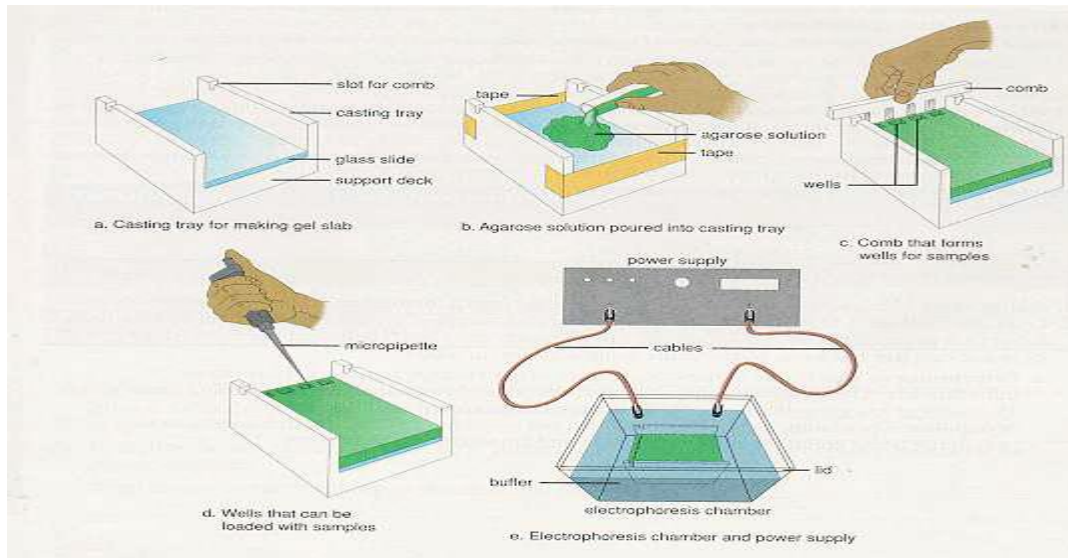


THEORY OF ELECTROPHORESIS

GEL ELECTROPHORESIS

- Separation is brought about through molecular sieving technique, based on the molecular size of the substances. Gel material acts as a "molecular sieve".
- Gel is a colloid in a solid form (99% is water).
- It is important that the support media is electrically neutral.
- Different types of gels which can be used are; Agar and Agarose gel, Starch, Sephadex, Polyacrylamide gels.
- A porous gel acts as a sieve by retarding or, in some cases, by completely obstructing the movement of macromolecules while allowing smaller molecules to migrate freely.



AGAR AND AGAROSE GEL

- Agar is a mixture of poly saccharides extracted from sea weeds.
- Agarose is a highly purified uncharged polysaccharide derived from agar.
- Agarose is chemically basic disaccharide repeating units of 3,6-anhydro-L-galactose.
- Agarose dissolves when added to boiling liquid. It remains in a liquid state until the temperature is lowered to about 40° C at which point it gels.
- The pore size may be predetermined by adjusting the concentration of agarose in the gel.
- Agarose gels are fragile. They are actually hydrocolloids, and they are held together by the formation of weak hydrogen and hydrophobic bonds.

- The pores of an agarose gel are large, agarose is used to separate macromolecules such as nucleic acids, large proteins and protein complexes.

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.

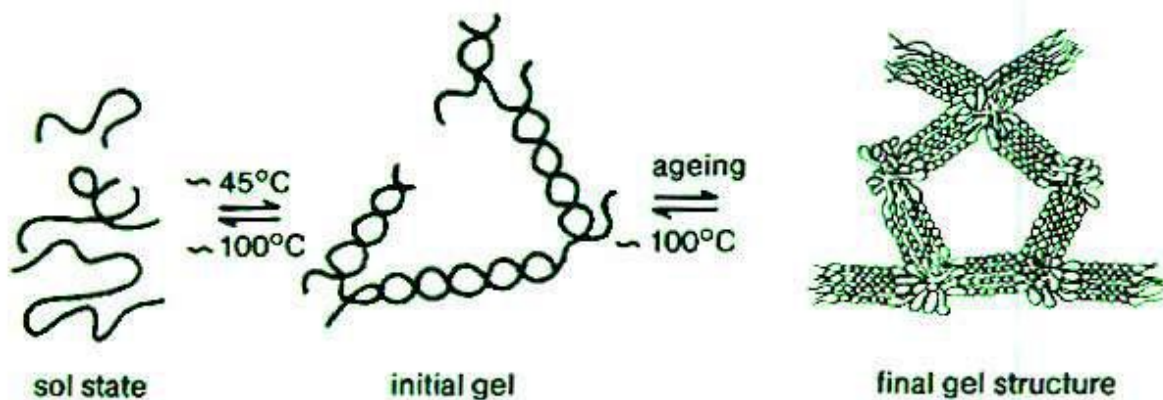
Disadvantages:

- 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:



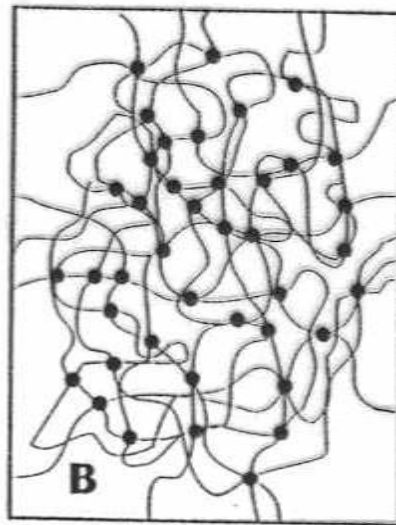
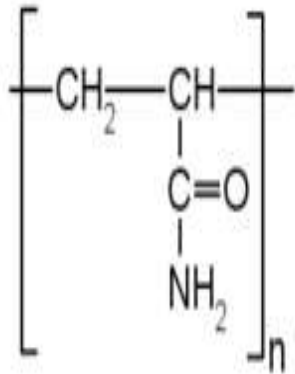
POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

It is prepared by polymerizing acryl amide monomers in the presence of methylene-bis-acrylamide to cross link the monomers.

- Structure of acrylamide ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$)
- Polyacrylamide gel structure held together by covalent cross-links.
- Polyacrylamide gels are tougher than agarose gels.
- It is thermostable, transparent, strong and relatively chemically inert.
- Gels are uncharged and are prepared in a variety of pore sizes.
- 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.



Types of PAGE

PAGE can be classified according the separation conditions into:

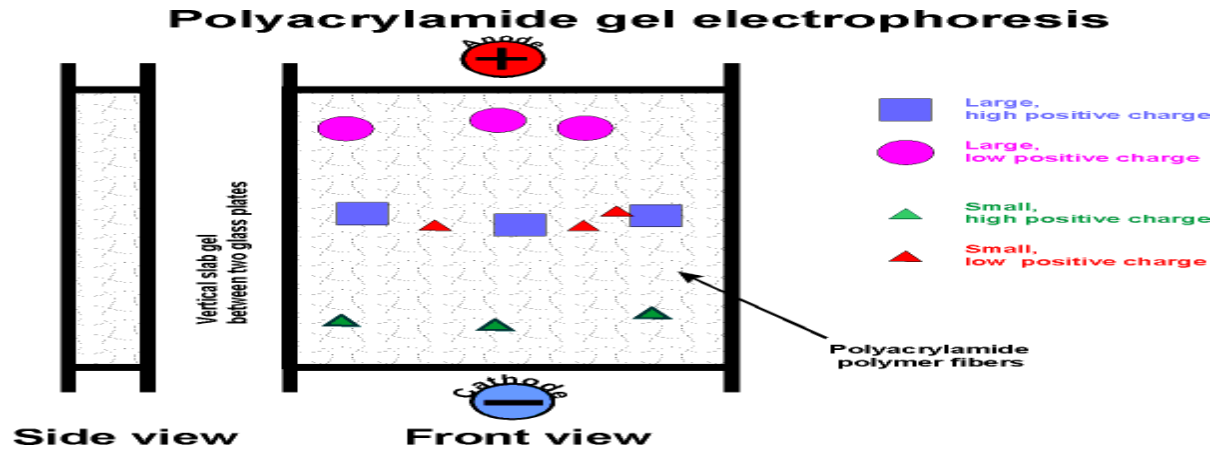
Native-PAGE:

- Native gels are run in non-denaturing conditions, so that the analyte's natural structure is maintained.
- Separation is based upon charge, size, and shape of macromolecules.
- Useful for separation or purification of mixture of proteins.
- This was the original mode of electrophoresis.

Denatured-PAGE or SDS-PAGE:

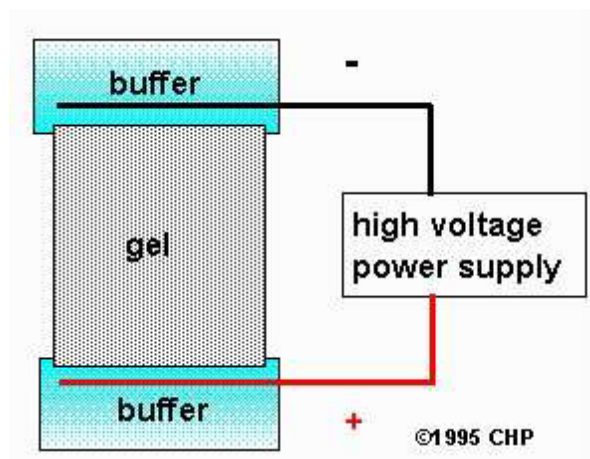
- Separation is based upon the molecular weight of proteins.

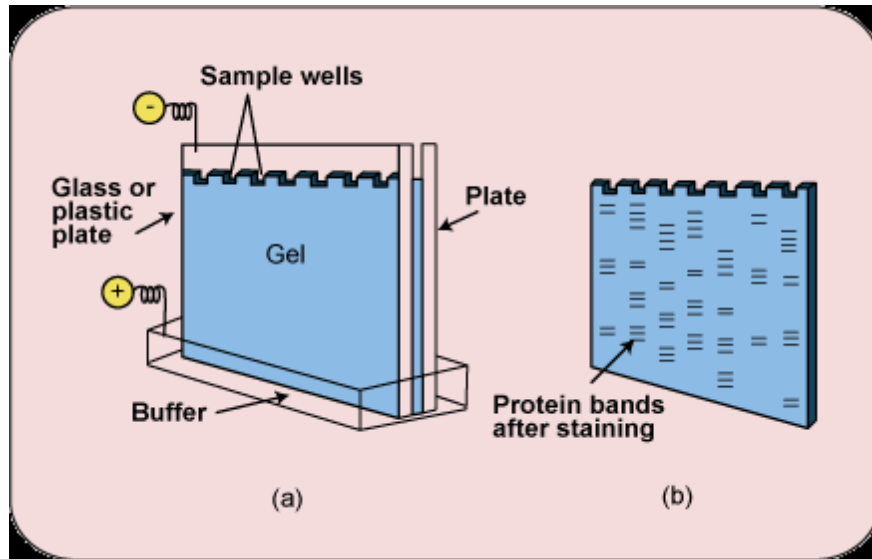
- The common method for determining MW of proteins.
- Very useful for checking purity of protein samples.



PAGE-Procedure

- The gel of different pore sizes is cast into a column inside a vertical tube, often with large pore gel at the top and small pore gel at the bottom.
- Microgram quantity of the sample is placed over the top of the gel column and covered by a buffer solution having such a pH so as to change sample components into anions.
- The foot of the gel column is made to dip in the same buffer in the bottom reservoir.
- Cathode and anode are kept above and below the column to impose an electric field through the column.
- Macromolecular anions move towards the anode down the gel column.
- There is no external solvent space, all the migratory particles have to pass through the gel pores.
- Rate of migration depends on the charge to mass ratio.
- Different sample components get separated into discrete migratory bands along the gel column on the basis of electrophoretic mobility and gel filtration effect.





Polyacrylamide Gel Electrophoresis (PAGE)

- a) The gel is poured vertically between two glass plates.
- b.) Protein bands are separated on the basis of relative molecular weight and visualized with stains.

SLAB PAGE

- The Polyacrylamide gel is cast as thin rectangular slab inside a plastic frame and this slab is placed vertically on a buffer solution taken in a reservoir.
- Several samples dissolved in dense sucrose solution or glycerol are placed in separate wells cut in to the upper edge of the slab and are covered by the same buffer solution. Cathode and anode are above and below to produce electric field effect. Different components migrate simultaneously down parallel lanes in the slab and get separated into bands.

Visualization:

- After the electrophoresis is complete, the molecules in the gel can be stained to make them visible.
- Ethidium bromide, silver, or coomassie blue dye may be used for this process.
- If the analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

- SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility.
- When a detergent SDS added to PAGE the combined procedure is termed as SDS PAGE.
- SDS coats protein molecules giving all proteins a constant charge-mass ratio.
- Due to masking of charges of proteins by the large negative charge on SDS binding with them, the proteins migrate along the gel in order of increasing sizes or molecular weights.
- SDS is an anionic detergent which denatures secondary and non-disulfide-linked tertiary structures by wrapping around the polypeptide backbone. In so doing, SDS confers a net negative charge to the polypeptide in proportion to its length.
- Molecules in solution with SDS have a net negative charge within a wide pH range.
- A polypeptide chain binds amounts of SDS in proportion to its relative molecular mass.
- The negative charges on SDS destroy most of the complex structure of proteins, and are strongly attracted toward an anode in an electric field.

Sodium Dodecyl Sulfate (SDS-PAGE)

- Native protein is unfolded by heating in the presence of β -mercaptoethanol and SDS.
- SDS binds to the protein so that it stays in solution and denatures.
- Large polypeptides bind more SDS than small polypeptides, so proteins end up with negative charge in relation to their size.
- When treated with SDS and a reducing agent, the polypeptides become rods of negative charges with equal "charge densities" or charge per unit length.
- Thus, we can separate the proteins based on their mass.

