Electrophoresis

For SEM IV

INTRODUCTION

- Electrophoresis is the migration of charged particles or molecules in a medium under the influence of an applied electric field.

Father of Electrophoresis

Arne Tiselius

(Sweden, 1902-1971)

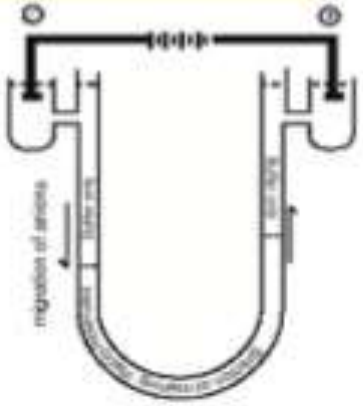
Wallach's Interpretation of Diagnostic Tests

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."



Electrophoresis

- a separation technique
- Simple, rapid and highly sensitive
- used in clinical laboratories to separate charged molecules from each other in presence of electric field

- – Proteins in body fluids: serum, urine, CSF
- – Proteins in erythrocytes: hemoglobin
- – Nucleic acids: DNA, RNA

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.

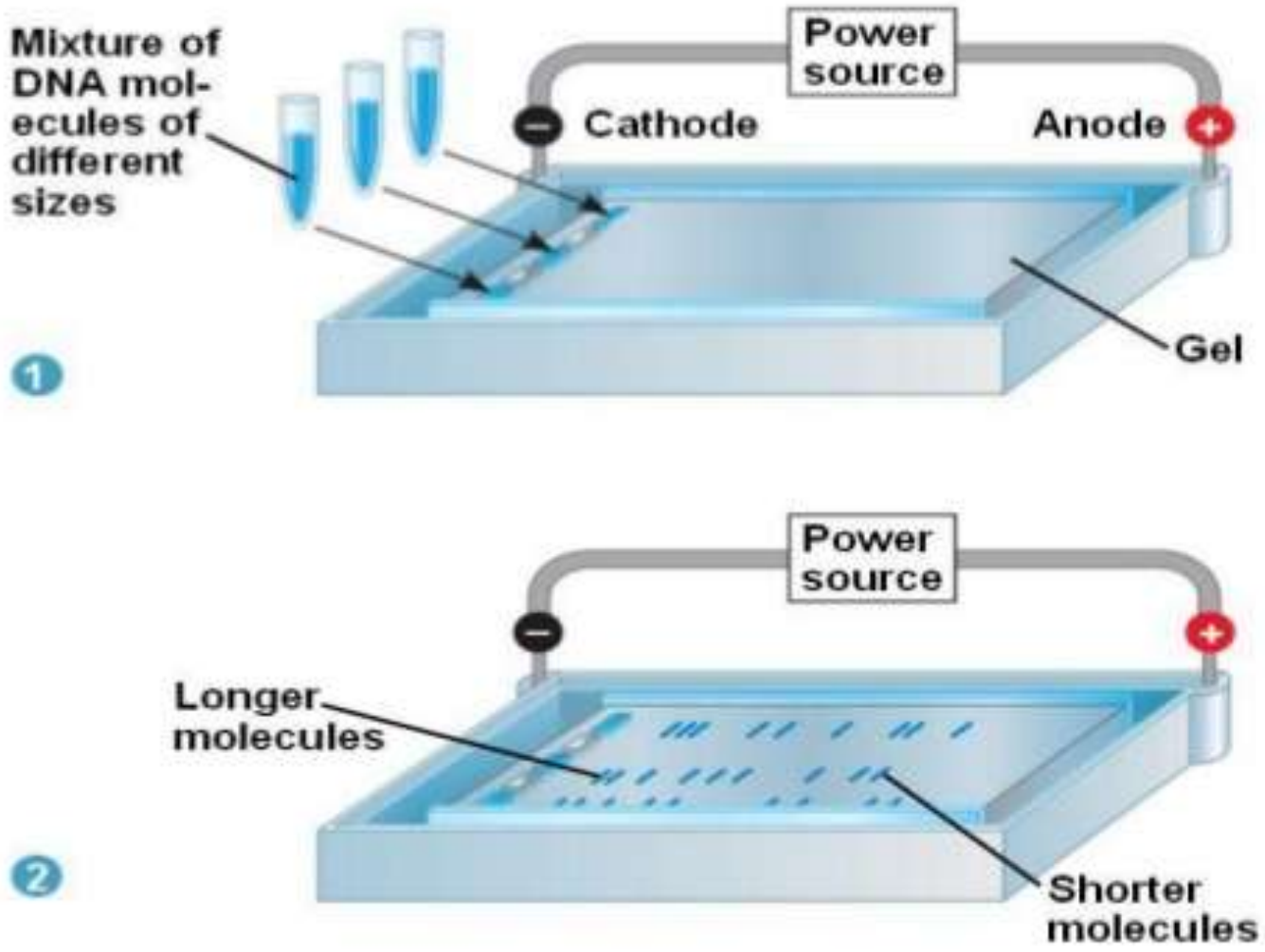
- Eg: as protein contain the ionizable amino and carboxyl group.

- 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.

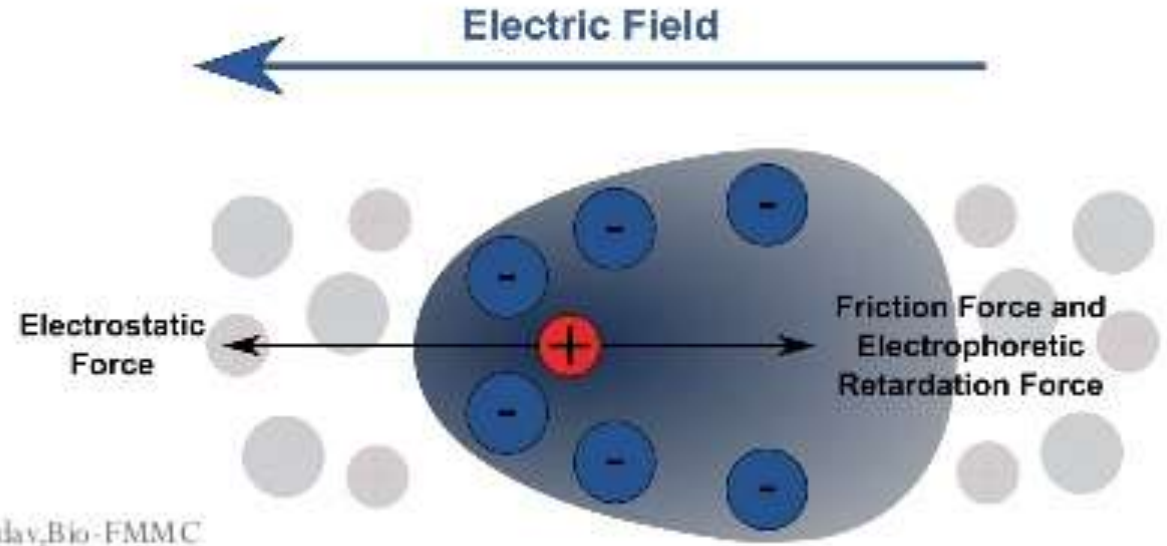

Mixture of DNA mol-ecules of different sizes
Power source
Cathode
Anode
Gel
1
Power source
Longer molecules
Shorter molecules
2

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.

Factors Affecting Electrophoresis

Electrophoretic velocity depends on:

Inherent Factors

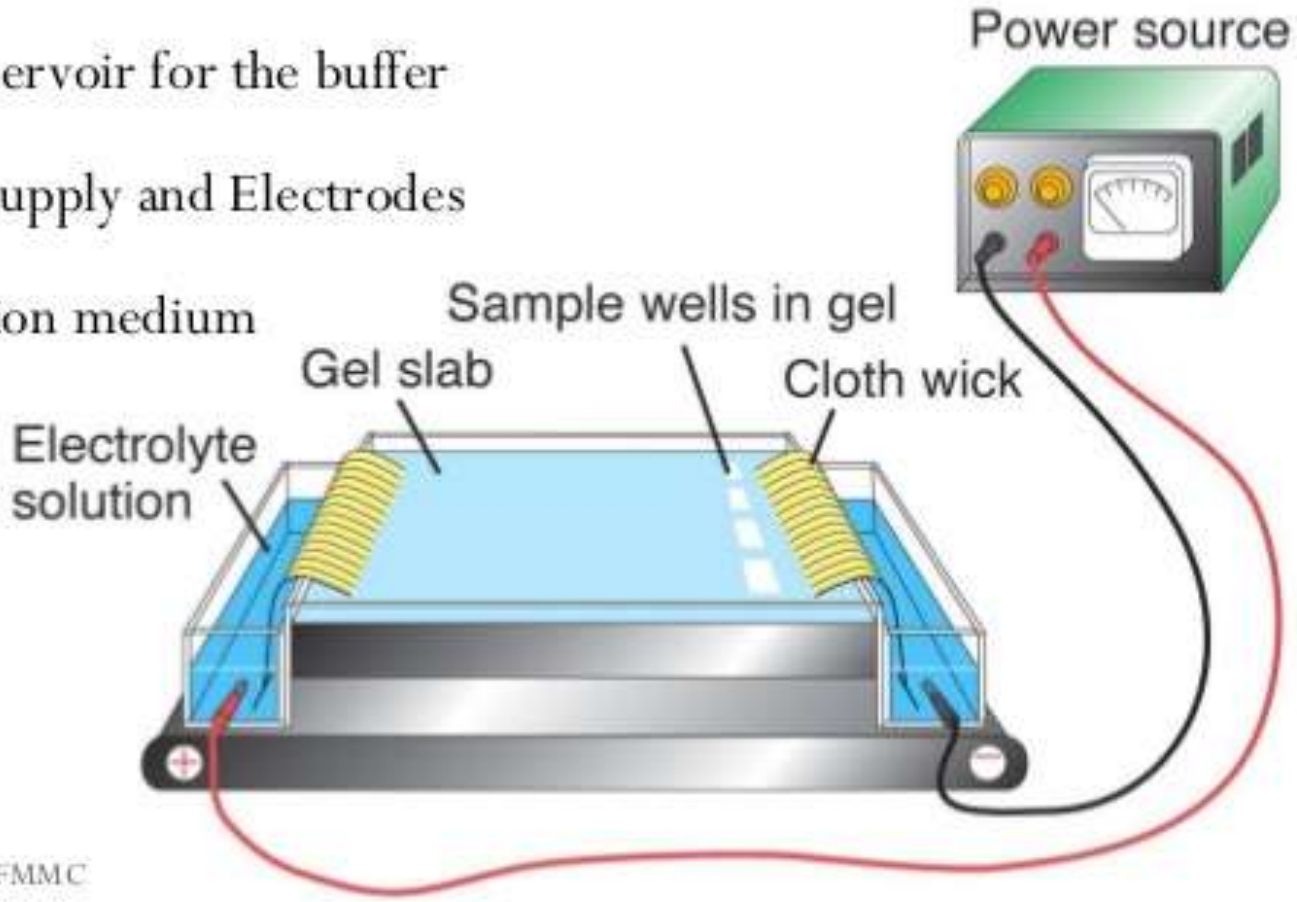
- Magnitude of its charge
- Charge density
- Molecular weight
- Tertiary or quaternary structure (i.e., its shape).

External Environment

- Solution pH
- Electric field
- Solution viscosity
- Temperature

Conventional electrophoresis

- Instrumentation :
  - Two reservoir for the buffer
  - Power supply and Electrodes
  - Separation medium



Commonly buffers used;

Buffer	pH value
Phosphate buffer	around 7.0
Tris-Borate-EDTA buffer (TBE)	around 8.0
Tris-Acetate EDTA buffer (TAE)	above 8.0
Tris Glycine buffer (TG)	more than 8.5
Tris -Citrate-EDTA buffer (TCE)	around 7.0
Tris -EDTA buffer (TE)	around 8.0
Tris -Maleic acid -EDTA buffer (TME)	around 7.5
Lithium Borate – buffer (LB)	around 8.6

Supporting medium

- Supporting medium is an matrix in which the protein separation takes place.
- Various type has been used for the separation either on slab or capillary form.
- Separation is based on to the charge to mass ratio of protein depending on the pore size of the medium, possibly the molecular size.

Properties:

Chemical nature	inert
Availability	easy
Electrical conductivity	high
Adsorptivity	low
Sieving effect	desirable
Porosity	controlled
Transparency	high
Electro-endosmosis (EEO)	low
Rigidity	moderate to high
Preservation	feasible
Toxicity	low
Preparation	easy

- Starch gel
- Cellulose acetate
- Agarose
- Polyacrylamide gel

Agarose Gel

- A linear polysaccharide (made-up of repeat unit of agarobiose-alternating unit of galactose and 3,6-anhydrogalactose).
- Used in conc as 1% and 3%.
- The gelling property are attributed to both inter- and intramolecular hydrogen bonding
- Pore size is controlled by the % of agarose used.
- Large pore size are formed with lower conc and vice versa.
- Purity of the agarose is based on the number of sulphate conc, lower the conc of sulphate higher is the purity of agarose.

ADVANTAGES:

- Easy to prepare and small concentration of agar is required.
- Resolution is superior to that of filter paper.
- Large quantities of proteins can be separated and recovered.
- Adsorption of negatively charged protein molecule is negligible.
- It adsorbs proteins relatively less when compared to other medium.
- Sharp zones are obtained due to less adsorption.
- Recovery of protein is good, good method for preparative purpose.

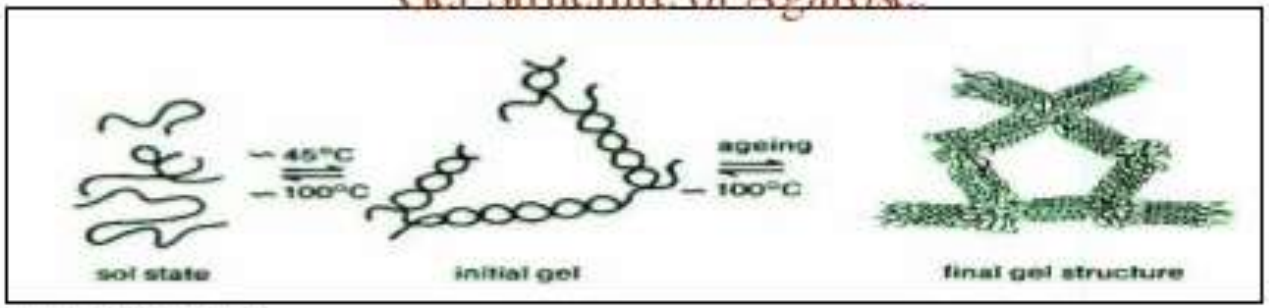
DISADVANTAGES:

- Electro osmosis is high.
- Resolution is less compared to polyacrylamide gels.
- Different sources and batches of agar tend to give different results and purification is often necessary.

APPLICATION:

- Widely used in Immuno electrophoresis.

Gel Structure of Agarose:



Cellulose acetate

- Thermoplastic resin made by treating cellulose with acetic anhydride to acetylate the hydroxyl group.
- When dry, membrane contain about 80% air space within fibers and brittle film.
- As the film is soak in buffer, the space are filled.
- Because of their opacity, the film has to be made transparent by soaking in 95:5 methanol:glacial acetic acid.
- It can be stored for longer duration.

Polyacrylamide

- Frequently referred to as PAGE.
- Cross-linked polyacrylamide gel are formed from the polymerization of the monomer in presence of small amount of N,N"-methylene-bisacrylamide.
- Bisacrylamide – two acrylamide linked by the methylene group.
- The polymerization of the acrylamide is an example for free radical catalysis.
- They are defined in terms of total percentage of acrylamide present, and pore size vary with conc.

- Made in conc between 3-30% acrylamide.
- Thus low % has large pore size and vice versa.

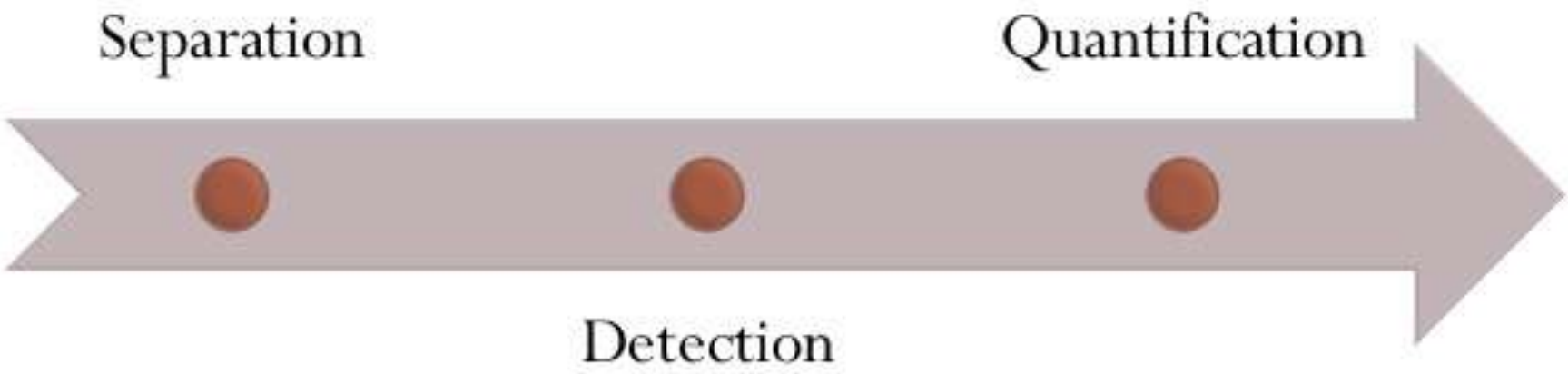
Proteins are separated on the basis of charge to mass ratio and molecular size, a phenomenon called Molecular sieving.

ADVANTAGES:

- Gels are stable over wide range of pH and temperature.
- Gels of different pore size can be formed.
- Simple and separation speed is good comparatively.

General Operation

- The general operation of the conventional electrophoresis include;



TYPES OF ELECTROPHORESIS

1) Zone Electrophoresis

a) Paper Electrophoresis

b) Gel Electrophoresis

c) Thin Layer Electrophoresis

d) Cellulose acetate Electrophoresis

2) Moving Boundary Electrophoresis

a) Capillary Electrophoresis

b) Isotachophoresis

c) Isoelectric Focussing

d) Immuno Electrophoresis

CLASSIFICATION

Slab gel electrophoresis	• Traditional methods, using a rectangular gel regardless of thickness
Disc electrophoresis	• DISContinuities in electrophoretic matrix caused by layers of polyacrylamide/starch gel that differ in composition & pore size

CLASSIFICATION

Isoelectric focusing electrophoresis	• IEF separates amphoteric compounds, such as proteins, with increased resolution in a medium possessing a stable pH gradient
Isotachophoresis	• Completely separates smaller ionic substances into adjacent zones tat contact one another with no overlap & all migrate at the same rate.

CLASSIFICATION

Pulse-Field electrophoresis

- Power is alternately applied to different pair of electrodes/ electrode arrays, so the electrophoretic field is cycled b/w 2 directions.

2-D electrophoresis

- Charge-dependent IEP in the first dimension.
- Molecular weight dependent electrophoresis in second.

SUPPORT MEDIA IN SEPERATION

Molecular size

- Gradient gels
- Gels containing denaturants

Molecular size & Charge

- Gel electrophoresis
- Immunoelectrophoresis
- 2D electrophoresis

Clinical applications of Electrophoresis

- Serum Protein Electrophoresis
- Lipoprotein Analysis
- Diagnosis of Haemoglobinopathies and Haemoglobin A1c
- Determination of Serum Protein Phenotypes and Micro heterogeneities eg. α1- 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 (determination of GNs)