

Electrophoresis

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INTRODUCTION:

Electrophoresis is a physical method of analysis which involves separation of the compounds that are capable of acquiring electric charge in conducting electrodes.

DEFINITION:

Electrophoresis may be defined as the **migration of the charged particle through a solution under the influence of an external electrical field.**

Ions that are suspended between two electrodes tends to travel towards the electrodes that bears opposite charges.



Father of Electrophoresis

Arne Tiselius

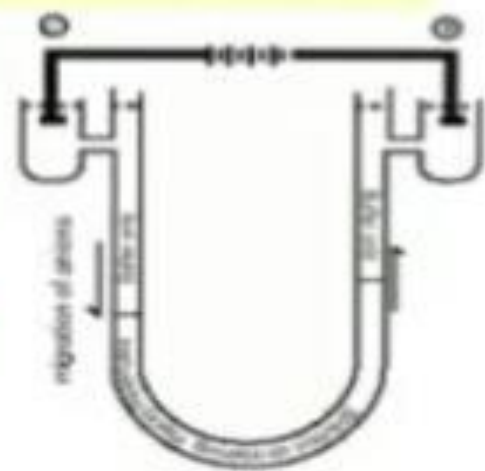
(Sweden, 1902-1971)

Wallach's Interpretation of Diagnostic Tests
The Nobel Prize in Chemistry 1948

*"for his research on **electrophoresis** and **adsorption** analysis, especially for his discoveries concerning the **complex nature of the serum proteins**"*

This type of cell is essentially a bent glass tube with electrolyte reservoirs containing the cathode and anode, and a buffer containing the macromolecules that need electrophoresed.

He tested **horse serum** in the apparatus and found **4 distinct bands** consisting of **albumin** and **3 globulin** components, which he named " **α** ," " **β** ," and " **γ** ."



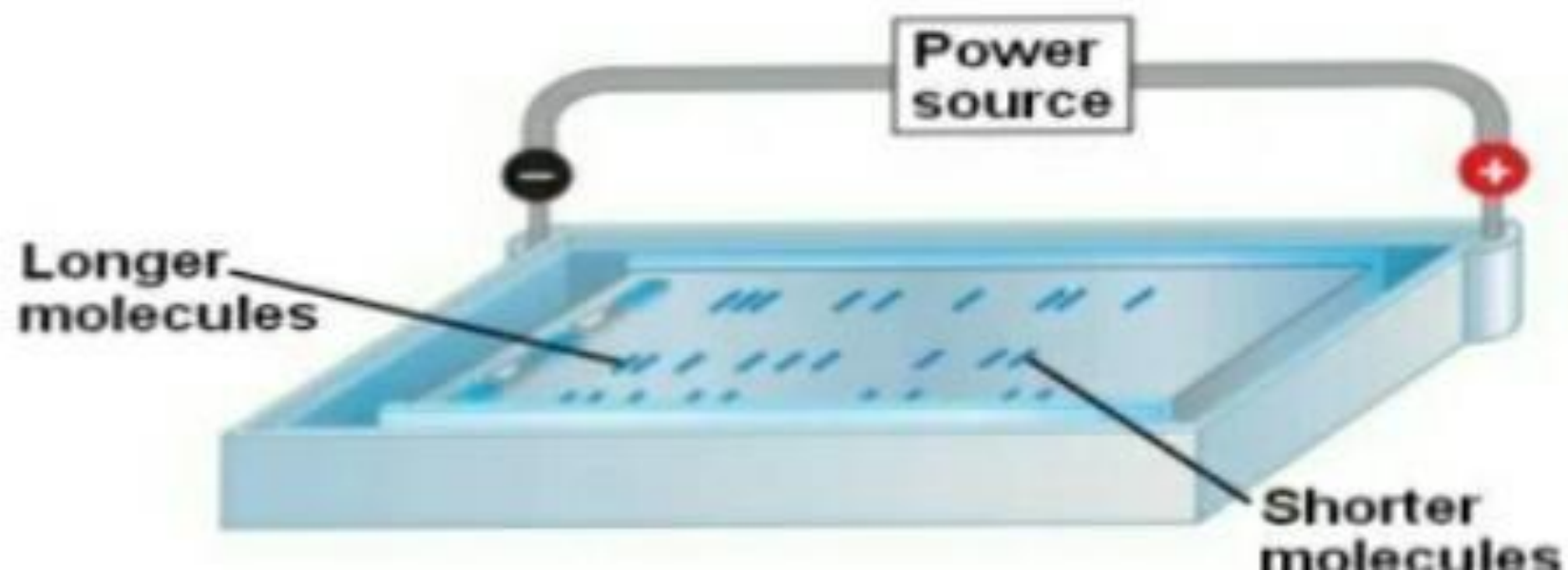
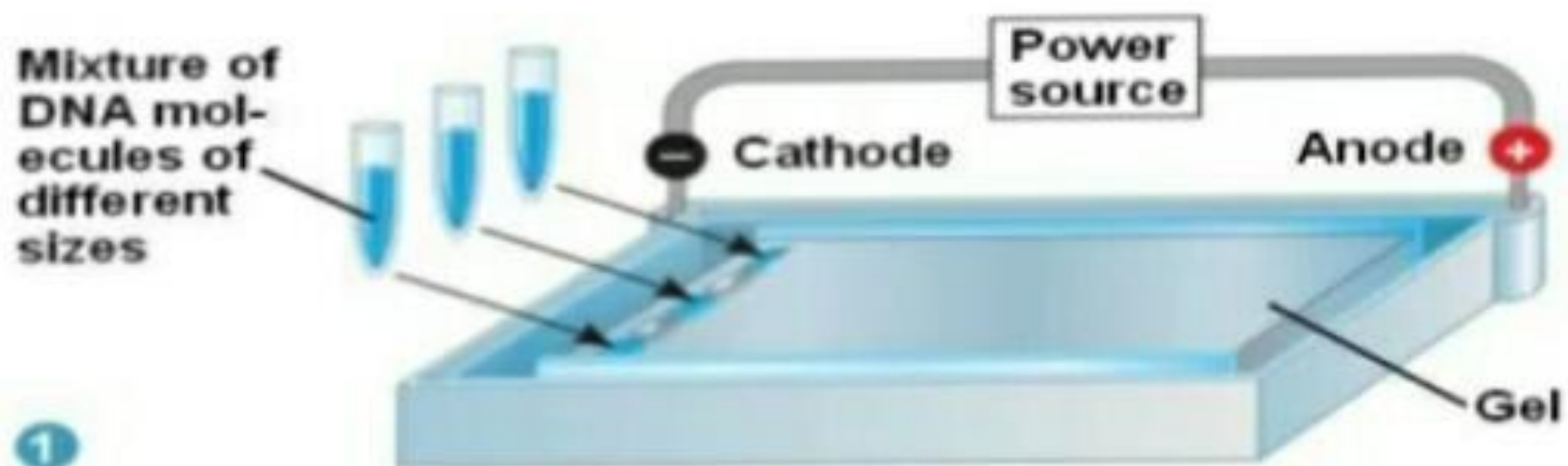
Principle :

- Comprehensive term that refers to the migration of charged particle of any size in liquid medium under the influence of an electric field.
- Depending on kind of charge the molecule carry, they move towards either
 - To cathode
 - Or to Anode
- An ampholyte become positively charged in acidic condition and migrate to cathode, in alkaline condition they become negatively charge and migrate to anode.

- Eg: as protein contain the ionizable amino and carboxyl group.
- The rate of migration of an ion in electrical field depend on factors,
 1. Net charge of molecule
 2. Size and shape of particle
 3. Strength of electrical field
 4. Properties of supporting medium
 5. Temperature of operation

1. Mobility

- Under the electrical field, the mobility of the particle is determined by two factors:
 - Its charge
 - Frictional coefficient
- Size and shape of the particle decide the velocity with which the particle will migrate under the given electrical field and the medium.

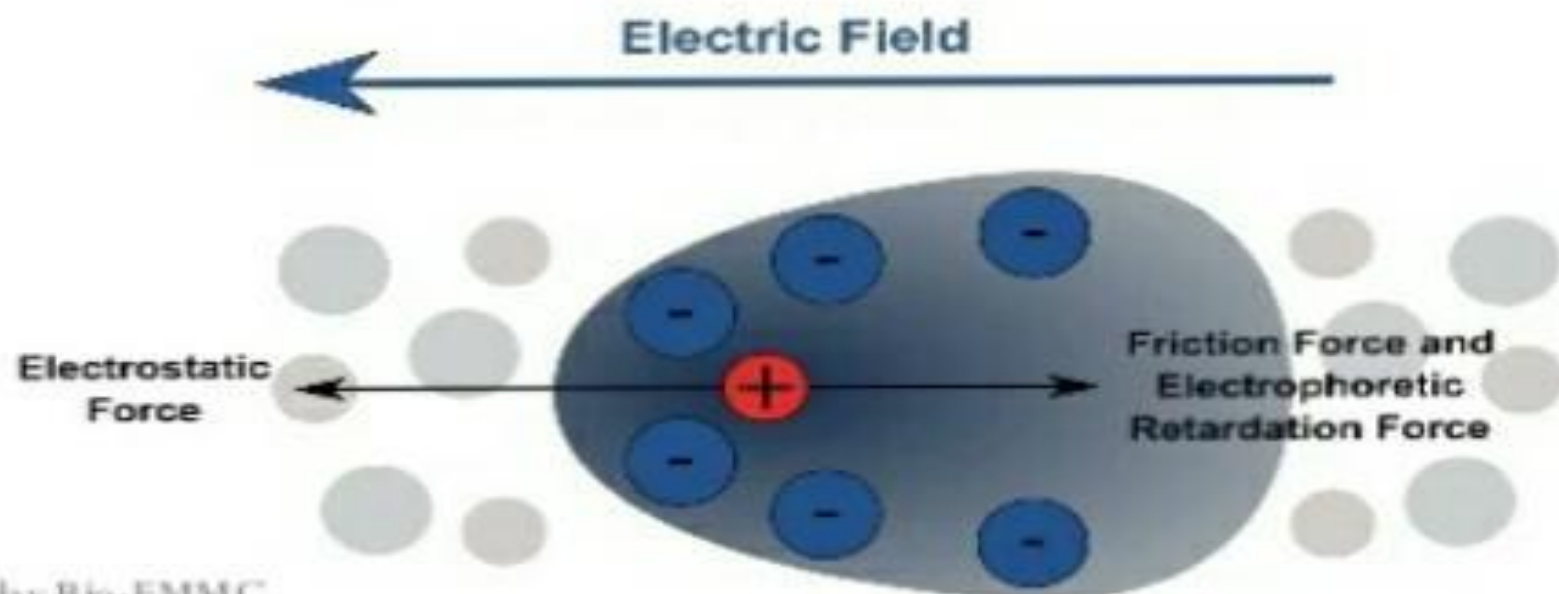


2. Strength of electrical field

- It determined by the force exerted on the particle, and the charge the particle carrying.

$$F=QV$$

when force is exerted on the particle it start moving, however the moment is restricted by the experience of the frictional force because of the viscosity.



Effect of pH on Mobility

- As the molecule exist as amphoteric , they will carry the charges based on the solvent pH.
- Their overall net charge is NEUTRAL when it is at zwitter ion state. And hence the mobility is retarded to zero.
- Mobility is directly proportional to the magnitude of the charge, which is functional of the pH of solvent.
- The pH is maintained by the use of Buffers of different pH.

TYPES OF ELECTROPHORESIS

Zone Electrophoresis

- a) Paper Electrophoresis
- b) Gel Electrophoresis
- c) Thin Layer Electrophoresis
- d) Cellulose acetate Electrophoresis

Moving Boundary Electrophoresis

- a) Capillary Electrophoresis
- b) Isotachopheresis
- c) Isoelectric Focusing
- d) Immuno Electrophoresis

Factors Affecting Electrophoresis

Electrophoretic velocity depends on:

Inherent Factors

- Magnitude of its charge
- Charge density
- Molecular weight
- Tertiary or quaternary structure (i.e., its shape).

External Environment

- Solution pH
- Electric field
- Solution viscosity
- Temperature

Clinical applications of Electrophoresis

- Serum Protein Electrophoresis
- Lipoprotein Analysis
- Diagnosis of Haemoglobinopathies and Haemoglobin A1c
- Determination of Serum Protein Phenotypes and Micro heterogeneities eg. α 1- antitrypsin deficiency, MM
- Genotyping of Proteins eg. ApoE analysis for Alzheimer's disease (polymorphic protein)
- Small Molecules (Drugs, Steroids) Monitoring
- Cerebrospinal Fluid Analysis

• Urine Analysis (diagnosis of GN)



THANK YOU

