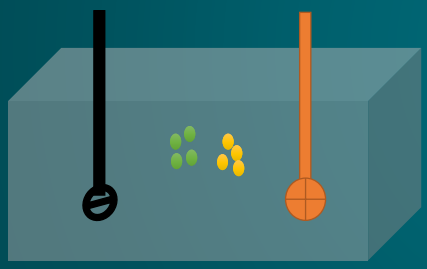
Electrophoresis Introduction (Part 1)

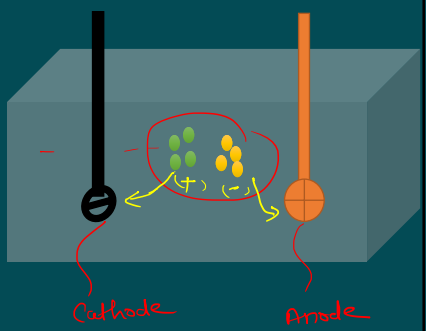


Chromatography
Instrumental Analysis

Electrophoresis

Introduction

- Tiselius a Swedish Biochemist won the NOBLE PRIZE in 1948. He first developed the electrophoresis technique and discovered the complex nature of serum protein
- **Electro- Electric Field** and **Phoresies- Separation of ions**
- The separation techniques in which the transport of particles through a solvent (aqueous buffer) by application of an electric field is called as electrophoresis
- Cations migrate toward the electric field's negatively charged cathode (Cataphoresis) and anions migrate toward the positively charged anode (Anaphoresis) and neutral species do not experience the electrical field and remain stationary.

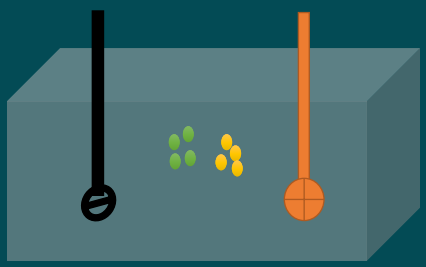


Electrophoresis

Introduction

- This technique also extended to ZONE ELECTROPHORESIS, it separates ions based on the migration of charged molecules through the cellulose acetate paper or agarose gel film.
- Most of the polymer are electrically charged macromolecules which can be separated by this technique.

- protein
Amino acids
Nucleic acid
DNA
RNA



Electrophoresis

Theory

- Movement of the charged particles depend upon frictional coefficient, which in turn depend upon function of some physical properties such as molecular weight, size.
- Electrostatic force is directly proportional to the electric field strength.
  - $Fe = qE$
- Electrostatic migration is opposed by frictional forces
  - $Ff = Vf$
- Where
  - Fe = Electrostatic force
  - q = Charge
  - E = Electric field strength
  - Ff = Frictional force
  - V = Velocity of migration
  - f = Frictional coefficient

Electrophoresis

Factors Affecting Electrophoresis

The rate of migration of the molecule

$$V = Eq/f$$

Where, V = molecule velocity

E = Electric field strength

q = molecular charge

f = friction coefficient of molecule

$$V/E = q/f$$

$$\mu = V/E = q/f$$

Where, μ = Electrophoretic Mobility

Electrophoresis

Factors Affecting Electrophoresis

- Rate of migration of ions directly proportional to the
  - Charge
  - Current
  - Voltage

"Faster Speed"

- Rate of migration of ions inversely proportional to the
  - Shape
  - Size
  - Frictional Resistance

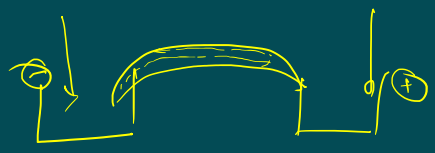
"Slower Speed"

Electrophoresis

Types of Electrophoresis

A. Moving Boundary Electrophoresis

1. Capillary Electrophoresis: Used to separate-
   - Proteins
   - Peptides & Amino acids
   - Inorganic ions
   - Organic acids and bases
   - Nucleic acid



B. Zone Electrophoresis

1. Paper Electrophoresis
2. Capillary Gel Electrophoresis
3. Gel Electrophoresis: Agarose Gel (DNA & Protein); Polyacrylamide Gel; SDS-PAGE

Paper Electrophoresis

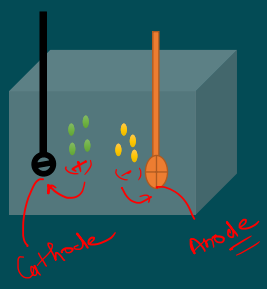
(Zone Electrophoresis)

Chromatography
Instrumental Analysis

Paper Electrophoresis

Introduction

- Electrophoresis: It is the separation techniques in which, charged molecules moves to opposite charged pole through a solvent by application of an electric field is called as electrophoresis.



Rate of migration of the molecule

$$V = Eq/f$$

Where, V = molecule velocity

E = Electric field strength

q = molecular charge

f = friction coefficient of molecule

$$\mu = V/E = q/f$$

Where, μ = Electrophoretic Mobility

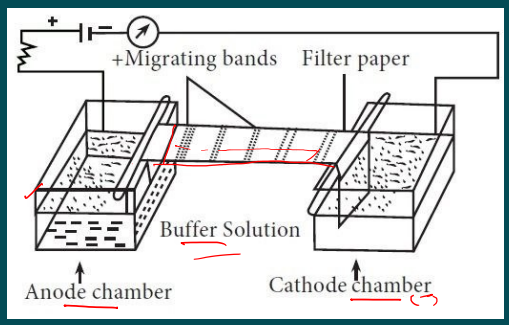
Paper Electrophoresis

Introduction

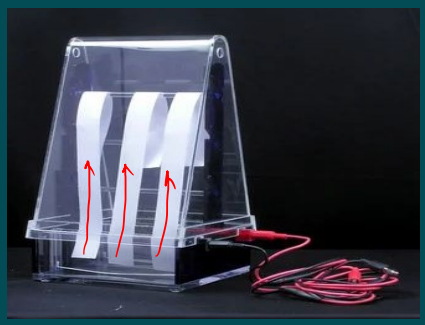
- Paper Electrophoresis: Electrophoresis done by chromatographic filter papers.
- Paper mainly contains 95% of alfa- cellulose
- It is mainly used to separate the small charged molecules like amino acids, peptides, etc.
- In this technique, the motion of colloidal particle of solution occurs leading to subsequent separation along the paper strip.
- PE is easier in comparison to gel electrophoresis.
- It does not require matrix preparation and it does not contain charges unlike the gel that interfere with the separation of compounds

Paper Electrophoresis

Apparatus



Horizontal Unit



Vertical Unit

Source: https://microbiologynotes.org/electrophoresis-overview-principles-and-types/

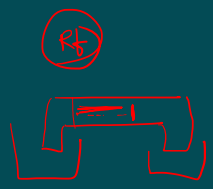
Paper Electrophoresis

Apparatus

- It consists basically of two items,
  - a power pack and
  - an electrophoretic cell.
- The **power pack** provides a stabilized direct current and has controls for both voltage and current output.
- Power packs, which have an output of 0-500 V and 0-150 mA are available and can be programmed to give either constant voltage or current.
- The **electrophoretic cell –** contains the electrodes, buffer solution reservoir, a support for paper and transparent insulating paper.
- Before using the paper, it

Paper Electrophoresis

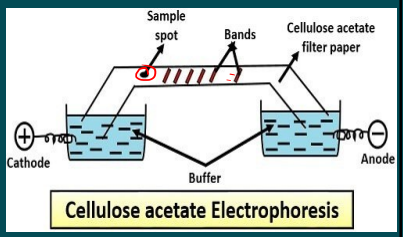
Procedure



- A strip of filter paper is moistened with buffer and the ends of the strip are immersed in to buffer reservoirs containing the electrodes.
- The samples are spotted in the centre of the paper and high voltage is applied.
- Application of high voltage causes less diffusion of small molecules giving better resolution and it take less time to complete the process.
- Spots migrate according to their charges.
- After electrophoresis, separated components can be detected by variety of staining techniques, depending upon their chemical composition

Paper Electrophoresis

Cellulose Acetate Paper Electrophoresis



Cellulose acetate Electrophoresis

- Kohn in 1958 introduced, Cellulose acetate as a medium for electrophoresis.
- It was developed from bacteriological cellulose acetate membrane filters and is commercially available as high purity cellulose acetate strips, which are thin and have a uniform micropore structure.
- Cellulose acetate is especially used for clinical investigations such as separation of hemoglobin's from blood, lipoproteins and glycoproteins.
- The adsorption is because of hydroxyl group present in the cellulose.
- Buffers used in both the electrophoresis i.e., in paper and cellulose acetate electrophoresis are same

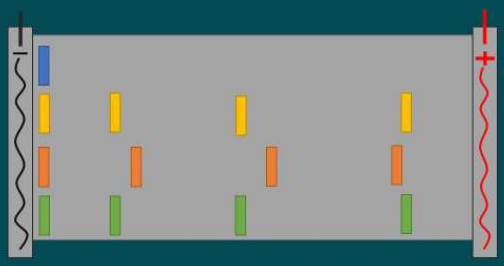
Paper Electrophoresis

Application

- Separation of biological materials like amino acids, proteins, and peptides, etc.
- Clinical investigation of biological sample
- Clinical investigation in sickle cell disease, hemoglobin abnormalities,
- Used in forensic department
- Used in separation and identification of alkaloid
- Used to quality control water and environment pollution

Gel Electrophoresis

(Zone Electrophoresis)

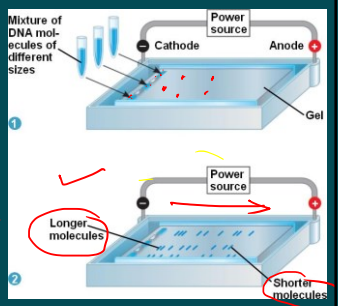


✓ Helping for identifying DNA base pair length

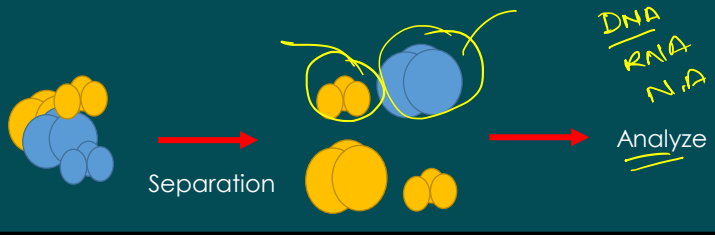
Chromatography
Instrumental Analysis

Gel Electrophoresis

- Electrophoresis: It is the separation techniques in which, charged molecules moves to opposite charged pole through a solvent by application of an electric field is called as electrophoresis

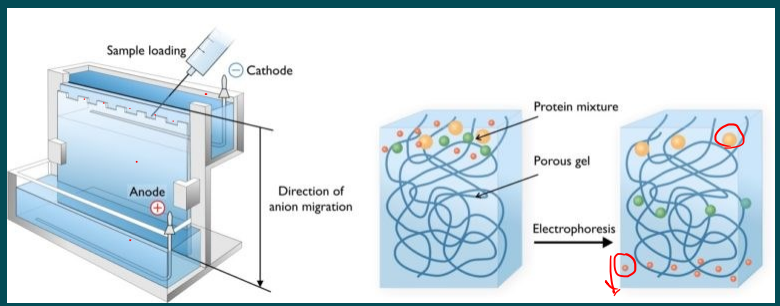


- **Gel Electrophoresis:**
  - Electrophoresis done by help of gel as a support matrix.
  - Most commonly used EP method.
  - Used for both analytical and preparative processes.
  - Charged molecules are separated based on their charge and size https://socratic.org/questions/why-is-gel-electrophoresis-used



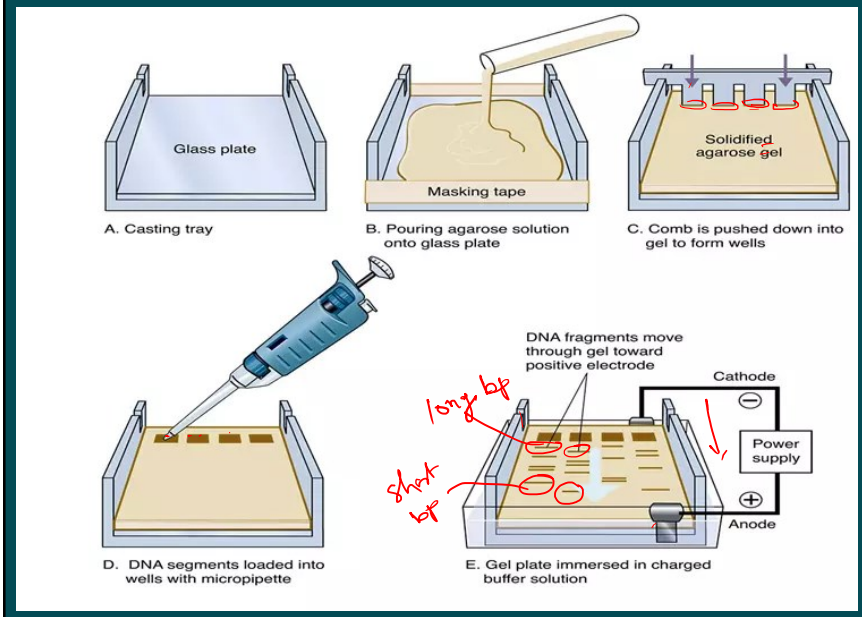
Gel Electrophoresis

- The porous **gel** matrix act as a sieve to separate the molecules.
- By adjusting the pH of the buffer, molecules being separated carry a net negative charge and will move towards the anode.
- As they move through the pores of the gel, the smaller molecules move faster than the larger molecules.



https://blog.biomall.in/gel-electrophoresis-the-separation-technique/

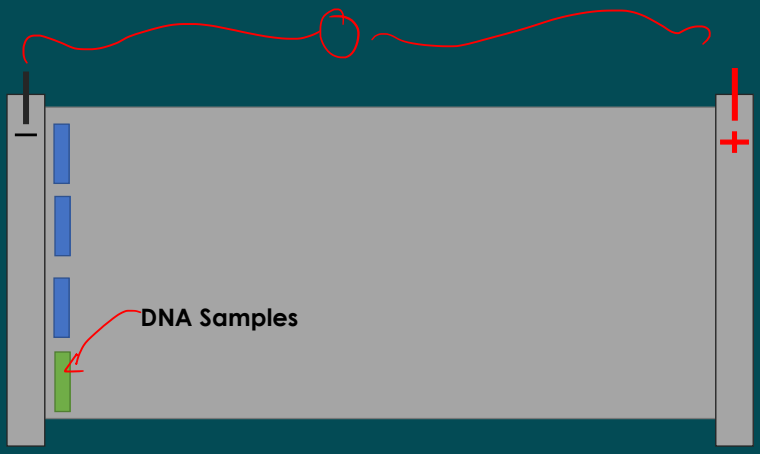
Gel Electrophoresis



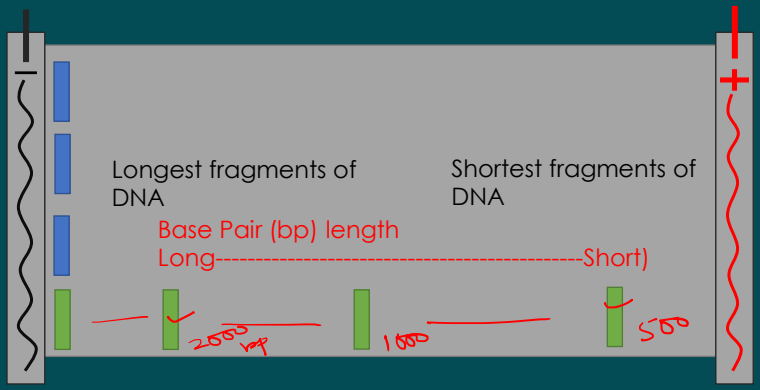
https://www.smacgigworld.com/blog/instrumentation-and-methodology-of-electrophoresis.php

Gel Electrophoresis

Gel Electrophoresis

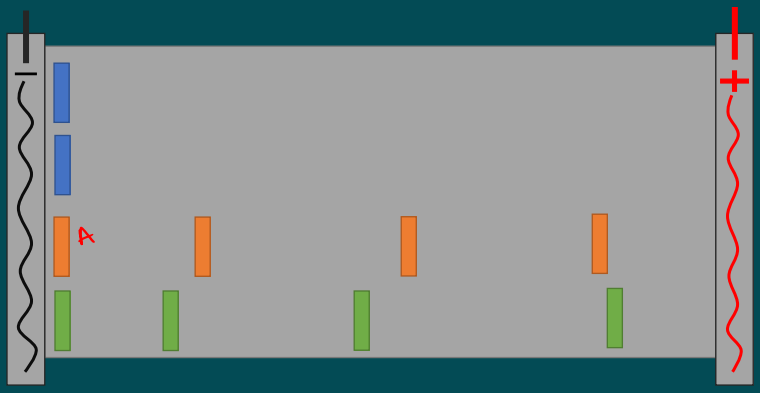


Gel Electrophoresis



- ✓ Helping for identifying DNA base pair length

Gel Electrophoresis



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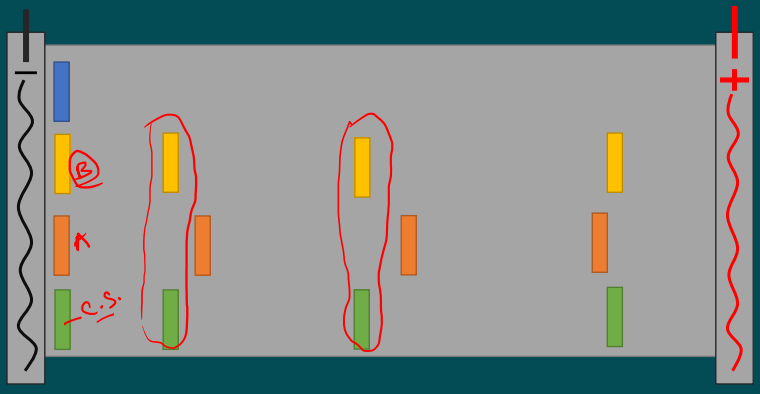
Gel Electrophoresis



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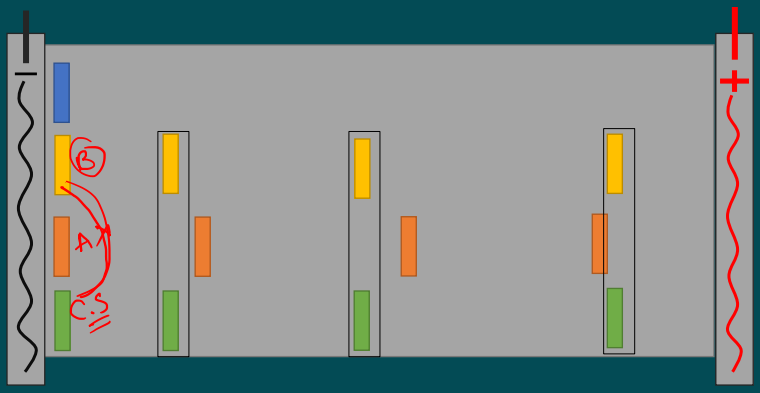
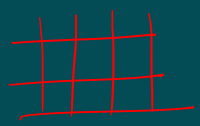
Gel Electrophoresis



✓ Helping for identifying DNA base pair length

Gel Electrophoresis

- Two forms of gel matrix are used, **cross-linked** and **non-cross linked.**
- Most common **cross-linked gels** are
  - agarose and
  - acrylamide
- **Agarose (Polysaccharide polymer, obtained form Red Seawead)**
  - It is a reversible matrix cross-linked by **hydrogen bonds**
  - Used in Horizontal Unit of EP
  - It has a large pore size good for separating large molecules.
  - Typically resolve 200 bp-20 kbp
  - Also used to separate **large proteins** and **protein complexes**



Gel Electrophoresis

- Two forms of gel matrix are used, **cross-linked** and **non-cross linked.**
- Most common **cross-linked gels** are
  - agarose and
  - acrylamide
- **Acrylamide** is a permanent matrix cross-linked with **methylene bridges**
  - Chain of acrylamide molecules.
  - It has a small pore size good for separating small molecules (DNA and Proteins).
  - Separates fragments < 200 bp
  - **Used in Vertical Unit of EP**

Gel Electrophoresis

APPLICATION OF GEL ELECTROPHORESIS

- Used to study the properties of a single charged species or mixtures of molecules.
- Used to separate organic bases, acids and inorganic ions.
- Used to identify amino acids, peptides and proteins.
- Used to separate very large proteins, nucleic acids and nucleoproteins etc.
- Used in Clinical Laboratory to separate proteins from each other
  - Proteins analysis in body fluids: Serum, Urine, CSF
  - Proteins in erythrocytes: Hemoglobin
  - Nucleic acids: DNA, RNA

Gel Electrophoresis

APPLICATION OF GEL ELECTROPHORESIS

- Agarose Gel electrophoresis is used to visualize:
  - Genomic DNA
  - RNA
  - PCR products
  - Plasmids
  - Restriction enzyme digest products

PC
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