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Unit-III

Electrophoresis

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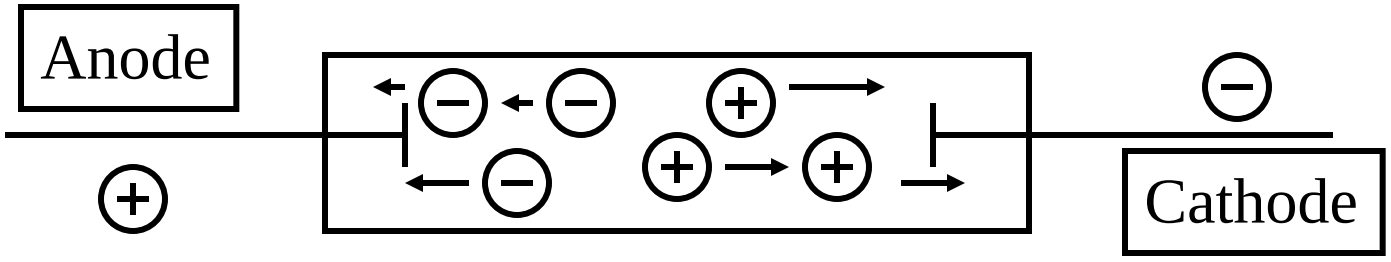
KEY CONCEPTS

- General principle
- Factors affecting Electrophoresis
- Types of Electrophoresis
- Gel Electrophoresis
- Applications

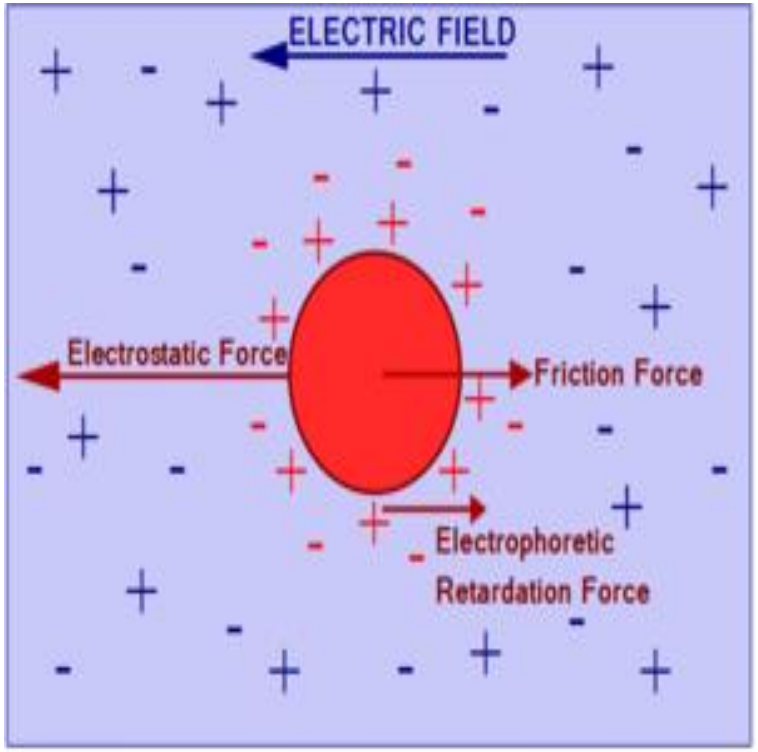
General principle

- Electrophoresis: Differential movement or migration of charged molecules (ions) in solution, with response to an electrical current.
- Separation of molecules according to size and/or charge.
- Negatively charged molecules (anions) will be attracted towards anode.
- Positively charged molecules (cations) will move towards cathode.



Basic Concept

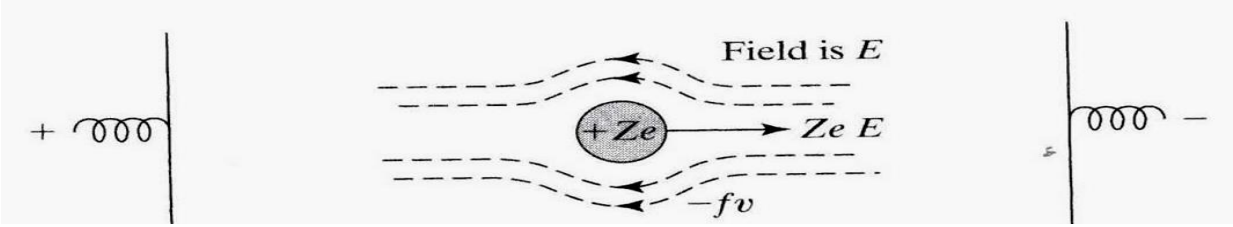
- As an analytical tool, electrophoresis is simple, rapid and highly sensitive.
- Rate of migration depends on:
  - ✓ Molecular charge (net charge)
  - ✓Molecular shape and size
  - ✓Strength of the electrical field,
  - ✓Ionic strength, viscosity, and temperature of the medium.



https://upload.wikimedia.org/wikipedia/commons/a/ab/Electrophoresis.svg

General principle

- An isolated charged particle in a non-conducting medium.



- The force ***(F)*** experienced by a particle in an electrical field is given by Coulomb's law,

$$\boldsymbol{F} = Ze\,E$$

(Where, ***E*** **-** *E*lectric field: potential per unit length)

- The viscous resistance of the medium to the motion: $-fv$

(Where, f - Frictional factor)

- The viscous resistance of the medium just balances the driving force.

$$-fv = F = Ze\,E$$

Electrophoretic Theory

- 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

- **Electrophoretic mobility value (μ):**

$$\mu = v/E = Eq/Ef = q/f$$

- From above equation, molecules move through gel based on charge to friction ratio
  - Since friction is based primarily upon mass, molecules migrate based upon charge to mass ratio
  - Therefore, differences in μ approximate differences in mass.

Electric Field

- Electric current is carried by buffers
  - Buffers keep the pH and charge surrounding analyte constant.
- **Effects of the electric field on the sample:**
  - In electrophoresis either current, voltage, or power, is always held constant.
  - Higher voltage causes greater migration speed.
  - Also leads to generation of heat.
    - May denature protein sample and destroy gel matrix
    - Also mixes samples through convection of buffer

Electrophoresis buffers

- Carry current and prevents analyte from being altered.
  - Common buffers are Tris-HCl and Tris-glycine.
  - Tris-Borate-EDTA (TBE), Tris-Acetate-EDTA (TAE), Tris-Phosphate-EDTA (TPE) used most often for DNA.
  - 10 mM sodium phosphate buffer used for RNA.

- Buffer additives modify sample molecules.
  - Formamide, urea (denaturing agents)

Net charge and pH

- The net charge of the molecule is determined by the pH of the medium.
- Proteins are amphoteric in nature (contain both acidic and basic residues).
- Each protein has its own characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups.
- Nucleic acids, unlike proteins, are not amphoteric. They remain negative at any given pH.

Factors Affecting Electrophoresis

The speed and direction of a moving charged particle influenced by

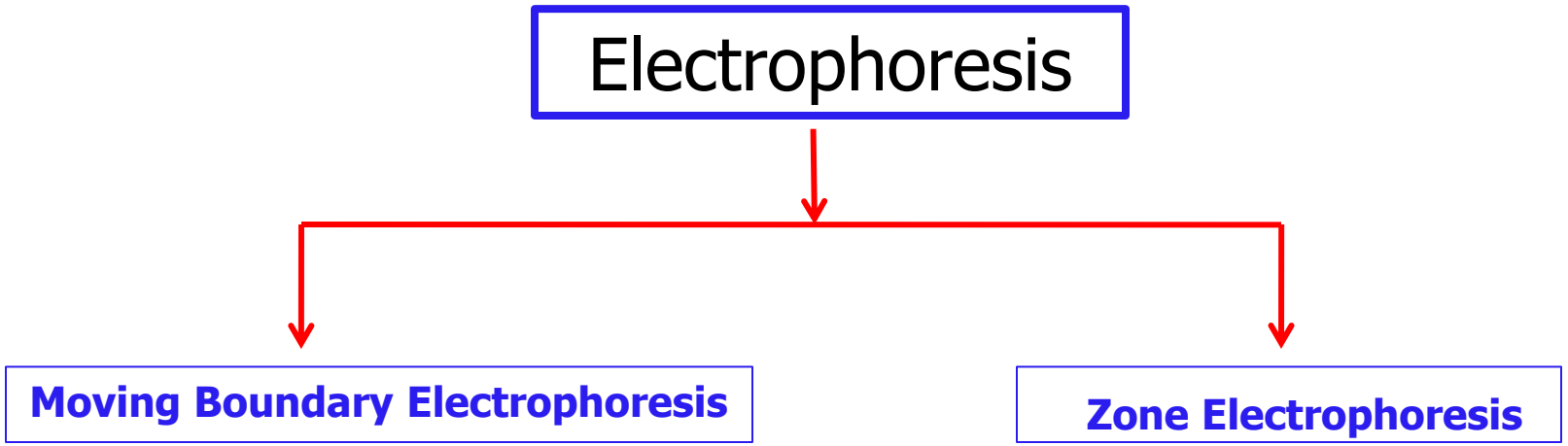
Internal Factors

- **Charge of the molecule**
  ↑ in charge = "faster speed"
- **Size and Shape**
  ↑ in size = "slower speed"

External Factors

- **Voltage & Temperature**
  ↑ in voltage & temperature
  = ↑ speed =↑ heat and
  leads to Protein denaturation
- **Buffer pH**
  pH determines net charge of the protein, hence direction of migration.
- **Supporting medium**
  - Protein interaction slows speed

Types of Electrophoresis



- **Capillary Electrophoresis (CE)**

  Used to separate:

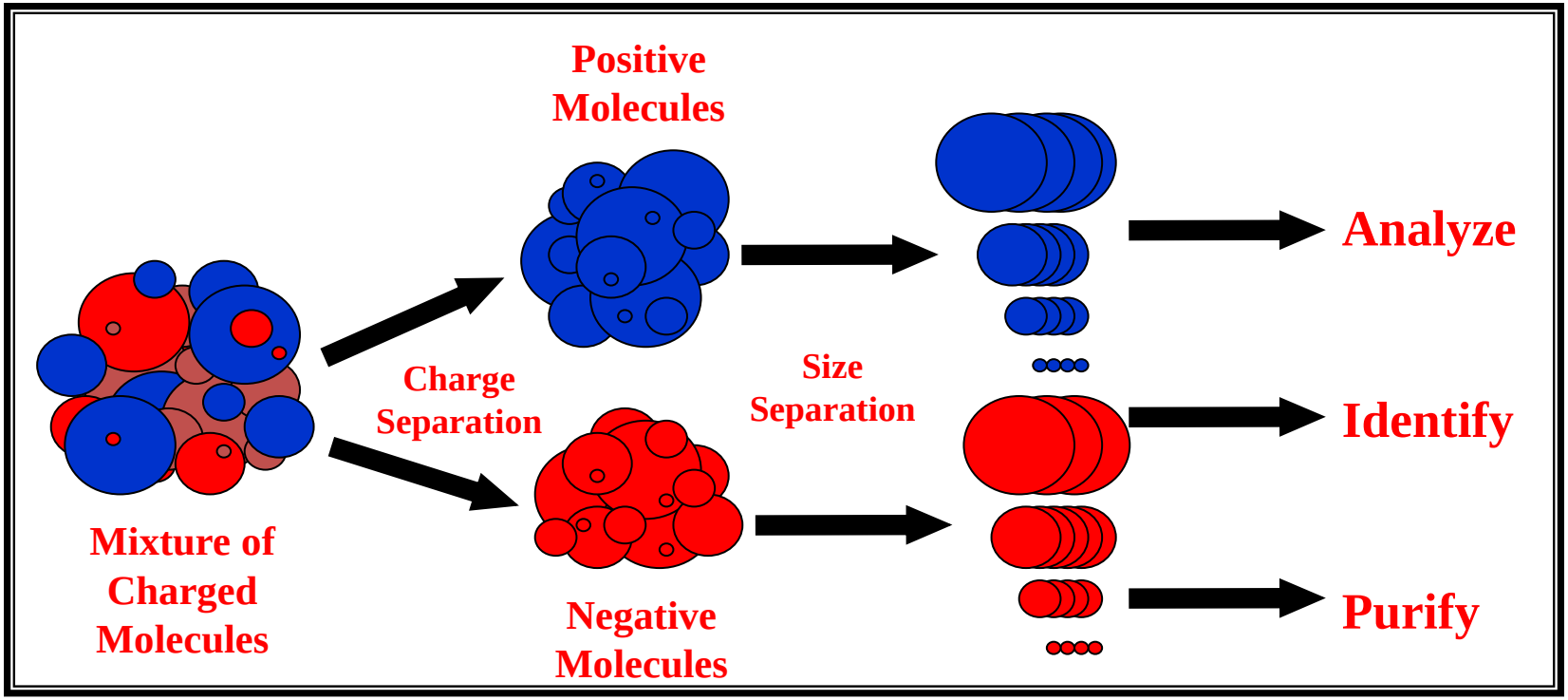
  - ✓Proteins
  - ✓Peptides & Amino acids
  - ✓Inorganic ions
  - ✓Organic bases & acids
  - ✓Whole cells
  - ✓Nucleic acids

- **Paper Electrophoresis**
- **Capillary Gel Electrophoresis**
- **Gel Electrophoresis**
  - Agarose gel (DNA & Protein)
  - Polyacrylamide gel (PAGE)
  - SDS-PAGE (Protein)

Gel Electrophoresis

Separation of a mixture of charged molecules

- A thin layer or zone of the macromolecule solution is electrophoresed through solid support matrix **(Gel).**
- Charged molecules are separated based on their charge and size.



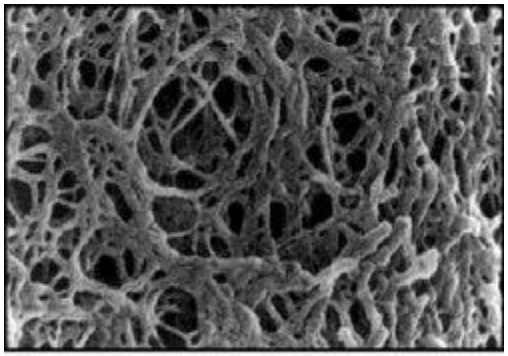
https://slideplayer.com/slide/8432364/26/images/4/Separation+of+a+Mixture+of+Charged+Molecules.jpg

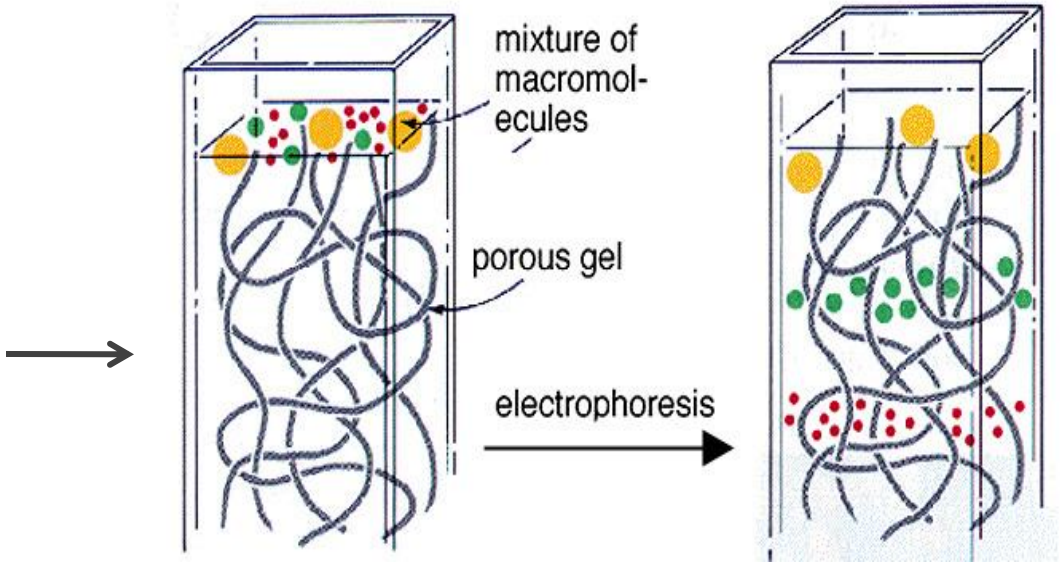
Gel Electrophoresis

Separation based on the Size:

- 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.

SEM photo of 1% Agarose (gel Matrix)





http://www.bioscience-beads.com/underivatized.html

http://stevegallik.org/cellbiologyolm_gelelectrophoresis.html

The property of Gels

- Can be **poured into slabs and columns**, can be drawn into capillaries.
- **Very stable**, allowing for post-separation manipulation.
- **Pore size can also be controlled** for, altering the migration properties of the gel.
- Two forms of gel matrices are used, **cross-linked** and **non-crosslinked.**
- Most common **cross-linked gels** are agarose and acylamide
  - **Agarose** is a reversible matrix cross-linked by **hydrogen bonds**
  - **Acrylamide** is a permanent matrix cross-linked with **methylene bridges**

Gel Electrophoresis

Gel Electrophoresis: Supporting medium is Gel

Gels are composed of polymers of sugars (Agarose or Polyacrylamide)

- **Agarose –** a complex sugar chain from red seaweed.
  - It has a large pore size good for separating large molecules.
- **Polyacrylamide –** chain of acrylamide molecules.
  - It has a small pore size good for separating small molecules.

- The kind of supporting **matrix used depends on the type of molecules** to be separated and on the desired basis for separation: charge, molecular weight, or both.
- Electrophoresis of biological macromolecules is at present carried out on either **polyacrylamide** or **agarose gels**

ELECTROPHORESIS OF DNA

Agarose Gel

- Separates fragments based on mass and charge.
- They have **large pore sizes** and are used for separating **larger DNA molecules** (RFLP Analysis) or RNA separation.
- Typically resolve 200 bp-20 kbp
- Also used to separate **large proteins** and **protein complexes**.

Polyacrylamide (PAGE)

- Used to obtain high resolution separations.
- Used for the separation of smaller DNA molecules (STR analysis and DNA sequence analysis.
- They have **small pore size gel,** is used to separate most proteins and small nucleotides.
- Separates fragments < 200 bp.

Gel Electrophoresis Apparatus and Types

- **Horizontal Gel Units** ("Submarine Gels")
  - Agarose gels
  - Most DNA and RNA gels
- **Vertical Gel Units**
  - Polyacrylamide gels (PAGE)
  - Sequencing gels
- **Pulse Field Gel Units**
  - Any electrophoresis process that uses more than one alternating electric field
  - Agarose
  - Large genomic DNA (Chromosomal)

Agarose Gel Electrophoresis

Preparation of Agarose gel
Melt, cool and add Ethidium bromide. Mix thoroughly.

↓

Pore into casting tray with comb and allow it to solidify

↓

Add running buffer. Load samples and DNA markers

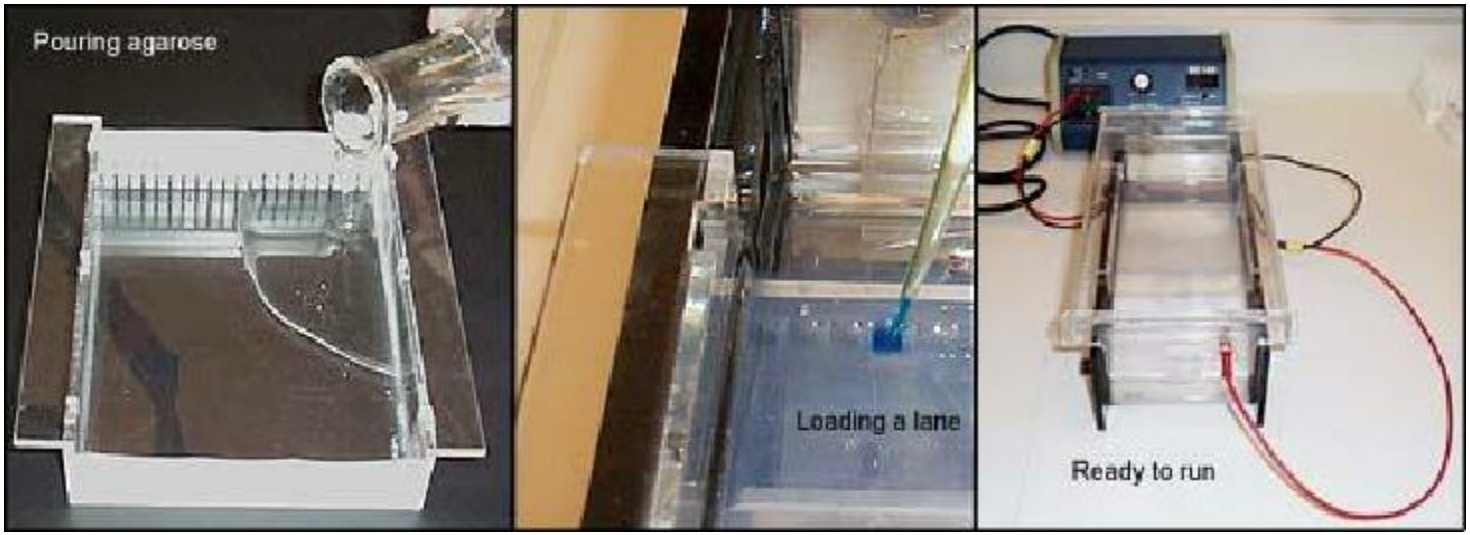
↓

Run gel at constant voltage until band separation occurs

↓

Observe the separated DNA bands in a UV chamber

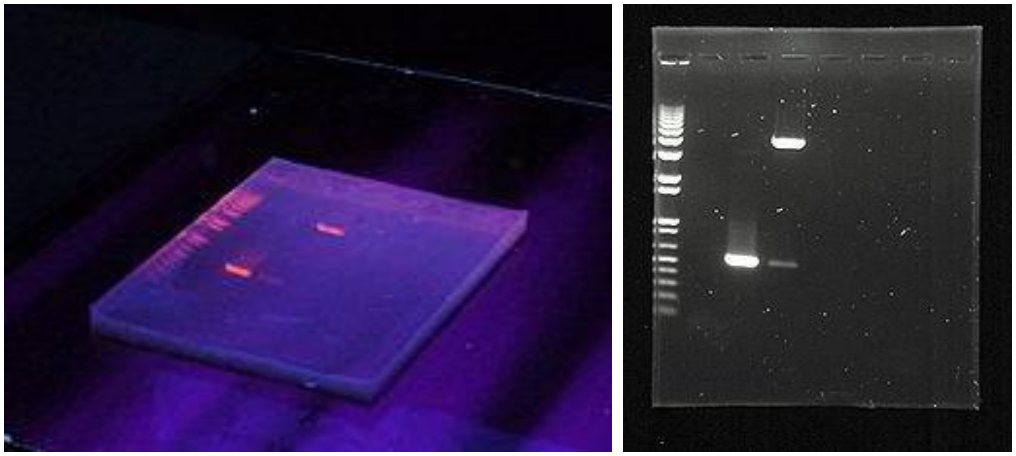
Preparation, Loading and Running of Gel



https://www.researchgate.net/figure/Preparation-loading-and-running-of-gel-in-electrophoresis_fig1_224829868

Image of the Gel

The Gel with UV illumination



https://en.wikipedia.org/wiki/Gel_electrophoresis_of_nucleic_acids

Electrophoresis - Applications

- 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

Electrophoresis - Applications

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