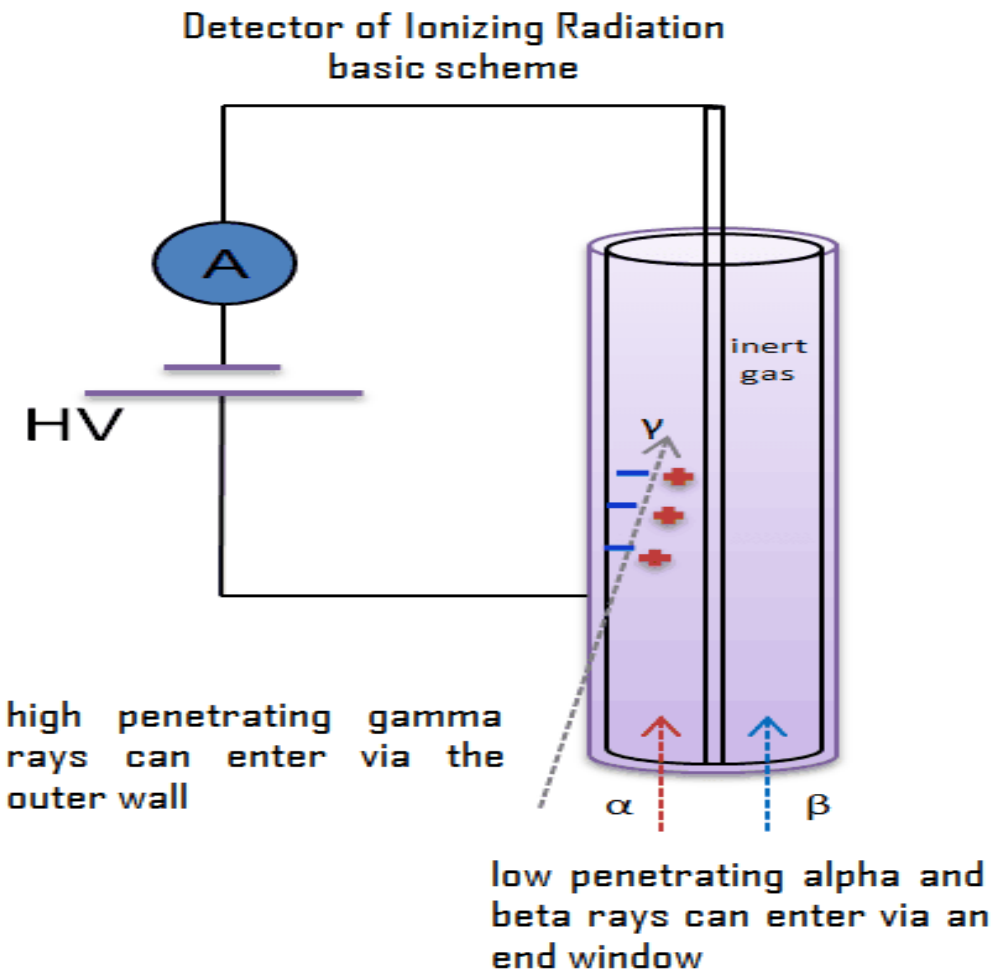
can set off a series of pulses. In practice the termination of the avalanche is improved by the use of "**quenching**" techniques.

The quenching gas molecules have a weaker affinity for electrons than the chamber gas does; therefore, the ionized atoms of the chamber gas readily take electrons from the quenching gas molecules. Thus, the ionized molecules of quenching gas reach the chamber wall instead of the chamber gas. The ionized molecules of the quenching gas are neutralized by gaining an electron, and the energy liberated does not cause further ionization, but causes dissociation of the molecule.

Advantages of Proportional Counters

Amplification: Gaseous proportional counters usually operate in high electric fields of the order of 10 kV/cm and achieve typical amplification factors of about 10^5. Since the amplification factor is strongly dependent on the applied voltage, the charge collected (output signal) is also dependent on the applied voltage and proportional counters require constant voltage. The high amplification factor of the proportional counter is the major advantage over the ionization chamber.

Sensitivity: The process of charge amplification greatly improves the signal-to-noise ratio of the detector and reduces the subsequent electronic amplification required. Since the process of charge amplification greatly improves the signal-to-noise ratio of the detector, the subsequent electronic amplification is usually not required. Proportional counter detection instruments are very sensitive to low levels of radiation. Moreover, when measuring current output, a proportional detector is useful for dose rates since the output signal is proportional to the energy deposited by ionization and therefore proportional to the dose rate.



Spectroscopy: By proper functional arrangements, modifications, and biasing, the proportional counter can be used to detect alpha, beta, gamma, or neutron radiation in mixed radiation fields. Moreover, proportional counters are capable of particle identification and energy measurement (spectroscopy). The pulse height reflects the energy deposited by the incident radiation in the detector gas. As such, it is possible to distinguish the larger pulses produced by alpha particles from the smaller pulses produced by beta particles or gamma rays.

Disadvantages of Proportional Counters

Constant Voltage: When instruments are operated in the proportional region, the **voltage must be kept constant**. If a voltage remains constant the gas amplification factor also does not change. The main drawback to using proportional counters in portable instruments is that they require a very stable power supply and amplifier to ensure constant operating conditions. This is difficult to provide in a portable

instrument, and that is why proportional counters tend to be used more in fixed or lab instruments.

Quenching: In practice the termination of the avalanche is improved by the use of "**quenching**" techniques.

Scintillation Counter

A scintillation counter or scintillation detector is a radiation detector which uses the effect known as scintillation. Scintillation is a flash of light produced in a transparent material by the passage of a particle (an electron, an alpha particle, an ion, or a high-energy photon). Scintillation occurs in the scintillator, which is a key part of a scintillation detector. In general, a scintillation detector consists of:

- Scintillator: A scintillator generates photons in response to incident radiation.
- Photo detector: A sensitive photo detector (usually a photomultiplier tube (PMT), a charge-coupled device (CCD) camera, or a photodiode), which converts the light to an electrical signal and electronics to process this signal.

The basic principle of operation involves the radiation reacting with a scintillator, which produces a series of flashes of varying intensity. The intensity of the flashes is proportional to the energy of the radiation. This feature is very important. These counters are suited to measure the energy of gamma radiation and, therefore, can be used to identify gamma emitting isotopes.

Scintillation counters are widely used in radiation protection, assay of radioactive materials and physics research because they can be made inexpensively yet with good efficiency, and can measure both the intensity and the energy of incident radiation. Hospitals all over the world have gamma cameras based on the scintillation effect and, therefore, they are also called scintillation cameras.

The advantages of a scintillation counter are its efficiency and the high precision and counting rates that are possible. These latter attributes are a consequence of the extremely short duration of the light flashes, from about 10^{-9} (organic scintillators) to 10^{-6} (inorganic scintillators) seconds. The intensity of the flashes and the amplitude of the output voltage pulse are proportional to the energy of the radiation. Therefore, scintillation counters can be used to determine the energy, as well as the number, of the exciting particles (or gamma photons). For gamma spectrometry, the most common detectors include sodium iodide (NaI) scintillation counters and high-purity germanium detectors.

Scintillation Counter – Principle of Operation.

Source: Ionizing radiation enters the scintillator and interacts with the scintillator material. This cause the electron to be raised to an excited state. For gamma rays, their energy is converted to an energetic electron via either the photoelectric effect, Compton scattering or pair production.

The excited atoms of the scintillator material de-excite and rapidly emit a photon in the visible (or near-visible) light range. The quantity is proportional to the energy deposited by the ionizing particle. The material is said to fluoresce.

Three classes of phosphors are used:

- inorganic crystals,
- organic crystals,
- plastic phosphors.

The light created in the scintillator strikes the photocathode of a photomultiplier tube, releasing at most one photoelectron per photon. Using a voltage potential, this group of primary electrons is electrostatically accelerated and focused so that they strike the first dynode with enough energy to release additional electrons.These secondary electrons are attracted and strike a second dynode releasing more electrons. This process occurs in the photomultiplier tube. Each subsequent dynode impact releases further electrons, and so there is a current amplifying effect at each dynode stage. Each stage is at a higher potential than the previous to provide the accelerating field. Primary signal is multiplied and this amplification continues through 10 to 12 stages.

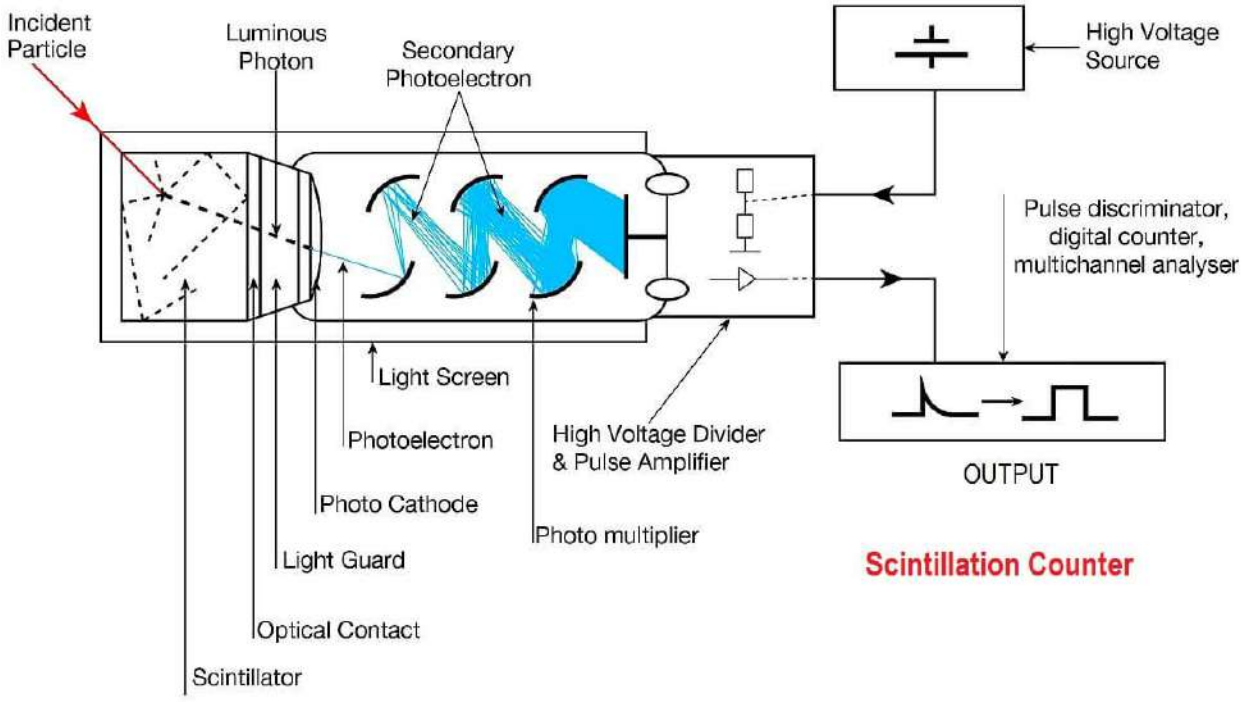
At the final dynode, sufficient electrons are available to produce a pulse of sufficient magnitude for further amplification. This pulse carries information about the energy of the original incident radiation. The number of such pulses per unit time also gives information about the intensity of the radiation. A scintillation detector or scintillation counter is obtained when a scintillator is coupled to an electronic light sensor such as:

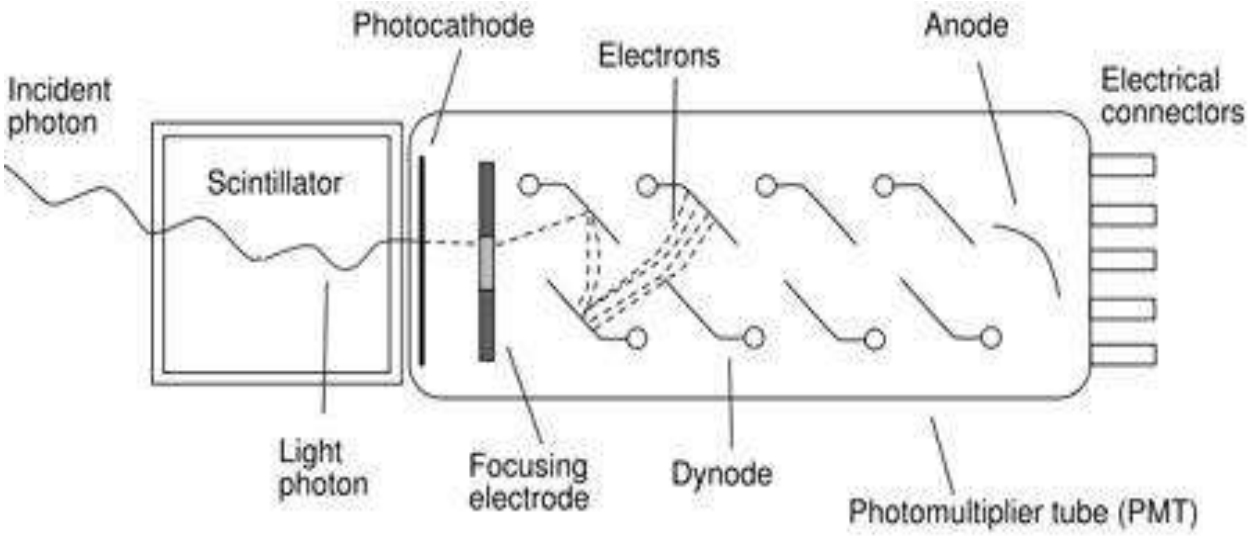
- a photomultiplier tube (PMT),
- a charge-coupled device (CCD) camera,
- photodiode

All these devices may be used in scintillation counters and all convert the light to an electrical signal and contain electronics to process this signal. A photomultiplier tube (PMT) absorbs the light emitted by the scintillator and re-emit it in the form of electrons via the photoelectric effect. The PMT has been the main choice for photon detection

ever since due to the fact that they have high quantum efficiency and high amplification. Lately however semiconductors have begun to compete with the PMT, the photodiode, for example, which has higher quantum efficiency in the visible range and above, lower power consumption and smaller size.

A number of portable gamma cameras for medical imaging use scintillator-CCD based detectors. In this case, a scintillator converts the incident radiation (X-rays usually) into visible wavelength photons, which can then be directly detected by the CCD camera.





Semiconductor Detector

A semiconductor detector is a radiation detector which is based on a semiconductor, such as silicon or germanium to measure the effect of incident charged particles or photons. In general, semiconductors are materials, inorganic or organic, which have the ability to control their conduction depending on chemical structure, temperature, illumination, and presence of dopants. The name semiconductor comes from the fact that these materials have an electrical conductivity between that of a metal, like copper, gold, etc. and an insulator, such as glass. They have an energy gap less than 4eV (about 1eV). In solid-state physics, this energy gap or band gap is an energy range between valence band and conduction band where electron states are forbidden. In contrast to conductors, electrons in a semiconductor must obtain energy (e.g. from ionizing radiation) to cross the band gap and to reach the conduction band.

Semiconductor detectors are very similar in operation as photovoltaic panels that generate electric current. In a similar way a current can be induced by ionizing radiation. As ionizing radiation enters the semiconductor, it interacts with the semiconductor material. It may excite an electron out of its energy level and consequently leave a hole. This process is known as electron–hole pair generation. In semiconductor detectors, the fundamental information carriers are these electron-hole pairs, which are produced along the path taken by the charged particle (primary or secondary) through the detector. By collecting electron-hole pairs, the detection signal is formed and recorded.

Principle of Operation of Semiconductor Detectors

Ionizing radiation enters the sensitive volume of the detector and interacts with the semiconductor material.

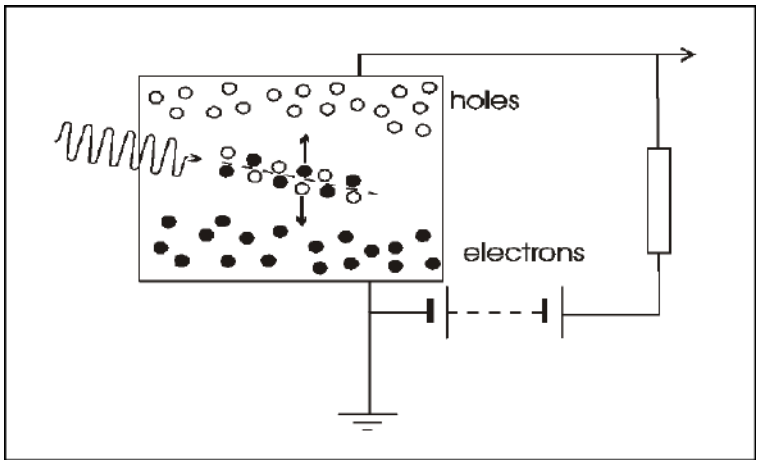
Particle passing through the detector ionizes the atoms of semiconductor, producing the electron-hole pairs. The number of electron-hole pairs is proportional to the energy of the radiation to the semiconductor. As a result, a number of electrons are transferred from the valence band to the conduction band, and an equal number of holes are created in the valence band.

Under the influence of an electric field, electrons and holes travel to the electrodes, where they result in a pulse that can be measured in an outer circuit,

This pulse carries information about the energy of the original incident radiation. The number of such pulses per unit time also gives information about the intensity of the radiation.

The energy required to produce electron-hole-pairs is very low compared to the energy required to produce paired ions in a gaseous ionization detector. In semiconductor detectors the statistical variation of the pulse height is smaller and the energy resolution is higher. As the electrons travel fast, the time resolution is also very good. Compared with gaseous ionization detectors, the density of a semiconductor detector is very high, and charged particles of high energy can give off their energy in a semiconductor of relatively small dimensions.

Silicon-based semiconductor detectors are mainly used for charged particle detectors (especially for tracking charged particles) and soft X-ray detectors while germanium is widely used for gamma ray spectroscopy. A large, clean and almost perfect semiconductor is ideal as a counter for radioactivity. However, it is difficult to make large crystals with sufficient purity. The semiconductor detectors have, therefore, low efficiency, but they do give a very precise measure of the energy. Detectors based on silicon have sufficiently low noise even by room temperature. This is caused by the large band gap of silicon (Egap= 1.12 eV), which allows us to operate the detector at room temperature, but cooling is preferred to reduce noise. The drawback is that silicon detectors are much more expensive than cloud chambers or wire chambers and require sophisticated cooling to reduce leakage currents (noise). They also suffer degradation over time from radiation; however this can be greatly reduced thanks to the Lazarus effect.



Application of Silicon Detectors

Since silicon-based detectors are very good for tracking charged particles, they constitute a substantial part of detection system at the LHC in CERN. Most silicon particle detectors work, in principle, by doping narrow (usually around 100 micrometers wide) strips of silicon to turn them into diodes, which are then reverse biased. As charged particles pass through these strips, they cause small ionization currents that can be detected and measured. Arranging thousands of these detectors around a collision point in a particle accelerator can yield an accurate picture of what paths particles take. For example, the Inner Tracking System (ITS) of a Large Ion Collider Experiment (ALICE) contains three layers of silicon-based detectors:

- Silicon Pixel Detector (SPD)
- Silicon Drift Detector (SDD)
- Silicon Strip Detector (SSD)

Reference:

[1]. https://www.creative-proteomics.com/

[2]. https://www.radiation-dosimetry.org/

[3]. https://chem.libretexts.org/

[4]. https://instrumentationtools.com/

[5]. http://www.premierbiosoft.com/

[6]. https://microbenotes.com/

[7]. https://www.shimadzu.com/

Post – Test:

1. Which among the following works with Evanescent Wave?
   a. **Attenuated Total Reflectance**
   b. UV Spectrometer
   c. IR Spectrometer
   d. Atomic Absorption Spectrometer
2. The Spectroscopy works with Interferogram and Total internal reflection are
   a. **FTIR and ATR**
   b. ATR and AAS
   c. FTIR and FES
   d. IR and UV
3. Which is the only detector used in Fourier Transform Infrared Spectrophotometer (FTIR) and why.
   a) **Pyroelectric transducer, Fast response**
   b) Photo detector, slower response
   c) PMT, High precision
   d) Golay cell, good sensitivity
4. In which of the following methods are liquid samples injected into the column in gas chromatography?
   a) Gas tight syringe
   b) **Micro-syringe**
   c) Rotary sample valve
   d) Solid injection syringes
5. Which of the following is not an ideal characteristic of a detector used in gas chromatography?
   a) Linear response to the solutes
   b) Short response time
   c) High reliability
   d) **Sensitive to the changes in the flow rate of a carrier gas**
6. Which of the following will improve the efficiency of the separation process in liquid chromatography?
   a) Increase in sample size, increase in column diameter
   b) Reduction in sample size, increase in column diameter
   c) Increase in sample size, reduction in column diameter
   d) **Reduction in sample size, reduction in column diameter**

7. In Laser opto-acoustic spectroscopy, the IR beam excites the molecules to higher states. In which of the following ways do the molecules return to the ground state?
   a) **Collision de-excitation**
   b) Random de-excitation
   c) By spontaneous emission
   d) By stimulated emission
8. Which of the following detectors are generally used for detection in NO analysis using CO laser?
   a) Photomultiplier tube
   b) Photovoltaic cell
   c) **Liquid nitrogen cooled Ge-Au element**
   d) Photo emissive tube
9. Which of the following occurs in Electrochemical cells used for the detection of hydrogen sulphide?
   a) Change in resistance
   b) Redox reaction
   c) **Oxidation-reduction reaction**
   d) Change in color
10. Calomel electrode can behave as which of the following components?
    a) Anode only
    b) Cathode only
    c) **Anode or cathode**
    d) Salt bridge
11. Which of the following is not the characteristic of silver/silver chloride electrode?
    a) These electrodes have good electrical and chemical stability
    b) It can be used in temperatures greater than $600^{o}C$
    c) **It can be used in places or solutions that have strong reducing agents**
    d) It should not be used in solutions that contain proteins, sulphide or bromide
12. Which of the following reference electrodes are used as internal and external reference electrodes in combination electrodes?
    a) **Silver/silver chloride electrode**
    b) Calomel electrode
    c) Mercury/mercury sulphate electrode
    d) Mercury/mercury chloride electrode

13. Which of the following is an ion optic device in which ions pass through a mirror and their flight is reversed?
    a) Reversal device
    b) **Reflectron**
    c) Mirror arrangement
    d) Separation chamber
14. Which of the following has to be done to increase the resolution of the quadrupole mass spectrometer?
    a) Increasing distance between detector and reflectron
    b) Increasing difference between the individual rf accelerating stages
    c) Increasing the length of the drift tube
    d) **Increasing the rod length of the electrode**
15. NMR is the study of the absorption of _____ by nuclei in a magnetic field.
    a) Radioactive radiation
    b) IR radiation
    c) **Radio frequency radiation**
    d) Microwaves