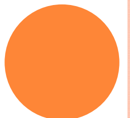
POLY ACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

ELECTROPHORESIS

- Electrophoresis is a separation technique that is based on the movement of charged particles in an electric field.

- The term electrophoresis was coined from a Greek word "Phoresis" which means "Being Carried Away".

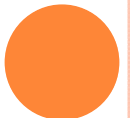
- Hence literal meaning of the word electrophoresis means "to carry with electricity."



PRINCIPLE

- Any charged ion or molecule migrates when placed in an electric field, the rate of migration depend upon its net charge, size, shape and the applied electric current.
- Can be represented by following eq.

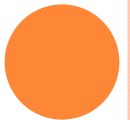
$$V = \frac{E*q}{f}$$



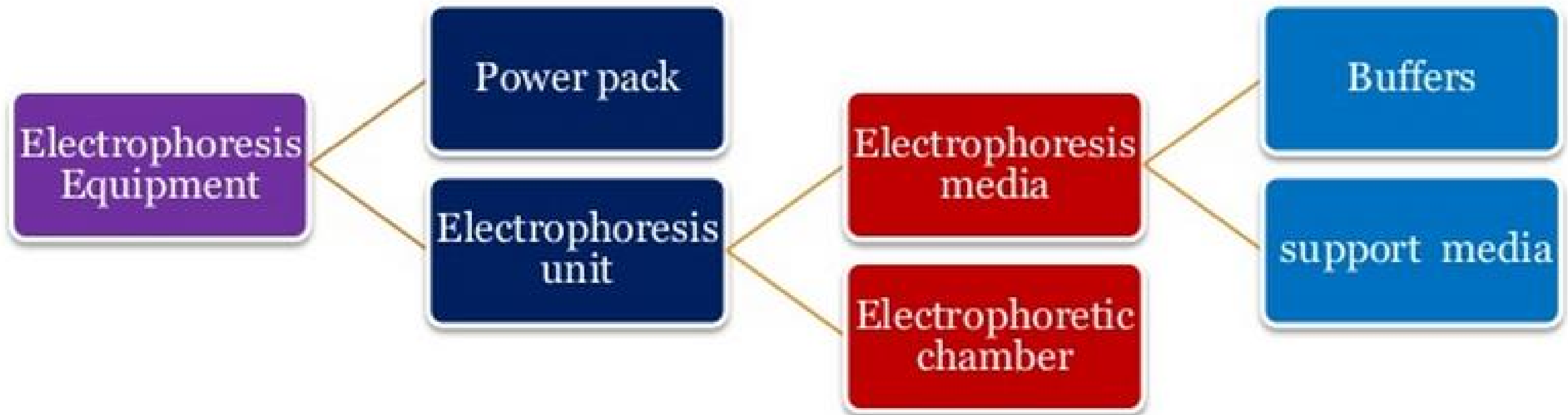
PRINCIPLE...CONT.

Whereby,

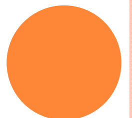
- v = velocity of migration of the molecule.
- E = electric field in volts per cm
- q = net electric charge on the molecule
- f = frictional coefficient



ELECTROPHORESIS INSTRUMENTATION



Electrophoretic Chamber

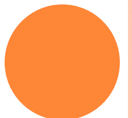


Buffers

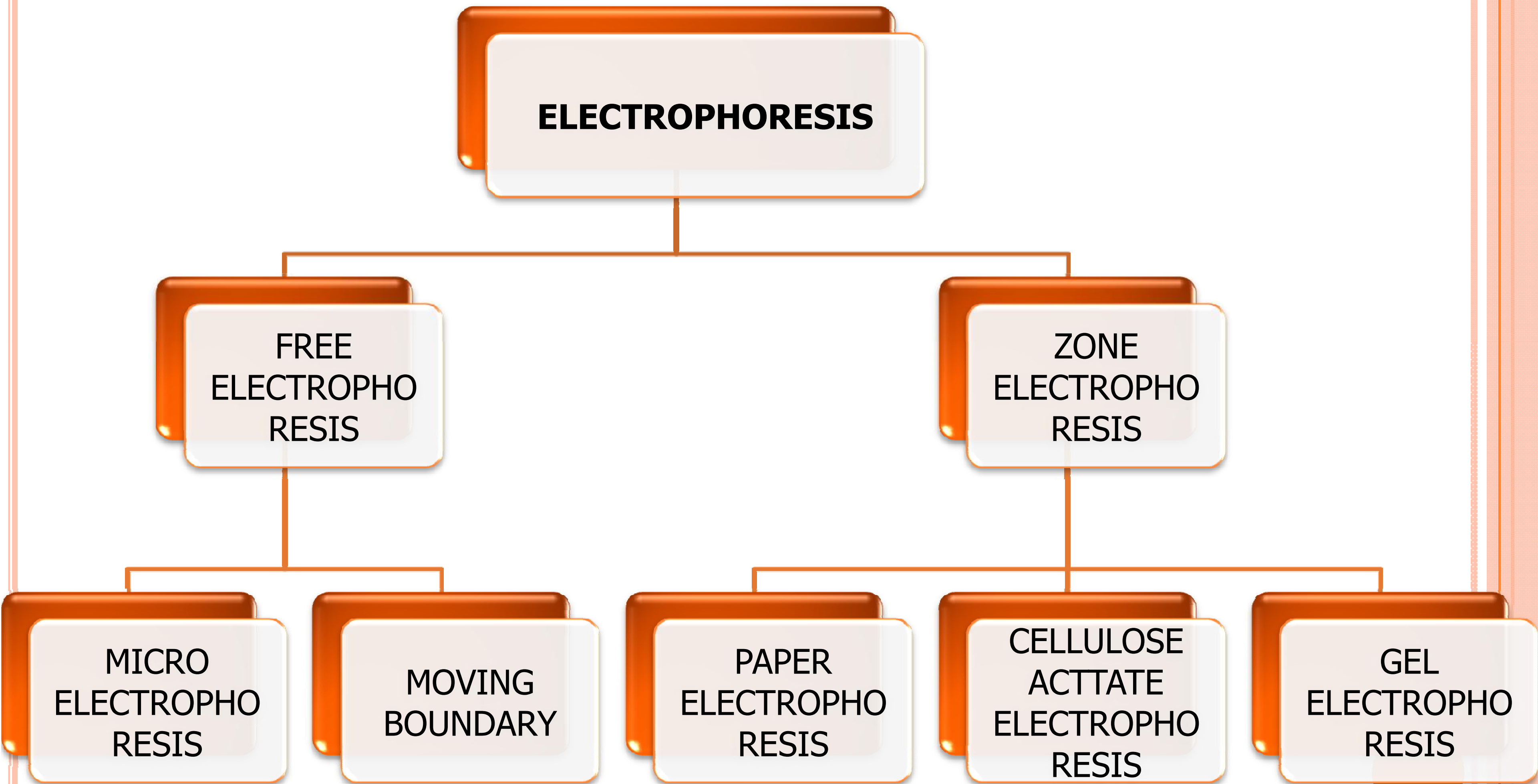
Buffer	Use / properties
Barbitone buffer – (around 8.0 pH)	• *serum protein separation* , • poor resolution, weak buffer.
Phosphate buffer- (around 7.0 pH)	• *Enzyme separation*, • low buffering capacity.- high conductivity
Tris – borate – EDTA buffer (TBE) -(pH around 8.0)	• *Nucleic acid Separation* • Good resolution , high buffering capacity , low conductivity.
Tris – acetate – EDTA buffer (TAE)- (pH around 8.0)	• *Nucleic acid separation* • high resolution , high buffering capacity , low conductivity.
Tris – glycine buffer - (pH more than 8.0)-	• *Protein separation* • high buffering capacity , low conductivity

SUPPORT MEDIA

- Filter Paper
- Cellulose acetate membrane
- Agar or Agarose gel
- Starch Gel
- Polyacrylamide gel

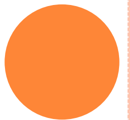


TYPES OF ELECTROPHORESIS

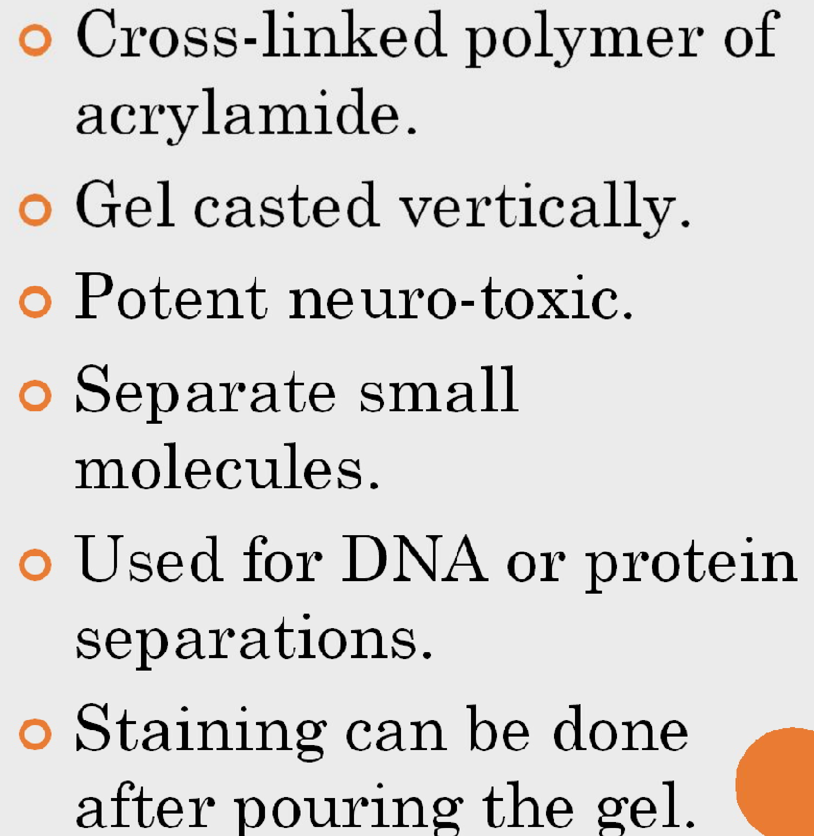


Gel Electrophoresis

- Agarose Gel electrophoresis
- Strach Gel Electrophoresis
- Poly acrylamide Gel Electrophoresis



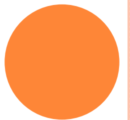
Gel Types



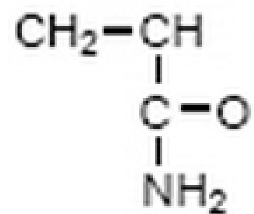
Agarose	Polyacrylamide Gel
Polysaccharide extracted from sea weed.	Cross-linked polymer of acrylamide.
Gel casted horizontally	Gel casted vertically.
Non-toxic.	Potent neuro-toxic.
Separate large molecules	Separate small molecules.
Commonly used for DNA separations.	Used for DNA or protein separations.
Staining can be done before or pouring the gel.	Staining can be done after pouring the gel.

Poly Acrylamide Gel Electrophoresis

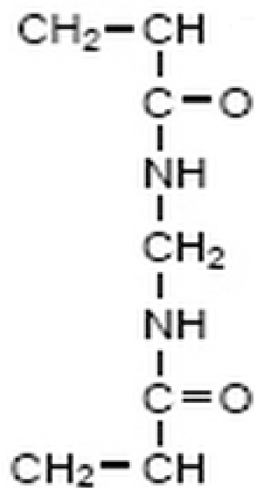
- It is a subtype of the gel electrophoresis whereby the normal gel is replaced with polyacrylamide gels used as support media.
- Gels are made by free radical-induced polymerization of acrylamide and N,N'-Methylenebisacrylamide.
- It is the most widely used technique of electrophoresis.

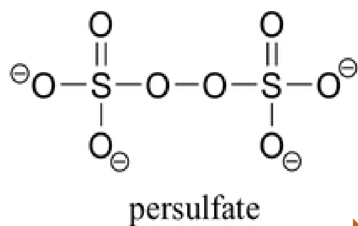


Acrylamide

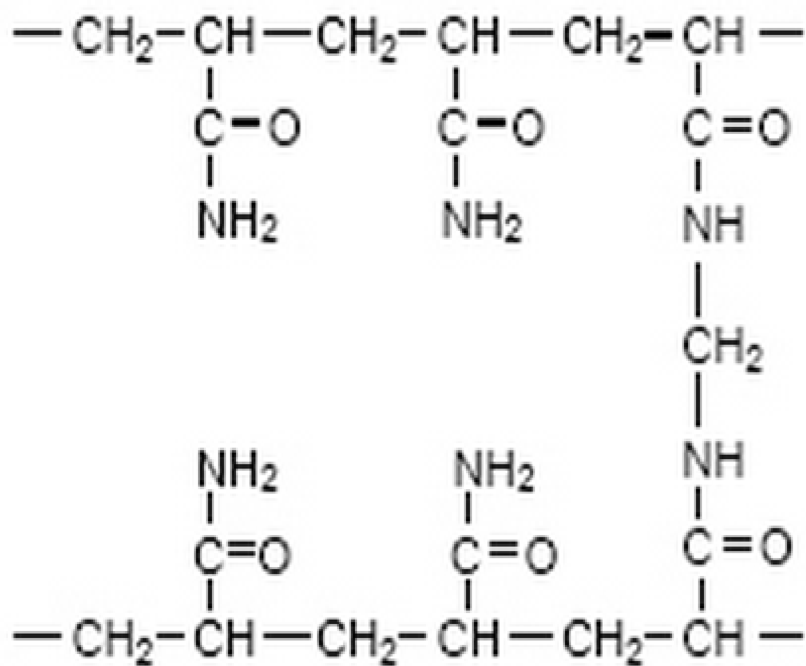


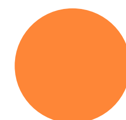
Bis





Polyacrylamide



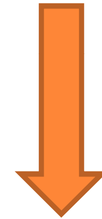


Procedure

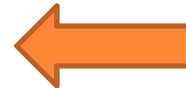
Set up gel

Load Buffer

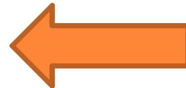
Get your sample obtained from previous purifying technique



Load Sample



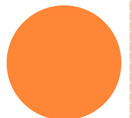
Run Gel



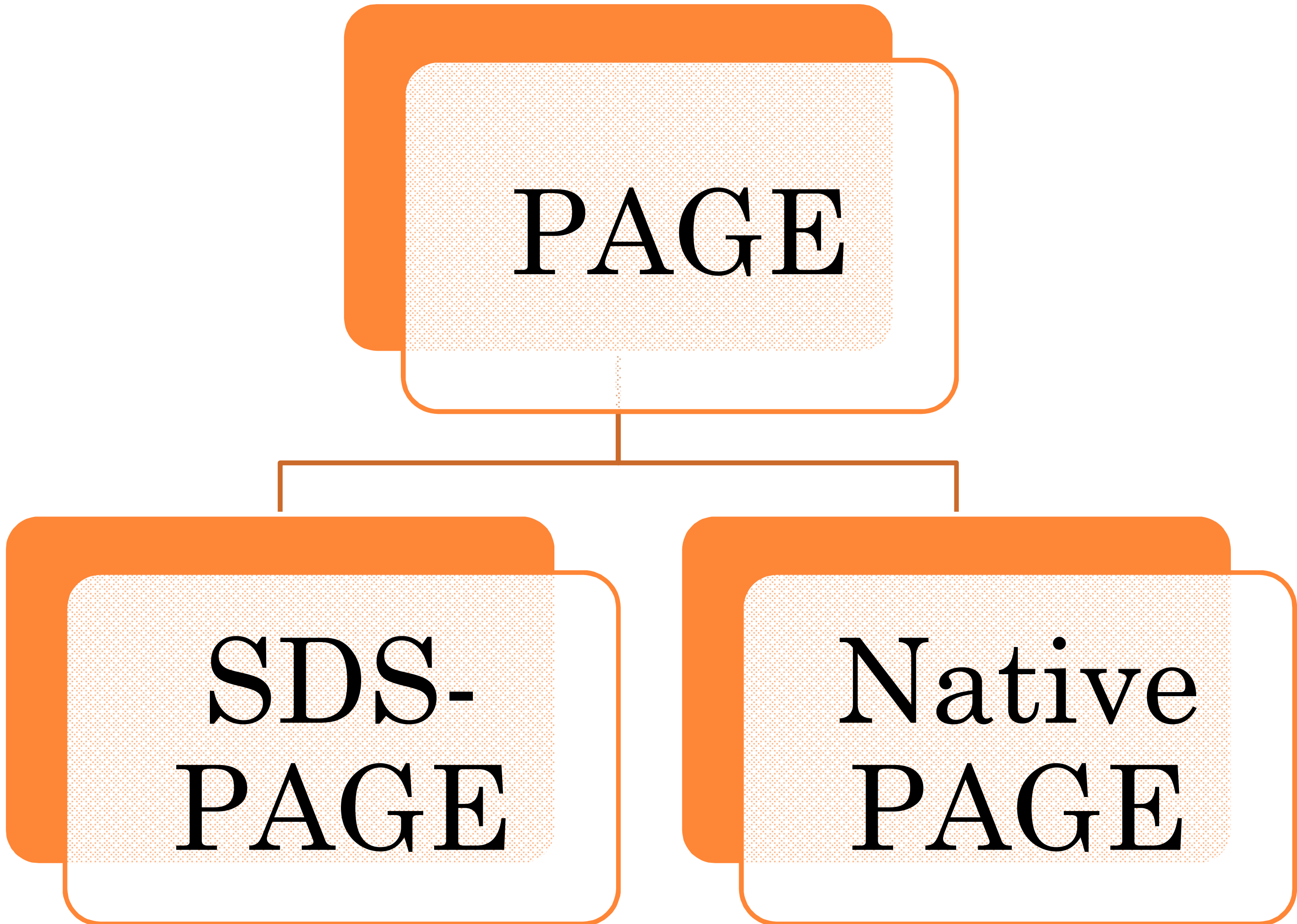
Stain and look at with UV light

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.
- Other methods may also be used to visualize the separation of the mixture's components on the gel.
- 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.

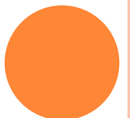


Types Of PAGE

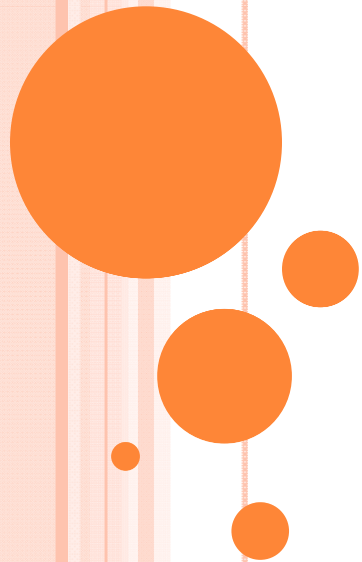


SDS - PAGE

- It is a modified version of PAGE whereby Sodium-dodecyl-sulphate(SDS) is used.
- SDS is an amphipathic surfactant.
- It denatures proteins by binding to the protein chain with its hydrocarbon 'tail', exposing normally buried regions and 'coating' the protein chain with surfactant molecules.
- The polar 'head' group of SDS adds an additional benefit to the use of this denaturant.

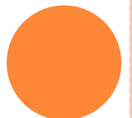


SDS-PAGE

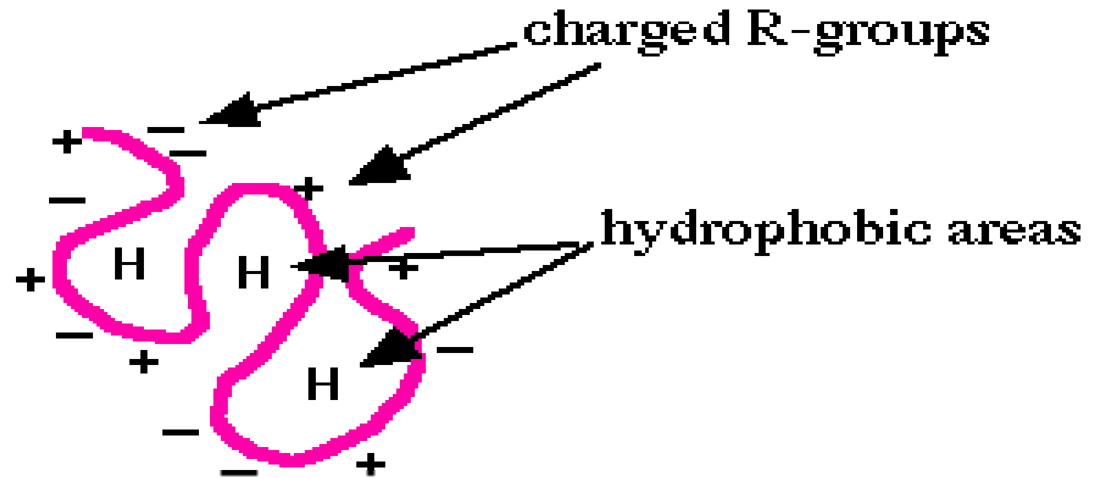


WHY ? ? ?

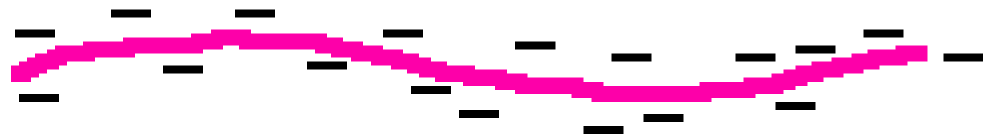
- In their native form, proteins fold into a variety of shapes, some compact, some elongated.
- The rate of migration of native proteins through a sieving medium is therefore more a reflection of their relative compactness, and less an accurate measure of molecular weight.
- Denaturing the proteins nullifies structural effects on mobility, allowing separation on a true charge/mass ratio basis.
- It also separates subunits in multimeric proteins, allowing analysis of large, complex aggregates.



BEFORE SDS



AFTER SDS



Negative Electrode

Load samples here

Buffer

well well well

Stacking Gel

Running Gel

big proteins

Glass Plates

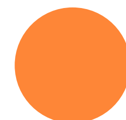
Positive Electrode

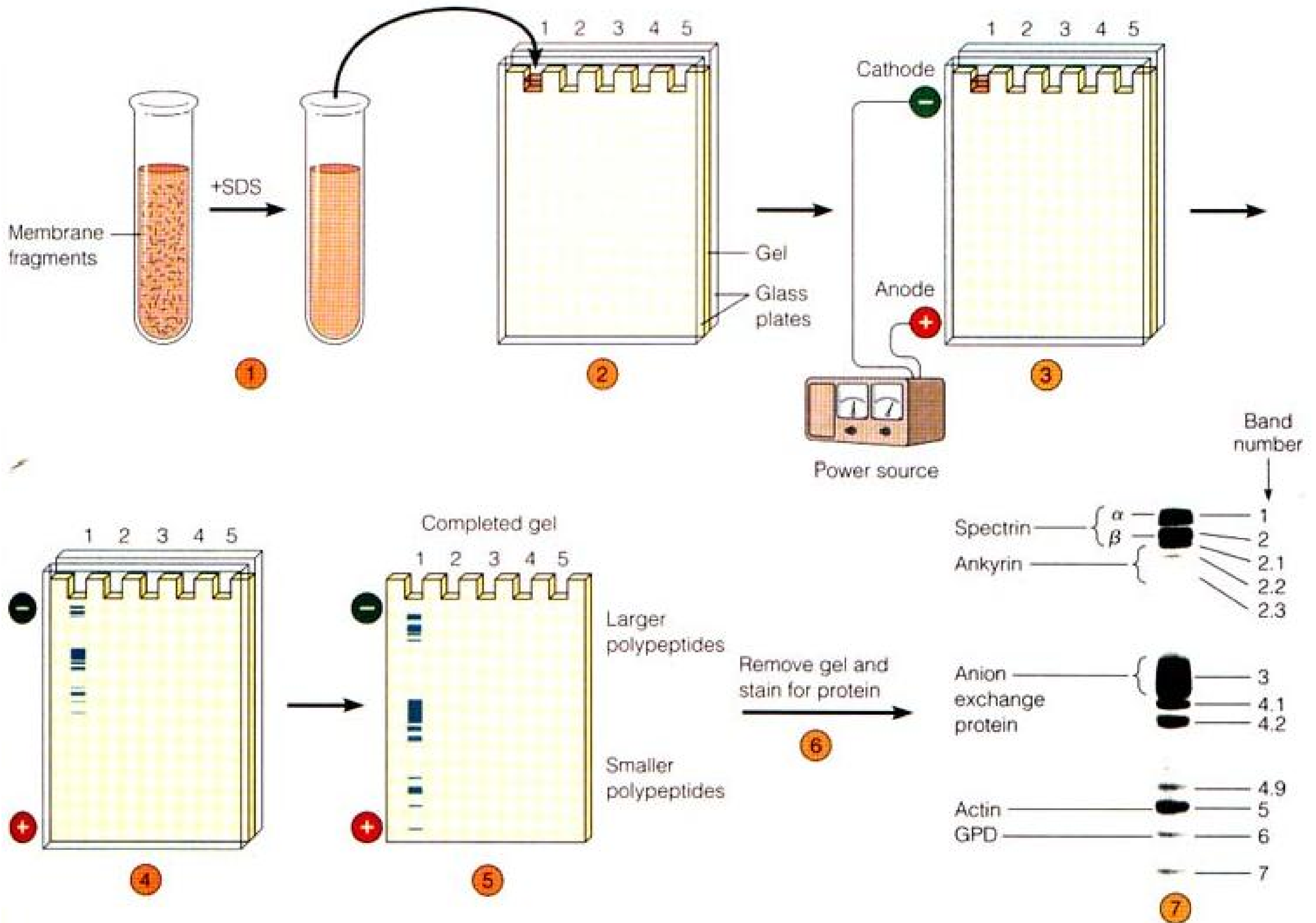
Spacer

small proteins

Buffer

An illustration of an apparatus used for SDS PAGE.





Membrane
fragments
+SDS
1
2
3
4
5
Gel
Glass
plates
Cathode
Anode
Power source
Band
number
Completed gel
Larger
polypeptides
Smaller
polypeptides
Remove gel and
stain for protein
Spectrin
α
β
Ankyrin
Anion
exchange
protein
Actin
GPD
1
2
2.1
2.2
2.3
3
4.1
4.2
4.9
5
6
7

Differences

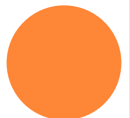
Native PAGE	SDS PAGE
• Separation is based upon charge, size, and shape of macromolecules. • Useful for separation and/or purification of mixture of proteins • This was the original mode of electrophoresis.	• Separation is based upon the molecular weight of proteins. • The most common method for determining MW of proteins • Very useful for checking purity of protein samples

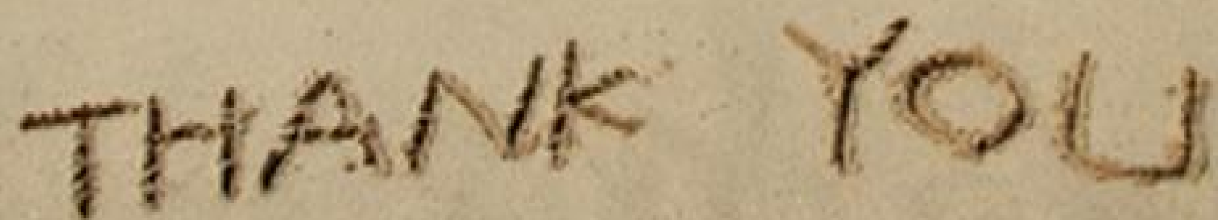
ADVANTAGES

- Chemically inert
- Hydrophilic and electrically neutral
- Transparent to light
- Stable over a wide range of pH, temperature, and ionic strength.
- Never bind to proteins.
- Available in wide range of pore sizes.
- Superior resolution

APPLICATIONS

- Used for estimation of molecular weight of proteins and nucleic acids.
- Determination of subunit structure of proteins.
- Purification of isolated proteins.
- Monitoring changes of protein content in body fluids.




THANK YOU