UNIT IV

ELECTROPHORESIS

Separation of charged molecules in a solution based on their tendency to move towards electrodes, by applying an electric field is called electrophoresis. This technique was first developed by Tiselvis and Longworth in 1937.

Electrophoresis is a combination of two words Electro means Electricity, Phoresies means Separation. Biomolecules such as nucleotides, amino acids, RNA, DNA and proteins are charged molecules. So, they can be separated by means of electrophoresis. If the molecules are not charged, as in sugars, they cannot be separated by electrophoresis.

Principle: -If a direct current is passed through a solution containing charged molecules, the positively charged cations (+) will migrate towards cathode (-) and the negatively charged anions (-) will move towards the anode (+). The mobility of the molecules, however, depends on the net charge, size and shape of the molecule, pH and composition of the suspending medium and applied current between the electrodes. DNA and RNA are negatively charged molecules so that they migrate towards the anode. The small molecules move faster through the supporting medium than the large molecules. This principle is used in the separation of RNAs and DNAs. The proteins also migrate through the supporting medium based on their net electric charge at specific pH and size. If two proteins have identical charge and similar shape, the electrophoretic separation would be difficult.

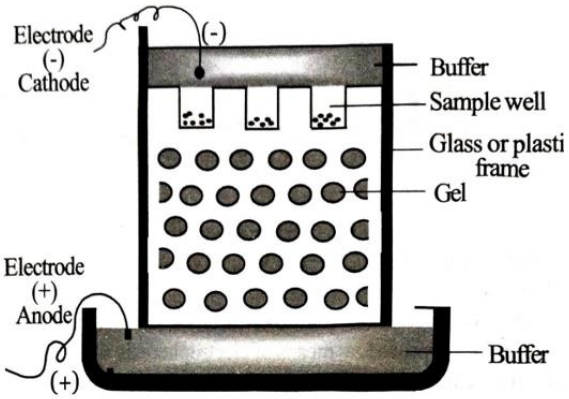


Fig.14.1: An electrophoresis set-up.

A supporting medium or matrix is required to separate molecules according to their size and charge. It should be an inert, non-toxic material with specific porosity. It should have good filtering effect to separate molecules based on their size. It should not interfere with electrical conductivity of buffer. Paper strips, cellulose acetate, starch, agarose, polyacrylamide, sephadox, etc. are used as supporting medium in electrophoresis

The pH change affects the movement of molecules through the pores of the supporting medium. In order to keep the medium at a constant pH, buffers are used in electrophoresis. Barbiton buffer, phosphate buffer, Tris-borate EDTA (TBE) buffer, Tris-acetate EDTA (TAE) buffe. Tris-glycine buffer, etc. are commonly employed in electrophoresis.An electrophoresis set-up consists of two buffer tanks, two platinum electrodes, supporting material (gel), buffer and sample A stable D.C. of 5-8 V/cm is suitable for the separation of charged molecules by using electrophoresis.

The rate of electrophoretic migration depends on the following factors:

1. pH of the medium.
2. Strength of electric field.
3. Size of the molecules.
4. Magnitude of the net charge on the molecule.
5. Temperature.
6. Concentration of sample.

Based on the supporting material used to separate biomolecules, electrophoresis is divided into the following types:

1. Paper electrophoresis
2. Agarose gel electrophoresis
3. Polyacrylamide Gel Electrophoresis (PAGE)
4. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)
5. Pulsed Field Gel Electrophoresis (PFGE)

Electrophoresis Media: -**The solid materials and the buffer solutions which favour the separation of solute particle is called electrophoresis media**. Electrophoresis has two types of media. i. Buffers ii. Supporting media

i. Buffers: -Buffer is a solution of a weak acid and one of its salts. It maintains a constant pH. It resists changes in H* and OH ion concentrations. If the pH of the medium is changed, it affects the migration of ions. The pH at which there is no migration of ions is known as isoelectric point. Barbitone Buffer, Phosphate Buffer, Trisborate EDTA Buffer (TBE), Tris-acetate EDTA Buffer (TAE) and Tris-Glycine buffer are some of the commonly used buffers.

ii. Supporting media:- Supporting medium is a matrix which charge. An ideal supporting medium has the following features:

An ideal supporting medium has the following features:

Chemical nature	- inert
Transparency	- high
Adsorptivity	- low
Sieving effect	- desirable
Preparation	- easy
Toxicity	- low
Electrical conductivity	- high
Electroendosmosis (EEO)	- low
Porosity	- controlled
Availability	- easy

Some of the commonly used supporting media: -

1) **Paper contains about 95% cellulose**. Cellulose is a poor conductor of electricity. It is not transparent. It absorbs proteins. So, it gives poor results.Various grades of Whatmann paper are available. Now-a-days, it has been used in the clinical investigations of serum and other body fluids. It is a good choice for demonstrations to students.
2) **Cellulose-acetate: -**The acetate group is non-absorbing. So, it gives better results. Moreover it is transparent and compounds can be separated easily and quickly. It is widely used in clinical medicine.
3) **Starch:** -Starch forms opaque gels on boiling and cooling. It is non-adsorbing and free of impurities. It gives better results in the separation of some isoenzymes. The resolving power of starch gel is remarkably good, but its pore size cannot be controlled. It is often contaminated by microbes. So it becomes opaque on staining. This is not suitable for the separation of proteins.
4) **Agarose**: -Agarose is a purified form of agar. It is a polysaccharide. It is highly transparent. It has a desirable sieving capacity and low electroendosmosis (EEO). It forms a gel with pores ranging from 100 to 300nm in diameter. It is widely used in various kinds of immunoelectrophoretic and nucleic acid separation.

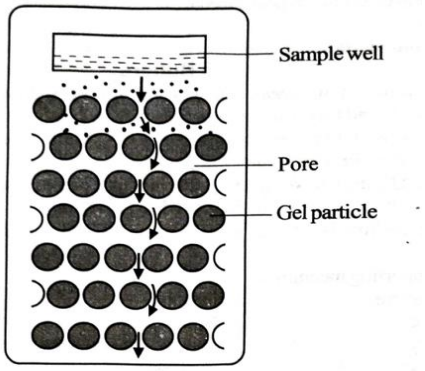


Fig.14.2: Gel used for electrophoresis.

5.Polyacrylamide Gel: -Polyacrylamide is a polymer composed of N,N-methylene, bisacrylamide, ammonium persulphate and tetramethylene diamine. It has low adsorption capacity and no osmotic

property. It forms a matrix when it is mixed with water. The pore size of the gel can be controlled suitably for the separation of nucleic acids and proteins. It is inert. It is highly transparent, so that it can be scanned with the UV light.

Applications

- Electrophoresis is used to separate proteins from a mixture. It is used to separate DNA from a mixture.
- Electrophoresis is used to separate RNA from a mixture. . It is used to separate immunoglobulins.
- Electrophoresis is used to separate plasma proteins.
- Electrophoresis is used to separate lipoproteins.
- Electrophoresis is used to determine molecular weight of proteins and DNA.
- Electrophoresis is used to determine the size of DNA fragments. It is used to resolve thousands of proteins and DNA in the biological sample at a time

1. Paper Electrophoresis: - It is type of Electrophoresis in which paper strip or cellulose acetate membrane is used as separating medium. Paper electrophoresis (PE) is useful for the separation of small-charged molecules, such as amino acids and small proteins using a strip of paper **This method was first developed by Tiselvis and Longsworth in 1937.**

Principle: -

If a direct current is passed through a wet paper containing charged molecules, the positively charged cations (+) will migrate towards cathode (-) and the negatively charged anions (-) will on the net charge, size and move towards the anode (+). Mobility of the molecules depends shape of the molecule, pH and composition of the suspending medium and the applied current between the electrodes. It does not require matrix preparation and it does not contain charges that interfere with the separation of compounds. Spots migrate according to their charges. After electrophoresis, separated components can be detected by variety of staining techniques, depending upon their chemical composition.

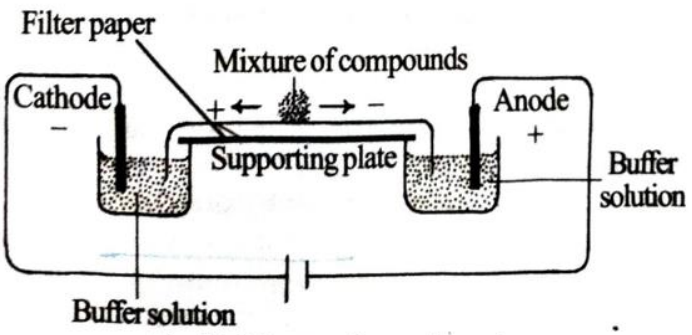


Fig.14.3:Paper electrophoresis.

Procedure: -

1. Fill both buffer tanks, of a horizontal electrophoresis apparatus, with tris-acetate buffe solution (0.07 mole/1 pH, 7.6). Check that the level should be the same in both tanks by arranging a siphon between them.
2. Remove the siphon and place the supporting plate (10×2.5cm) with its edge touching the buffer solution.
3. Streak the paper with amino acid mixture (aspartic acid, histidine and lysine) in tris. acetate buffer containing 10gm/1 glucose. Simultaneously, streak 3 paper strips with only one amino acid in each and run.
4. Place the filter papers on the supporting plate and dip their ends in the buffer solutions. 5. Wet the papers upto a few centimetres from the tips and leave the rest to be wetted by capillary attraction.
6. Switch on the current from the power pack (8v/cm).
7. Carry out electrophoresis for 3 hrs.
8. Remove the strips and dry them in an oven at 110°C.
9. Dip the strips in a solution containing aniline diphenylamide and then dip them in freshly prepared ninhydrin (0.2g in 100ml of acetone).
10. Allow acetone to evaporate by heating the strips in the oven for a few minutes.
11. Identify the amino acids after drying by comparing the positions of respective amino acids in the strips.

Applications

1. Paper electrophoresis is used to distinguish solutions containing micro molecules in different proportions.
2. This is used to demonstrate any change in the charge of molecules in relation to pH change.
3. It is also used in the separation of small biomolecules in a solution.
4. Low voltage paper electrophoresis is used for the separation of proteins, carbohydrates and nucleic acids.
5. High voltage paper electrophoresis (200v/cm for 10-100 minutes) is used in the separation of amino acids in a mixture.

Agarose Gel Electrophoresis: -Separation of molecules based on their charges and size using agarose gel as a supporting medium, is called agarose gel electrophoresis.

Principle:-If a direct current is passed through an agarose gel block containing charged molecules, the positively charged cations (+) will migrate towards the cathode (-) and the negatively charged anions (-) will move towards the anode (+). Proteins and nucleic acids are negatively charged molecules, with the result that they tend to move towards the anode. Mobility of the molecules depends on the net charge, size and shape of the molecule, pH and composition of the suspending medium and electric current. Agarose is a linear polymer of D-galactose and L-galactose. When it is mixed with water, it undergoes cross-linking between the agarose units and forms a porous matrix called gel. If a solution is dispersed

within the gel, the pores have sieving capacity. As a result smaller molecules move towards the anode faster than the larger ones. The pore size is determined by the concentration of agarose. DNA and mRNA are negatively charged molecules. So, the electric field helps them to move towards the anodic end.

Procedure: -

1)Agarose (0.8g/100ml of 1× TAE buffer) is dissolved and heated by keeping it in a microwave oven. It is then transferred into the trough of electrophoresis apparatus upto the height of 75mm thickness. Prior to solidification, a comb is fixed at one end to prepare loading wells. The comb is removed to have loading wells in the gel.

2) Buffer reservoirs are filled with 1×TAE buffer.

3) The DNA sample is digested with a restriction enzyme. The DNA sample is heated to 65°C for 2 minutes to remove aggregates into individual fragments of DNA. To observe the progress of electrophoresis, a dye bromophenol blue of known migration rate, is mixed with the DNA sample.

4)The sample is filled in the wells and suitable molecular markers are loaded in one well. The electrophoresis unit is connected with a power supply and is switched on to start the movement of DNA fragments from the cathodic end to anodic end.

5)After about 1 hour, the current is switched off and the gel is transferred to a container containing ethidium bromide. The stained gel is washed with distilled water to remove unbound ethidium bromide from the gel.

6)This gel is exposed to UV light by keeping it under an UV transilluminator to observe light bands that indicate DNA bands. The size of the DNA fragments can be estimated by comparing their locations in relation to the positions of the molecular markers.

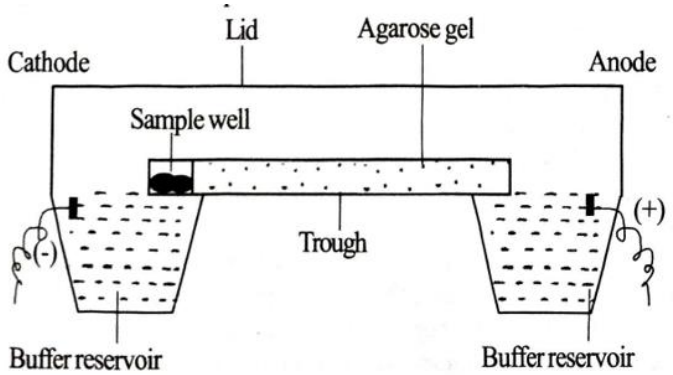


Fig.14.4: Set-up of horizontal agarose gel electrophoresis.

Applications

1. It is used for the separation of DNA fragments in a sample.
2. . It is used to detect the purity of DNA fragments.
3. It is used to determine the size of DNA fragments.
4. It is also used in the separation of proteins in a solution.

5. Estimation of the size of DNA molecules
6. Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
7. Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
8. Agarose gels allow purification of DNA fragments

Polyacrylamide Gel Electrophoresis (PAGE):-Separation of molecules based on their charges and size using polyacrylamide gel as a separating medium, is called polyacrylamide gel electrophoresis (PAGE). This technique was developed in 1970 by Ulrich K.

Principle: -If a direct current is passed through a polyacrylamide gel containing charged molecules the cations (+) will migrate towards cathode (-) and the anions (-) will move towards the anode (+). Since proteins and nucleic acids are negatively charged molecules, they tend to move towards the anode. Small molecules move more rapidly through the gel than the large molecules. Mobility of the molecules depends on the net charge, size and shape of the molecule, pH and composition of the suspending medium and current. Polymerization of acrylamide in the presence of methylene bisacrylamide and ammonium persulphate, gives polyacrylamide gel. When the gel is allowed to polymerize in small tubes sealed at the bottom with a rubber cap and a layer of water on the top, it forms a column of crosslinked matrix with uniform pores. Water at the top layer excludes air that interferes with polymerization. The pore size can be changed by altering the concentration of acrylamide in the solution. 2.5% acrylamide is suitable for the separation of nucleic acids whereas 7.5% acrylamide is of much use in the separation of different proteins.

Procedure: -

Rubber caps are placed over the bottom of hollow glass tubes. Acrylamide solution containing 7.5% acrylamide, ammonium persulphate and small pore solution, are filled in the glass tube. Water is added on the top of the acrylamide solution and kept as such for 25-40 minutes for solidification.

The water layer is removed and 2.5% acrylamide solution containing a large pore solution is added to the top of the solidified gel. This gel is covered with tris buffer of pH 8.3. About 50ml of albumin (a sample protein) and 40% w/v sucrose are added to the top of the gel. Bromophenol blue is added to it to indicate the progress of electrophoresis. The rubber caps are removed and the glass tubes are attached to the buffer reservoirs. The reservoirs are filled with tris buffer.

Electrophoresis unit is switched ONN to run the gel till the bromophenol blue reaches the opposite end of the gel rod. With the help of a syringe water between the gel and glass tube is taken out and the gel

rods.are collected from the glass tubes. If the sample contains proteins, the rods are stained with Coomassie blue. After washing the gel with acetic acid, the colour bands are visualized for proteins.

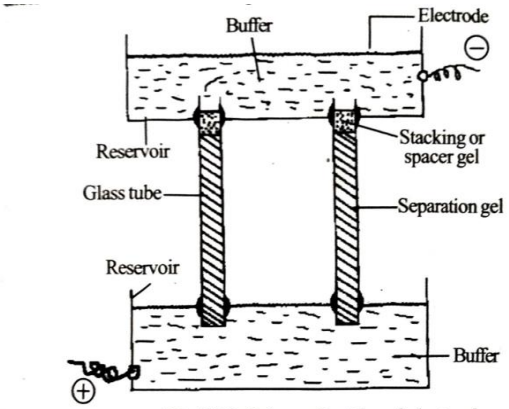


Fig.14.5: Polyacrylamide gel electrophoresis .

Applications

1. PAGE is used for the separation and purification of DNAs and proteins in biological samples.

2. It is used to separate proteins based on charge, but not mass.

3. It is used to determine the molecular weight of proteins and DNAs.

4. It is also employed for quantifying proteins and DNAs.

5. It is used for PCR.

6.It is used for DNA sequencing

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE):-It is Separation of molecules based on size using polyacrylamide gel as a separating medium and sodium dodecyl sulphate (SDS) as a detergent to neutralize the charge of proteins, is called SDS-PAGE.

Principle: -Sodium dodecyl sulphate (SDS) is an anionic detergent. When SDS is added to a solution containing proteins, the detergent molecules bind with the polypeptide of the proteins. Thus, number of SDS molecules bound to the protein is nearly half of the number of amino acid residues in the protein. The SDS-protein complex has some net negative charge. Therefore, proteins move towards the anode depending on their size. Mobility of the protein molecules depends on the net charge, size and shape of the molecule. Acrylamide solution in a suitable concentration and in the presence of ammonium persulphate, solidifies into a gel. Addition of bisacrylamide into this solution produces cross linking and a sieving gel is formed. Since the separation of molecules depends on these sieves, the degree of sieving can be controlled by regulating the amount of these chemicals.

Procedure: -

1. Two dry clean glass plates are taken and a spacer plate is kept between them along three sides. This setup is held together with bulldog clips. Petroleum jelly or 2% agar or silicon grease is spread around the edges of the spacer. This set up is called slider.

2.The separating gel is prepared and poured into the space between the two glass plates. Some amount of water is poured on the top of the gel and the gel is left as such for 30-60 minutes.

3. Stacking gel is prepared. Water is removed from the separating gel and the gel is once again washed with stacking gel. Then stacking gel is poured on the separating gel in the slider. A few drops of butanol is added to create an even gel slab. Then the gel is dried by blotting with filter paper.

4. The comb is inserted in the stacking gel. It is allowed for solidification for 30-60 minutes.

5. The comb is removed carefully without disturbing the well-shape

6. The slider is fixed into the lower tank. Necessary quantity of running buffer is poured into the tank of the electrophoresis apparatus.

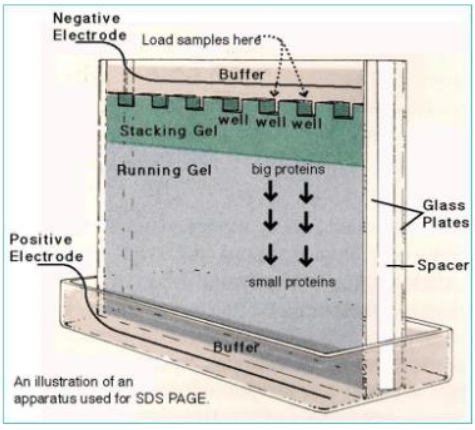
7. The protein sample is boiled with SDS buffer for 3 min and cooled to room temperature. The denatured protein sample is loaded into the wells at different concentrations. A few wells are loaded with standard markers. Bromo phenol blue is added to each well as a marker to locate the molecular movements.

8. The upper tank is filled with upper gel buffer.

9. The electrodes connected to the power pack and 80V current are given for 7-8 hrs.

10. When the samples reach 1 cm above the lower surface of separating gel, the power is switched off.

11.The slider is removed from the chamber. The gel is carefully removed and kept into the Stainer for 2 hours. Then it is washed with a detainer until the background is clear. Clear blue Sands can be observed in the gel. They are compared to the standards for confirmation.



An illustration of an apparatus used for SDS PAGE.

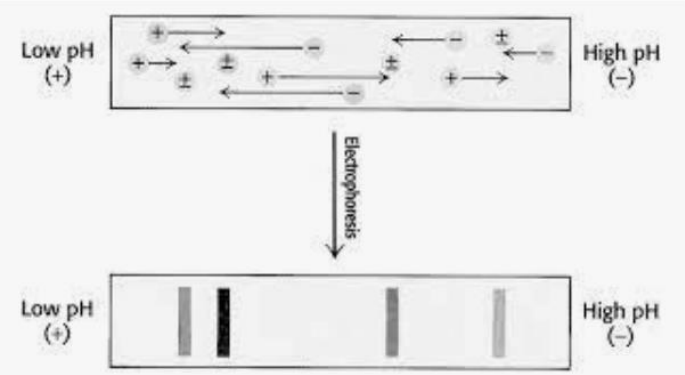
Applications

1) SDS-PAGE is used to separate proteins based on their mass and size.

2) It is useful to determine the molecular weight of proteins.
3) It is used to separate the different subunits of complex proteins.
4) This method can resolve thousands of proteins in a biological sample.
5) It is used to measure the molecular weight of the molecules.
6) It is used to estimate the size of the protein.
7) Used in peptide mapping
8) It is used to compare the polypeptide composition of different structures.
9) It is used to estimate the purity of the proteins.
10) It is used in Western Blotting and protein ubiquitination.
11) It is used in HIV test to separate the HIV proteins.
12) Analysing the size and number of polypeptide subunits.
13) To analyse post-translational modifications.

Isoelectric focussing/ Electro focusing: -Electrophoresis along the gradient of pH values in the supporting medium is called electro focusing. In this method, electric current forces the protein molecules to reach the isoelectric point in the pH gradient. There is no further movement of proteins after reaching the same pH as their isoelectric point. Hence, this method is also called isoelectric focusing (IEF). The IEF is very useful for the separation of closely related proteins having the same size and weight but different isoelectric points

Principle: -Proteins contain many positively and negatively charged groups on their surface. Therefore, each protein has some net ionic charge. Solutions also have some ionic charge depending on the cations and anions in them. This ionic charge of solutions varies with pH values. The pH at which the ionic charge of a solution is equal to the ionic charge of a protein is called isoelectric point of the protein. At the isoelectric point, the number of negative charges on the protein is equal to the number of positive ions in the solution. So, the net electric charge is zero.Since the electrical potential difference is zero, proteins do not move further while moving across the pH gradient. Here, proteins are focused to their isoelectric pH of the medium. Proteins exist as cations at pH values below the isoelectric point. They exist as anions at pH range above the isoelectric point. If proteins are allowed to move along a pH gradient across the gel under the influence of electric current, they move to the isoelectric pH and they do not move further due to electric current. Many closely related proteins differ in their isoelectric pH because of the presence of different acidic and basic groups. Hence, such proteins can be separated by electro focusing. The apparatus consists of a lower buffer tank, an upper buffer tank and a glass tube. The glass tube is fixed in between the two tanks. One electrode is fixed in each buffer tank



Procedure: -

1. A short glass tube with open ends is taken and cleaned well.
2. The lower end of the tube is closed with a cork.
3. A sucrose density gradient column with pH gradient is prepared in the tube. 4.Ampholyte containing 1.94g acrylamide, 0.06g bisacrylamide, 5g sucrose, 0.25ml riboflavin solution and 40ml of water is prepared. The ampholyte solution is poured slowly along the wall of the tube until it reaches the level of the column inside.
5. The tube is kept undisturbed for 3hrs for the polymerization of polyacrylamide gel with pH gradient.
6. After polymerization of the gel, the cork at the base is removed.
7. A sample containing proteins is poured on the top of the pH gradient column.
8. The tube is attached with two buffer reservoirs.
9. A solution with more acidic pH is filled in one buffer reservoir and another solution with more alkaline pH is filled in the other buffer reservoir.
10. Electrodes are connected and the electrophoresis unit is switched on.
11. Proteins move along the gel under the influence of electric current. After reaching the isoelectric pH, there is no further movement of the protein. This movement can be seen by the movement of bromophenol blue dye added to the protein solution.
12. After disconnection, the particular band is taken from the gel to isolate the protein.

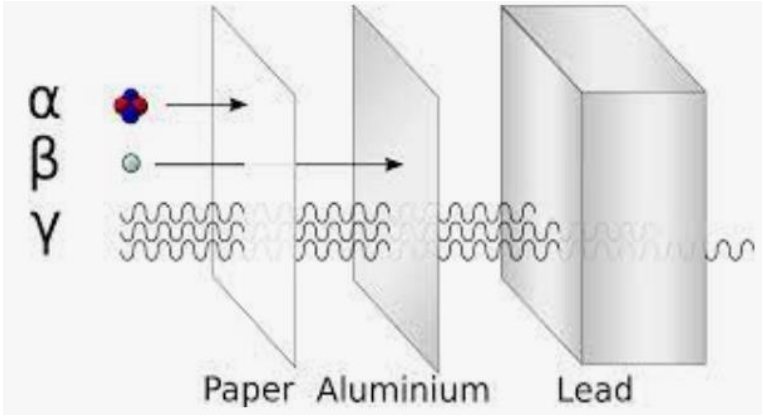
Applications: -

1. The IEF is very useful for the isolation and purification of proteins having the same mass but different isoelectric points.
2. It is a sensitive technique to resolve closely related proteins
3. Widely used for separation and identification of serum proteins.
4. Used in food and agricultural industries, forensic and human genetics laboratories.
5. Used in enzymology, immunology and membrane biochemistry.
6. Used in 2D Gel electrophoresis is an application of IEF.

Radioisotope techniques

Radioactivity: -The property of radioactive isotopes to emit some radiations is known as radioactivity. It occurs as a result of disintegration of the nucleus of the isotope through emission of atomic particles such as protons or neutrons. **Radioactivity was first discovered by Henri Becquerel in 1896**.Radioactive disintegration occurs in the nucleus of radioactive atom which contains an excess of either neutrons or protons. In such cases, the nucleus tends to makeup a balance by emitting radiations. Radioactivity persists until the number of neutrons and protons becomes equal. During disintegration, **the radioactive isotopes emit alpha (α)-radiation , beta(β)-radiation ,gamma (γ)-radiation depending on the nature of nucleus**.

1) **alpha (α)-radiation: -**The α-radiation consists of a particle. The α -particle is the largest particle emitted from the atom. It is composed of 2 protons and one neutron, as in the helium nucleus. It is emitted by nucleus which has a greater number of protons than neutrons. The α- particle is positively charged and moves at the speed of 16130 Km per second. It is readily stopped by skin or paper; so, it cannot penetrate deeper tissues. On emission of alpha particle, the nucleus will contain two protons and one neutron less than in the original nucleus. The element will be converted into another element.



Penetrating power of different particulate radiations through paper, aluminium and lead plates

2) **Beta(β)-radiation: -** The β-radiation is composed of β-particles. It is composed of protons or electrons. The β particles are known as positrons. They are positively charged and equal to the charge of an electron but with opposite charge. Positron is emitted by the nucleus which has one proton more than in the stable nucleus. Therefore, on emission of a particle, the atom becomes one proton less than its previous form. The β-particles composed of electrons are called negatrons (ß-). The speed of ß- particle is equal to the speed of electrons. The penetrating power of β-particles is

little more than the α particle. It has low ionizing power but strongly affects photographic plates. It can penetrate paper but it is stopped by aluminium plate.

3) **Gamma (γ)-radiation: -**The γ radiation is composed of γ particles. It has no mass and charge. The permeating capacity (10000 arbitrary units) of γ particles is 100 times more than that of a particle. It can affect photographic plates and produce fluorescence. It is so powerful that it can penetrate a lead plate. Radiations affect photographic plates, ionize gases, cause scintillation effects, produce heat and can also effect some chemical changes.

   Chemical property of element has not been changed due to radioactivity. For example, chemical properties of radioactive C^{14} are similar to those of stable isotope of carbon, C^{12}.Heavy elements like radium, uranium and thorium discharge a, ß, γ and X-rays spontaneously in nature. This type of radioactivity is called natural radioactivity. There is no need for bombardment to initiate this radioactivity

Units of Radioactivity: - The activity of a quantity of radioactive material where one decay takes place per second. **The SI unit of radioactivity is becquerel (Bq) and this term is named after Henri Becquerel.**

1 becquerel = 1 radioactive decay per second = 2.703×10^{-11} Ci

Other units of radioactivity: -

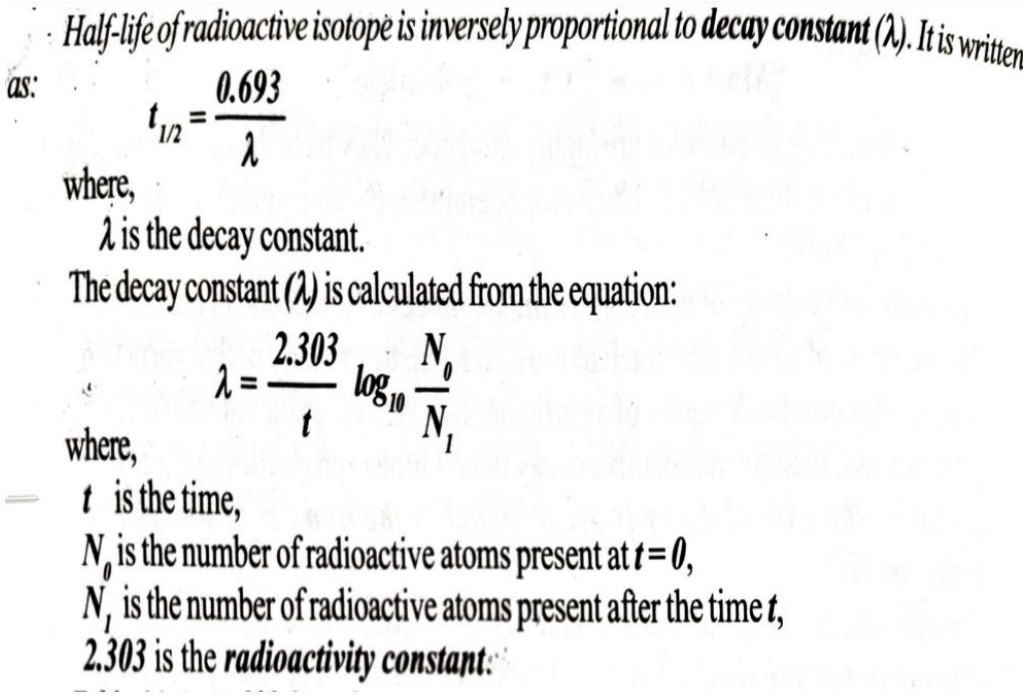
Radioactivity is measured in terms of **Curie (Ci).** It is defined as 3.7×10^{10} disintegrations per second (d.p.s). It is equal to the amount of disintegration of 1 gram of pure radium per second.

1 curie = 3.7×10^{10} radioactive decays per second
1 becquerel = 1 radioactive decay per second = 2.703×10^{-11} Ci.
1 rutherford = 1.106 radionuclide decays per second

Half-life: -

The time taken for the disintegration of one half of radioactive substance is called half-life ($t_{1/2}$) of radioactive isotope. In other words, half-life is the time required for the radioactivity of a substance to half of its original level. It is independent of concentration of the substance and Age of fossils and others are measured on the basis of the half-life of radioactive substances. In a radioactive sample, all atoms do not emit radiation at a time. The emission of radiation is a spontaneous process. That is, disintegration of one atom is followed by another atom, then by another atom and so on until the final

radioactive nucleus gets disintegrated. At the end of half-life, about 50% of atoms in the sample have lost their radioactivity. Half-life of radioactive isotope is inversely proportional to decay constant It is written as:



Half-life of radioactive isotope is inversely proportional to **decay constant** (λ). It is written as:

$$t_{1/2} = \frac{0.693}{\lambda}$$

where,

λ is the decay constant.

The decay constant (λ) is calculated from the equation:

$$\lambda = \frac{2.303}{t} \log_{10} \frac{N_0}{N_1}$$

where,

t is the time,

N_0 is the number of radioactive atoms present at $t = 0$,

N_1 is the number of radioactive atoms present after the time t,

2.303 is the ***radioactivity constant.***

Radioactive Isotopes: -Atoms of the same element differing in the number of neutrons but having the same atomic number are called isotopes.

For example, 0^1 and 0^18 are isotopes of oxygen. Isotopes are designated by writing the symbol of the element followed by a superscript of mass number. For example, carbon exists in three isotopic forms. One form has 6 protons, 6 neutrons and 6 electrons and hence its mass number is 12 (6protons + 6 neutrons). This isotopic form is written as C^{12}. Since its protons to neutrons ratio is exactly 1, it remains as a stable isotope. Another isotopic form of carbon has 6 protons, 7 neutrons and 6 electrons, so that its mass number is 13 (6 protons and 7 neutrons). It is designated as C^{13}.

Radioactive isotopes may emit α,β,γ rays during their spontaneous decomposition. An element seems to be radioactive if its proton to neutron ratio exceeds 1. It remains radioactive until the proton to neutron ratio becomes 1 (equal number of protons and neutrons).

Measurement of radioactivity: -The quantitative determination of radioactivity in samples using a suitable instrument is called radiation dosimetry or measurement of radioactivity. The instruments being used for measuring the radioactivity are popularly known as radiation dosimeters. The radiation dosimeter and its reader are together called dosimetry system. Radiation dosimeters measure the

duration of exposure, absorbed dose, rate of emission of radiation per unit time and other related quantities of radiations accurately. There are several types of radiation dosimeters in

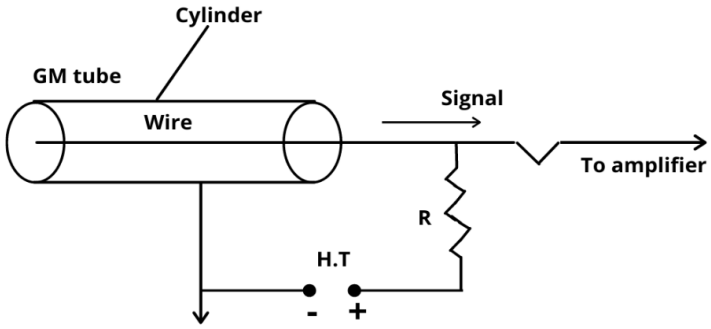
common use. They are:

1.Geiger muller counter

2. Scintillation counter

1.Geiger muller counter: -

Instruments which measure the radioactivity of substances by measuring the rate of ionization of gas filled in a glass chamber are called ionization chamber radiation dosimeters (ICRD). They are simply called ionization chambers. They measure ß-radiation emitted by radioactive substances. The ionization chambers are available in different sizes and shapes for diagnostic purposes and radiotherapy. Geiger-Muller counter (also known as GM tube). The GM tube was first devised by Hans Geiger in 1909. It consists of a small glass tube with sealed ends and two electrodes. This tube is filled with an ionizable gas such as argon or neon or helium. It acts as an ionization chamber. Radiation emitted from the radioactive substance ionizes the gas and generates some current. The current flow is directly proportional to the intensity of emitted radiation.



Instrumental setup of Geiger-Muller counter.

It has two electrodes. They are inner electrode and outer electrode. The inner electrode is fixed along the axis of the glass tube. It is made of a fine tungsten wire with 0.1-0.5 mm thickness. It serves as the anode. (+). The outer electrode is a metal tube around the glass tube. It may be made of brass or nickel with 1-5 cm thickness and suitable length. It serves as the cathode. The two electrodes are separated by a high-quality insulating material at one end of the tube to prevent leakage of electric current. One end of the tube has a small mica window. The two electrodes are connected with a volt meter called electrometer. A potential difference is maintained between the electrodes by applying some low voltage current that is not enough to ionize the internal gas. This is called polarizing voltage. When a radioactive sample is brought near the window, the β-particles, emitted from it, enter the ionization chamber through the window. These β-particles collide with gas molecules and ionize them. As a result,

anions (-ve) and cations (+ve) are produced inside the ionization chamber. The cations move towards the anode (+ve electrode) while the anions move towards the cathode (-ve electrode). Since the ions accumulate around the oppositely charged electrodes, the potential difference (polarizing voltage) is neutralized. The electric pulse is measured by the electrometer

Applications of Geiger Muller counter:-

1. To detect radioactive rocks and minerals in the course of mineral prospecting.
2. For Fire responders for making an initial determination of radiation risk.
3. For Hazard Management personnel in checking for radiation danger in an emergency situation.
4. To check for environmental levels of radioactivity near a nuclear power facility.
5. To test for danger amidst a nuclear accident or leakage of radioactive coolant.
6. To check for radioactive contamination of clothing and shoes in your workplace.
7. Radiation detection in the scrap metal processing business.
8. To check possible leakage or exposure to X-rays in a medical facility
9. To check for radiation in areas where depleted uranium ammunition shells have been used.
10. To check for irradiated gemstones in the jewellery trade.
11. To check the levels of iodine 131 in cancer patients undergoing radiation therapy.
12. You are in close proximity to a uranium mine and want to test the soil and environment for dangerous levels of radioactivity.
13. To test for radioactive contamination of food.
14. To check materials in your anthropology or archaeology field.
15. To check for radioactivity in metal objects in your home or office that could be made of recycled radioactive materials.

Scintillation Counter: - This apparatus is more sensitive and efficient than the GM tube and is mostly used in detecting y -rays. The process of giving sparkles of brilliant light by certain substances on receiving particulate radiations is known as scintillation. The instrument that measures the flashes of light produced by the fluorescent crystals on receiving radiations is called as scintillation counter. **Scintillation counter was invented by Sir Samuel Curran** in the early 1944 to measure the radioactivity of uranium salts. **It works on the principle that fluorescent compounds produce flashes of brilliant light when they are exposed to particulate radiations.**

The substance that produces flashes of light on receiving radiation is called a scintillator or phosphor. It may be a plastic containing anthracene or crystals of zinc sulphide or calcium tungstate or sodium iodide or organic liquids. When these substances are exposed to ionizing radiations, they absorb the radiation and go to excited state having more energy. The excited substances lose the extra energy in the form of light (photons). These photons are measured with a photomultiplier tube.
It uses certain fluorescent crystals, which emit light in the form of scintillations on absorbing radiation. The main parts of this counter are schematically shown in **Figure** Phosphor is usually used as

scintillating substance. Natural inorganic crystals (Zinc sulphide or calcium tungstate) and certain organic liquids as well as plastics are also used as phosphors. Inorganic phosphors are mainly used to detect protons, deuterons and y rays whereas organic phosphors are used to detect a particles.These substances when exposed to radiation scintillate and emit flashes of light. These light pulses are converted into electric pulses, which are amplified and recorded.

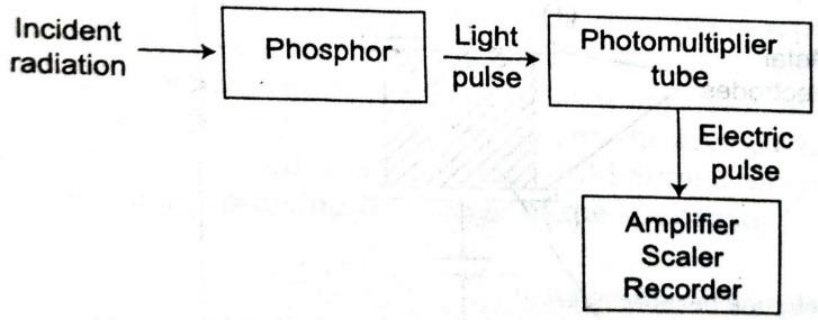


Figure 9.2. Schematic representation of scintillation counter.

Applications of Scintillation Counter

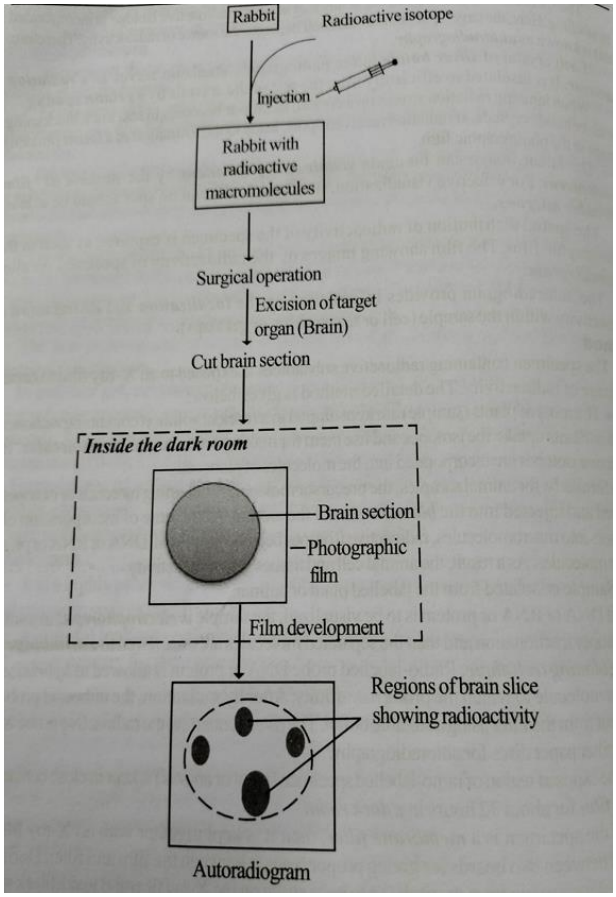
1. As the pulse height is proportional to the energy of the incident radiation, it is used for the investigation of the energy distribution of nuclear radiations. Scintillation Counters are widely used in radioactive contamination, radiation survey meters, radiometric assay, nuclear plant safety, and medical imaging, which are used to measure radiation.
2. There are several counters mounted on helicopters and some pickup trucks for rapid response in case of a security situation due to radioactive waste or dirty bombs.
3. Scintillation counters are designed for weighbridge applications, freight terminals, scrap metal yards, border security, contamination monitoring of nuclear waste, and ports.
4. It is widely used in screening technologies, In vivo and ELISA alternative technologies, cancer research, epigenetics, and Cellular research.
5. It also has its applications in protein interaction and detection, academic research, and pharmaceuticals.
6. A liquid Scintillation Counter is a type of scintillation counter that is used for measuring the beta emission from the nuclides. It is most efficient for γ-ray counting.
7. With its large size and highly transparent phosphor, it displays very high efficiency.

Autoradiography

The visualization of the pattern of radiation distribution of a specimen labelled with radioactive substance is called autoradiography. Autoradiography is a suitable technique for identifying DNA, RNA and proteins in a sample. It is best for tracing the route of conversion of molecules in biochemical

pathways. **The technique of autoradiography was first developed by Mary Curie in 1898. The photographic emulsion and film stripping methods are later invented by Roger in 1979**.

The radioactive isotopes are stable only for definite periods and then they decompose to form other atoms or fragments of atoms. During this radioactive decay, many atoms produce radioactive emissions, which may be measured by special devices. In this technique, the specimen itself is the source of the radiation because of the incorporation of radioactive isotope that can emit a-, or -rays. Hence it is called autoradiography. The images of radioactivity are captured on a photographic emulsion. In this technique, the sample containing radioactive substance (a chromatogram or a tissue slice or a section of a plant or animal) is kept in close contact with a photographic plate or an X-ray film. That is, the cells or tissues already exposed to radioactive isotopes are made to contact with a photographic emulsion for specific period as shown in the figure. The emitting radiations affect the emulsion in the same way like that of light. On development, the areas, which have been in contact with radioactivity, could appear as spots on the film, thus producing the image of the specimen



1. To find and investigate the various properties of DNA
2. To find the location and amount of particular substance within a cell including cell organelle, metabolites
3. Tissue localization of radioactive substance.
4. To find the site and performance of targeted drug. And To locate the metabolic activity site in the cell.

5. Autoradiography is used to localize the position and size of a particular molecule in a sample.
6. Demonstration the rate of production and life span of blood cells in animal's body.
7. It is useful for the study of movement of solutes in whole plants or animals.
8. Diagnosis of genetic diseases in man based on blotting and nucleic acid techniques.
9. It is a poweful determination to accumulate specific molecules in certain organs

Biosaftey:-

1. The operating voltage must correspond to the midpoint of flat plateau region of plateau graph.
2. If the continuous discharge is produced, the voltage should be lowered.
3. The applied voltage must be relatively stabilized.
4. Introduction of light should be prevented to avoid photo electric effect.
5. Ensure that the instrument has been calibrated within the last 12 months,
6. Ensure that the batteries are working properly
7. Do not ever apply a high voltage beyond the plateau region, as the tube will be damaged.
8. **Required PPE:** For any work with an open radioactive source, wear: disposable gloves made up latex or nitrile gloves are generally suitable),a full-length lab coat (worn closed with sleeves rolled down)
9. close-toed shoes. Never wear sandals or other open-toed shoes while working with radioactivity.
10. **Safety Glasses:** You should wear safety glasses for any radioisotope procedure, but it is especially important whenever there is a potential for the build-up of pressure that could release a spray of material.
11. **Petroleum-Based Hand Creams:** Avoid using petroleum-based hand creams when wearing gloves because petroleum-based hand creams may increase glove permeability.
12. **No Eating or Drinking:** Do not eat or drink in any room labeled with a Caution: Radioactive Materials sign on the door.
13. **Never pipette anything by mouth.** Always use rubber or plastic gloves when handling radioactive material