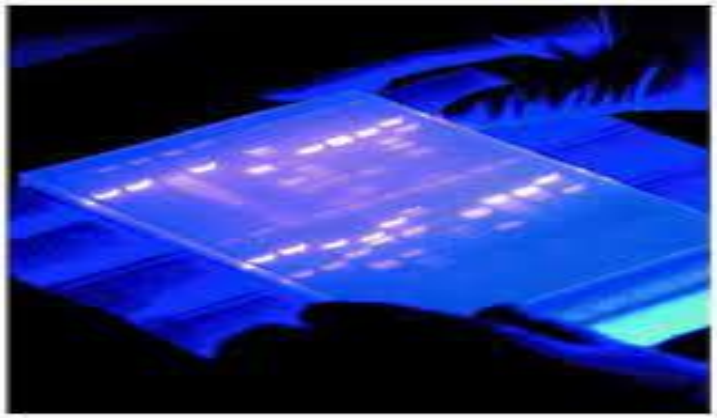
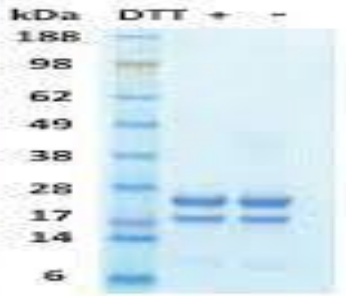




Electrophoresis principles

Post graduate MS. Course
Lecture-1
Dr.Najwa

INTRODUCTION

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

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.

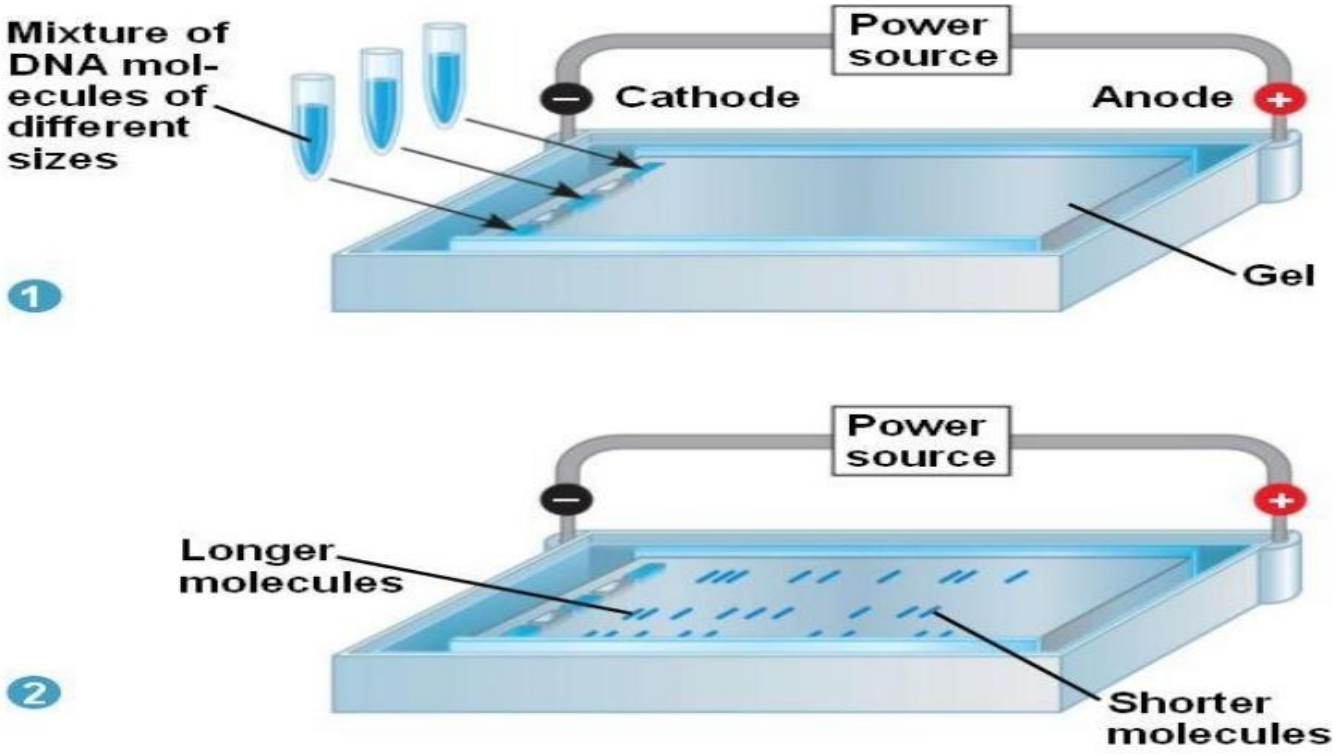
- Eg: as protein contain the ionizable amino and carboxyl group.

- 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.

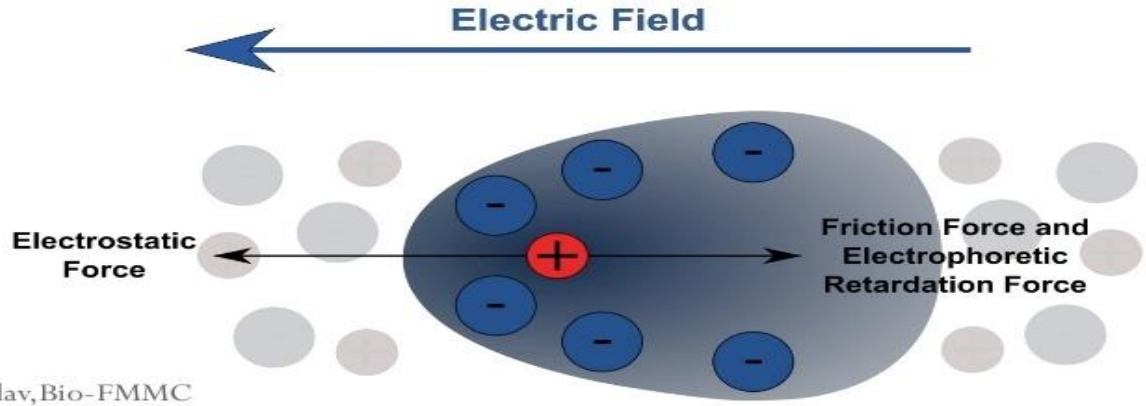

Mixture of DNA mol-ecules of different sizes
Power source
Cathode
Anode
Gel
1
Power source
Longer molecules
Shorter molecules
2

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

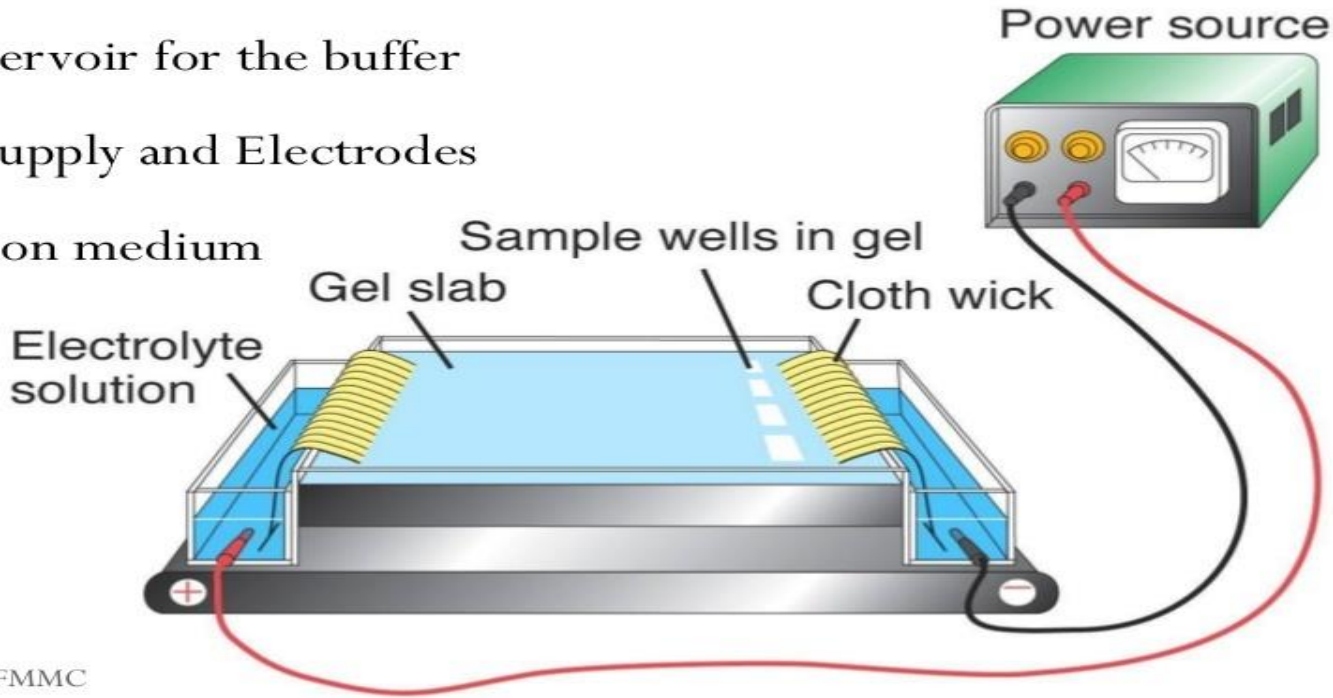
- Magnitude of its charge
- Charge density
- Molecular weight
- Tertiary or quaternary structure (i.e., its shape).

External Environment

- Solution pH
- Electric field
- Solution viscosity
- Temperature

Conventional electrophoresis

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



Power supply

- Drives the moment of ionic species in the medium and allow the adjustment and control of the current or voltage.
- Constant delivery is required.
- Pulsed power can also be applied.

Buffer

- The buffer in electrophoresis has twofold purpose:
  - Carry applied electrical current
  - They set the pH as which electrophoresis is carried out.
- Thus they determine;
  - Type of charge on solute.
  - Extent of ionization of solute
  - Electrode towards which the solute will migrate.
- The buffer ionic strength will determine the thickness of the ionic cloud.

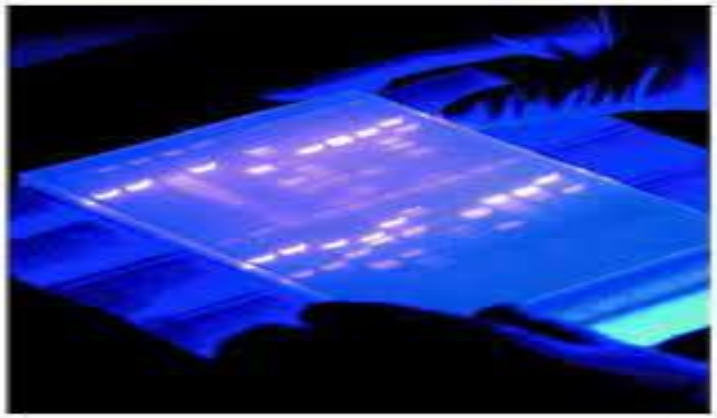
Commonly buffers used;

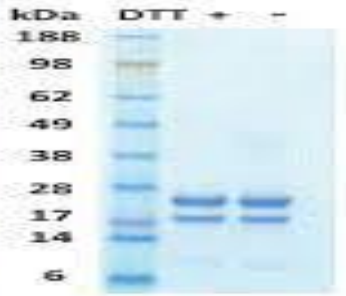
Buffer	pH value
Phosphate buffer	around 7.0
Tris-Borate-EDTA buffer (TBE)	around 8.0
Tris-Acetate EDTA buffer (TAE)	above 8.0
Tris Glycine buffer (TG)	more than 8.5
Tris -Citrate-EDTA buffer (TCE)	around 7.0
Tris -EDTA buffer (TE)	around 8.0
Tris -Maleic acid -EDTA buffer (TME)	around 7.5
Lithium Borate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

- Starch gel
- Cellulose acetate
- Agarose
- Polyacrylamide gel





Electrophoresis methods

Post graduate MS. Course
Lecture-2
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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.

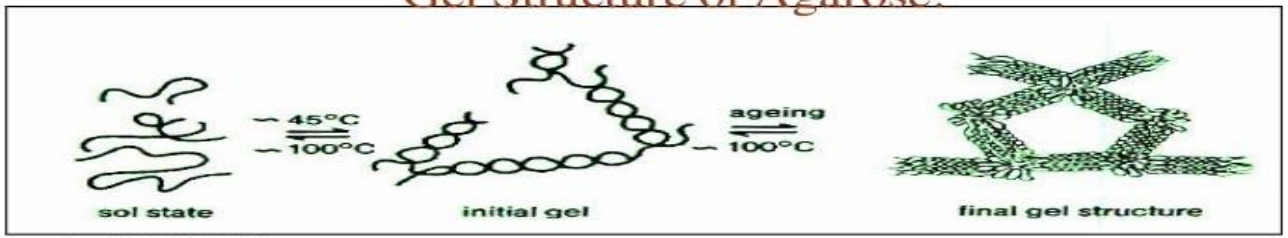
DISADVANTAGES:

- 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:

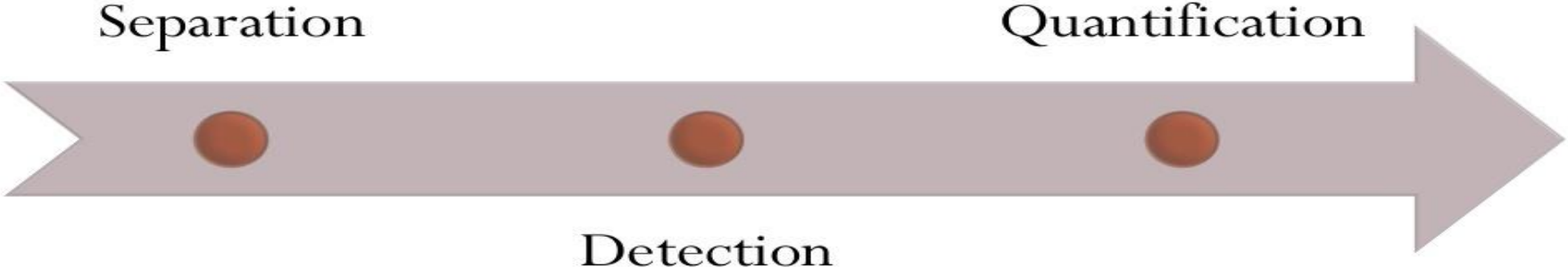
- Widely used in Immuno electrophoresis.

Gel Structure of Agarose:



General Operation

- The general operation of the conventional electrophoresis include;



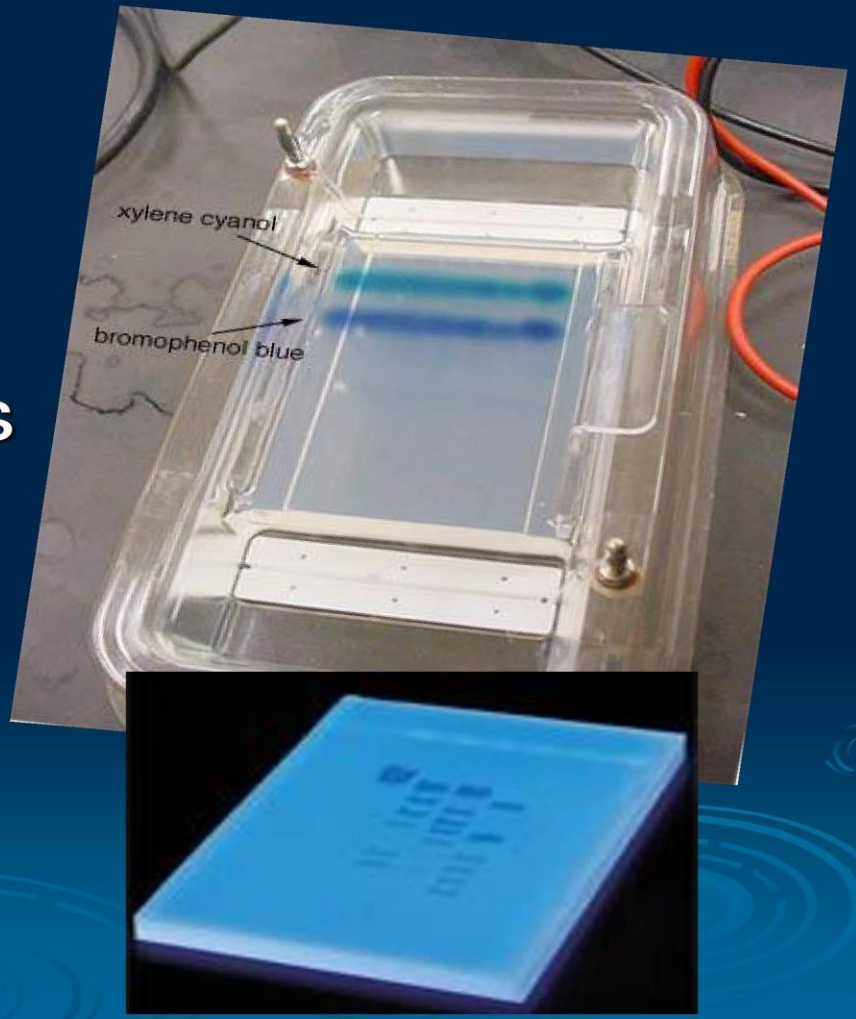
a. Electrophoresis Separation

- When performed on precast or agarose gel, following steps are followed;

- Excess buffer removed
- 5-7 µL sample
- Placed in electrode chamber
- Current application
- Gel is rinsed, fixed and dried
- Stained
- Scanned under densitometry

DNA Staining

- Allows DNA visualization after gel electrophoresis
- Ethidium Bromide
- Bio-Safe DNA stains
  - In gel staining



Common effect of variables on separation

pH	Changes charge of analyte, effective mobility; structure of analyte-denaturing or dissociating a protein.
Ionic strength	Changes in voltage; increased ionic strength reduces migration velocity and increase heating.
Ions present	Change migration speed; cause tailing of bands.
Current	Too high current cause overheating.
Temperature	Overheating cause denature protein; lower temp reduce diffusion but also migration; there is no effect on resolution.
Time	Separation of bands increases linearly with time, but dilution of bands increase with square root of time.
Medium	Major factors are endosmosis and pore size effect, which effect migration velocities.

THANK YOU