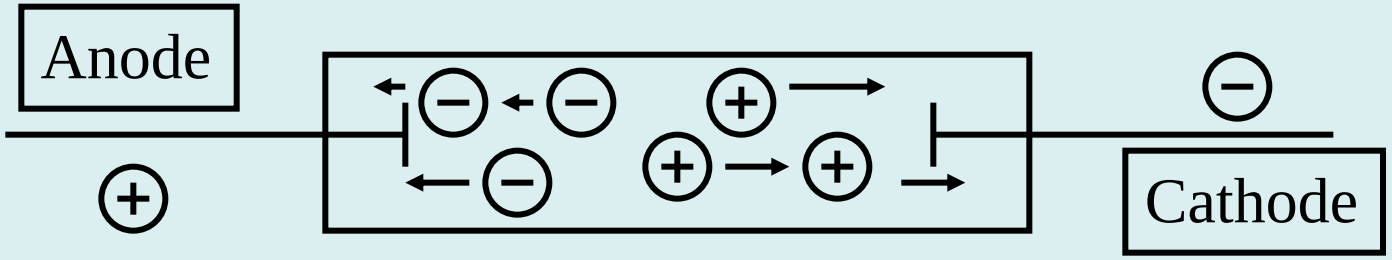
ELECTROPHORESIS

ELECTROPHORESIS

- Electrophoresis (Greek) = borne by electricity
- Migration of charged particles or molecules in a medium under the influence of an electric field.
- First observed by a Russian physicist, Alexander Reuss in 1807 on a study related to colloidal structure



PRINCIPLE OF ELECTROPHORESIS

Migration Velocity:

The rate of migration of a particular molecule depends on its net charge, size, shape and the applied current.

$$v = \mu_{ep} E = \mu_{ep} \frac{V}{L}$$

Where:

v = migration velocity of charged particle in the potential field (cm sec $^{-1}$)

μ_{ep} = electrophoretic mobility (cm^2 V^{-1} sec^{-1})

E = field strength (V cm $^{-1}$)

V = applied voltage (V)

L = length of capillary (cm)

PRINCIPLE OF ELECTROPHORESIS

Electrophoretic mobility:

- The movement of a charged molecule in an electric field is often expressed in terms of electrophoretic mobility

$$\mu_{ep} = \frac{q}{6\pi\eta r}$$

Where:

q = charge on ion

η = viscosity

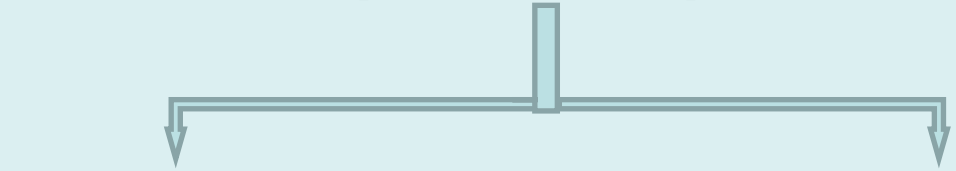
r = ion radius

TYPES OF MOLECULES THAT CAN BE SEPARATED BY ELECTROPHORESIS

- Nucleic acids (RNA and DNA)
- Proteins
- Peptides
- Whole cells

TYPES OF ELECTROPHORESIS

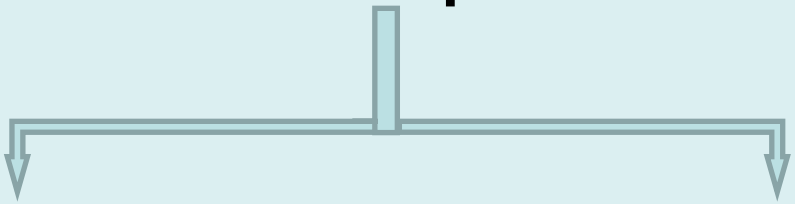
Paper electrophoresis



Horizontal type

Vertical type

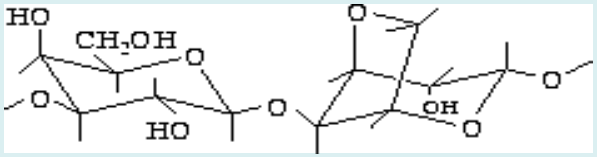
Gel electrophoresis



- Horizontal gel electrophoresis
  - Starch gel
  - Agar gel
  - Agarose gel
  - Submerged gel
- Vertical gel electrophoresis
  - SDS-PAGE
  - Native PAGE

AGAROSE GEL ELECTROPHORESIS

Agarose



D-galactose 3,6-anhydro L-galactose

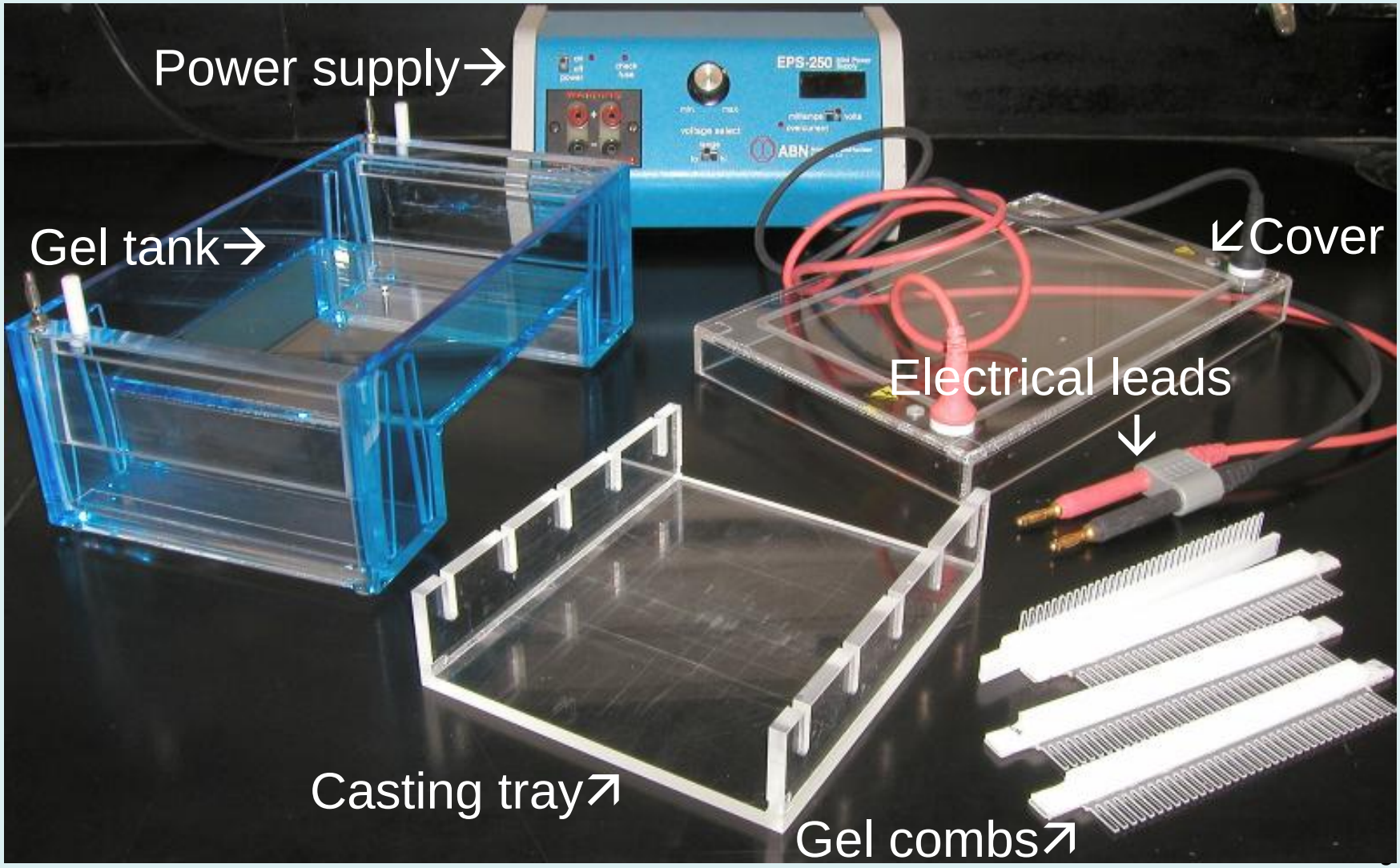
- Agarose is a linear polysaccharide made up of the basic repeat unit agarobiose
- Agarobiose comprises alternating units of D-galactose and L-galactose joined by α-(1→3) and β-(1→4) glycosidic linkages
- Lina Hesse, technician and illustrator for a colleague of Koch was the first to suggest agar for use in culturing bacteria
- Agarose was first used in biology when Robert Koch used it as a culture medium for Tuberculosis bacteria in 1882

AGAROSE GEL ELECTROPHORESIS

TYPES OF AGAROSE

- High melting temperature agarose (standard)
  - Manufactured from 2 seaweeds – *Gelidium* and *Gracilaria*
- Low Melting Temperature Agarose
  - This has been modified by hydroxyethylation and therefore melt at lower temperatures
  - The degree of substitution determines the exact melting temperature

ELECTROPHORESIS EQUIPMENTS

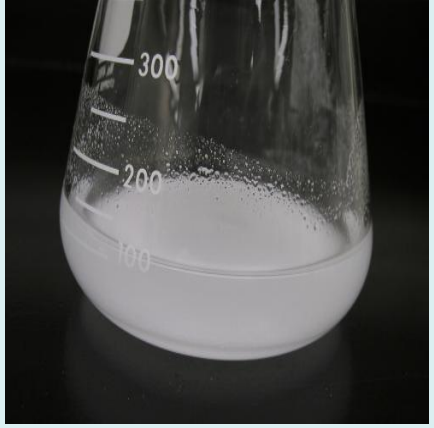


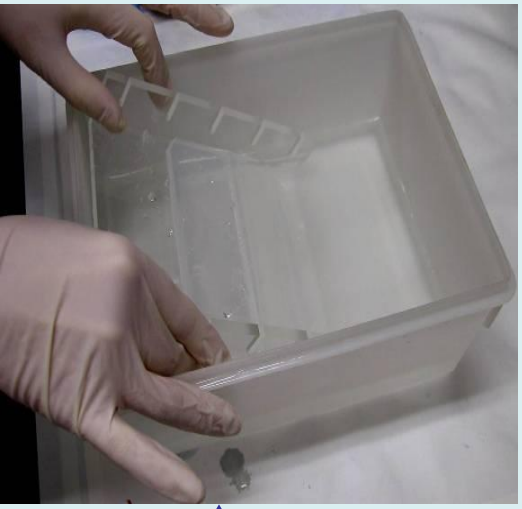
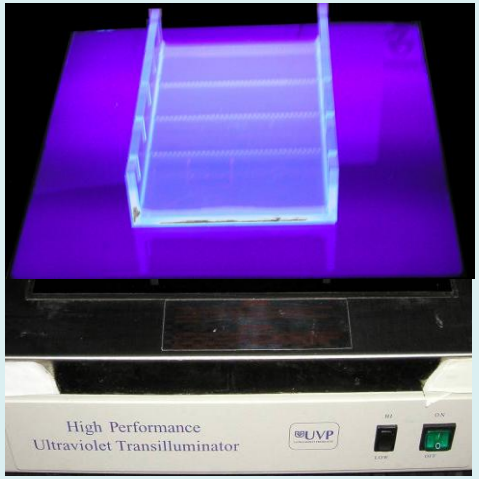
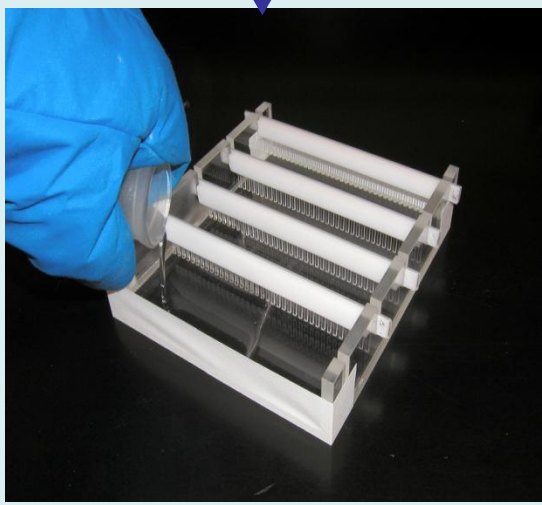
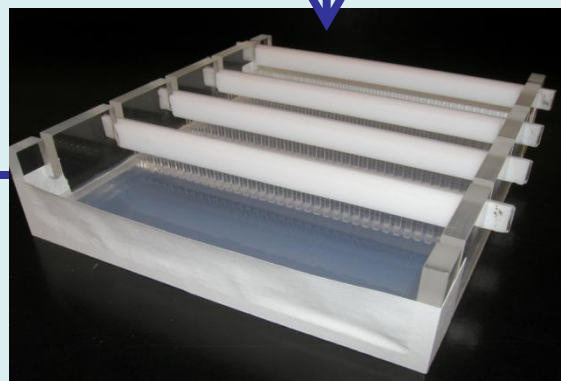
AGAROSE GEL ELECTROPHORESIS

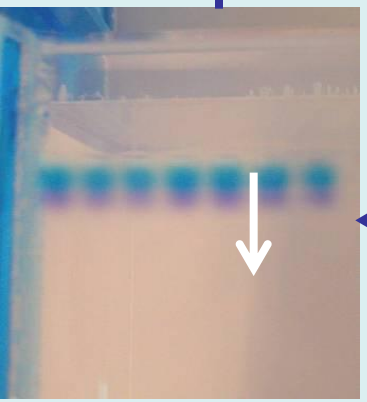
Performing Agarose Gel Electrophoresis

- Seal the platform with a tape and insert the comb it is correct position
- Weigh 1 gm of agarose and dissolve in 100ml of the Buffer by heating in a hot plate or in a microwave oven
- Pour the gel slowly into the tank and push any air bubbles away to the side using a disposable tip
- Leave to set for at least 30 minutes, preferably 1 hour, with the lid on if possible
- Remove the comb and place the platform into the buffer tank
- Load the sample added with required amount of gel loading dye
- Run at 50V/100V till the dye front reaches the end of the gel
- Observe under UV Trans-illuminator




300
200
100

300
200
100


High Performance
Ultraviolet Transilluminator
UVP



AGAROSE GEL ELECTROPHORESIS

- **Percentage of Gel**: 0.8 – 3%
- Ethidium Bromide (EtBr) can be added along with the gel {1µL(10mg/mL)}/ with running buffer (0.075µg/ml) / can be stained after running (0.5µg/ml)
- A band is easily visible if it contains about 20ng of DNA.
  - **Too much DNA** : The band appears to run fast (implying that it is smaller than it really is) and in extreme cases can mess up the electrical field for the other bands, making them appear the wrong size also.
  - **Too little DNA:** The only problem in that we can not be able to see the smallest bands because they are too faint.

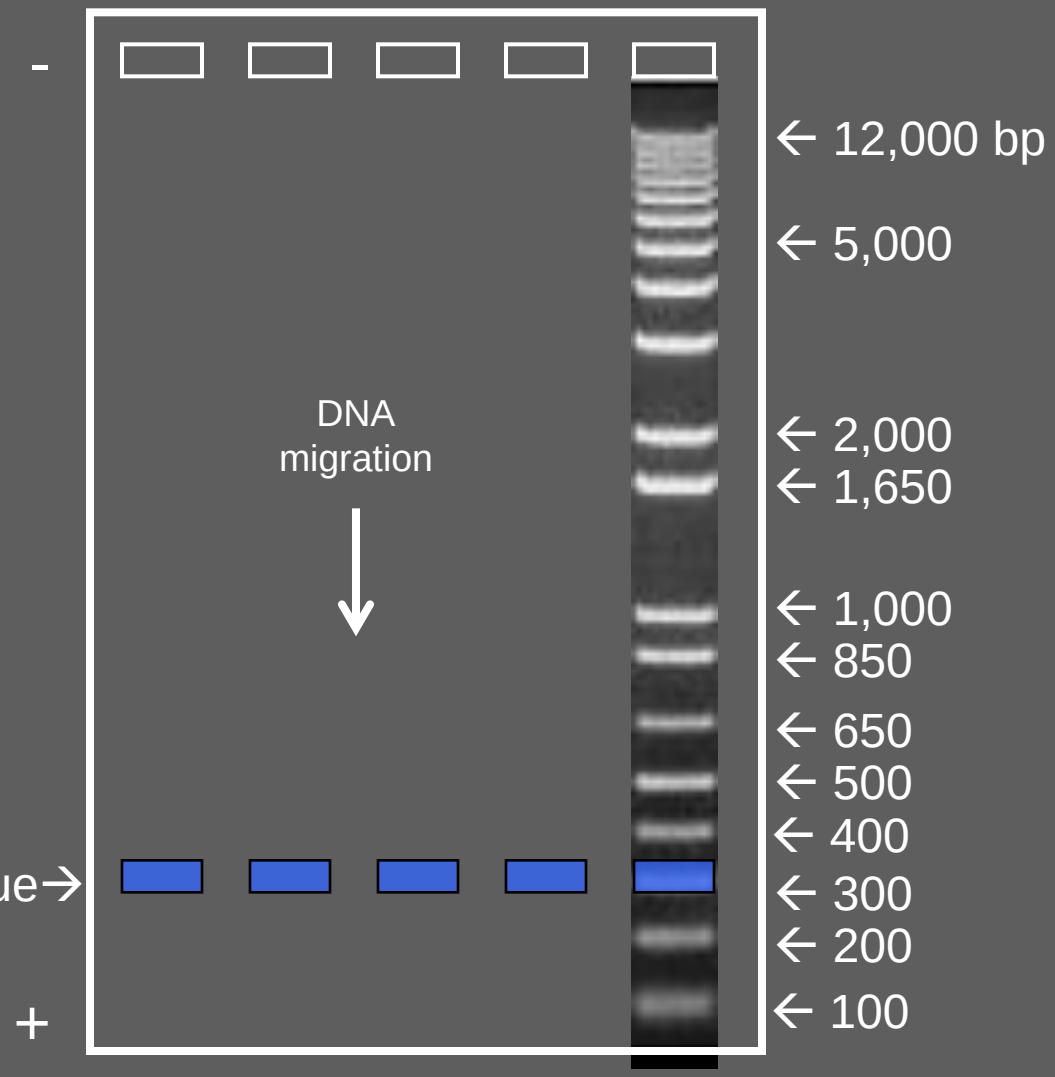
AGAROSE GEL ELECTROPHORESIS

- **Gel Loading Buffer/Dye**: 2µl of 6X stock solution: contains
  - 10mM Tris-HCl (pH 7.6)
  - 0.03% bromophenol blue
  - 0.03% xylene cyanol
  - 60mM EDTA
  - 60% glycerol

  – Bromophenol blue migrates at a rate equivalent to 200–400bp DNA
  – Xylene cyanol migrates at approximately 4kb equivalence

- Running Buffer: 1x
- TBE: Tris Borate EDTA
- TAE: Tris Acetate EDTA

DNA Ladder Standard



Note: bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule

Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

APPLICATIONS OF AGAROSE GEL ELECTROPHORESIS

- DNA
  - Criminal investigations
    - Blood
    - Saliva
    - Hair
    - Semen

ELECTROPHORESIS OF PROTEINS

- Separate proteins based on
  - Size (Molecular Weight - MW)
  - Isoelectric Point

- Allows us to
  - characterize (degradation, MW)
  - quantify
  - determine purity of sample
  - compare proteins from different sources

ELECTROPHORESIS OF PROTEINS

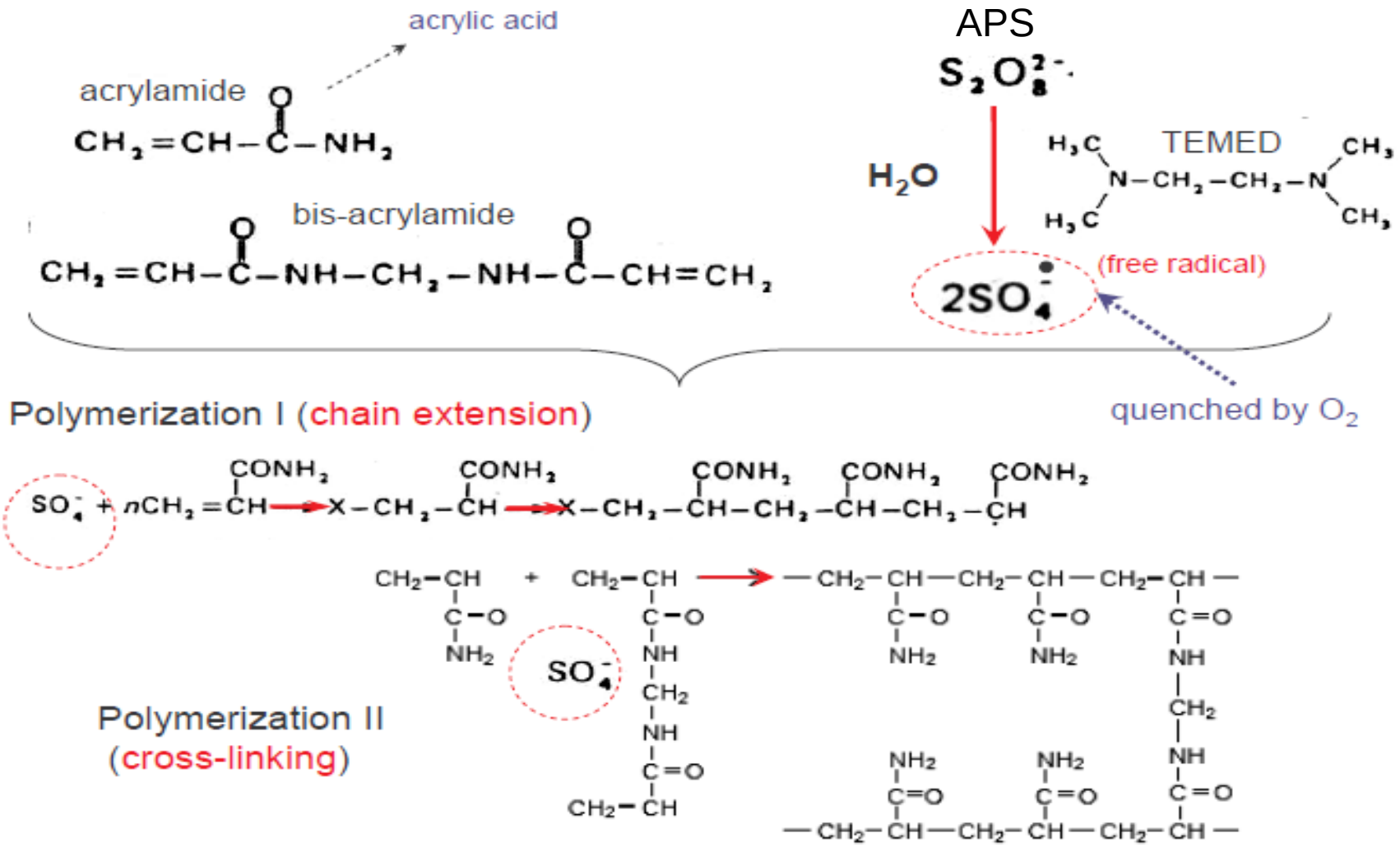
- Native continuous PAGE
- Native discontinuous PAGE
- SDS PAGE (nonreducing)
- SDS PAGE (reducing)
- Urea PAGE
- Isoelectrofocusing
- 2D PAGE
- Immunoelectrophoresis

ELECTROPHORESIS OF PROTEINS

Polyacrylamide gel electrophoresis (PAGE)

- Polyacrylamide gel (PAG) is a synthetic gel prepared from pure acrylamide monomer and crosslinkers.
- PAG is a nonionic, thermostable, transparent, strong and relatively inert (chemically).
- PAG can withstand high voltage gradients, buffers with extreme pH and other chemicals and reagents.
- PAG is feasible to various staining and destaining procedures.

ELECTROPHORESIS OF PROTEINS



ELECTROPHORESIS OF PROTEINS

Native continuous PAGE

- Proteins retain their native conformation and biological activity.
- Separation according to the electrophoretic mobility and the sieving effect of the gel.
- Used to detect a particular protein, often an enzyme on the basis of their biological activity.
- The enzyme of interest can be identified by incubating the gel in an appropriate substrate solution such that a colour product is formed at the site of enzyme.

ELECTROPHORESIS OF PROTEINS

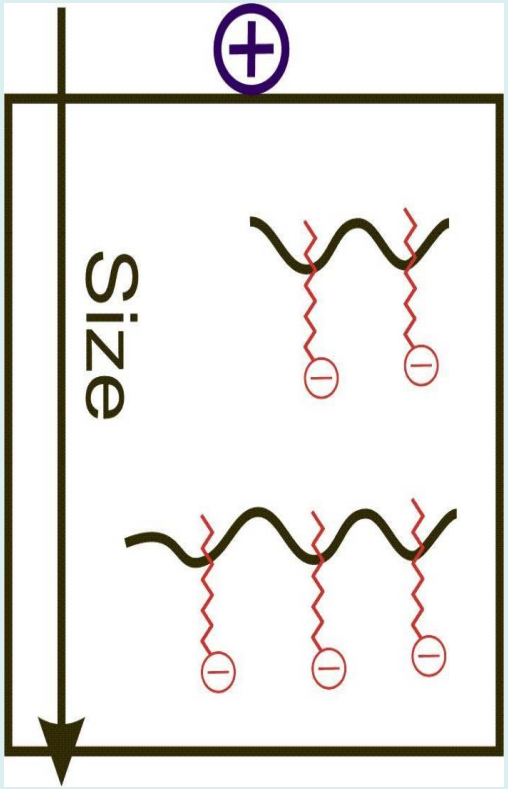
Native discontinuous PAGE

- Consists of two types of gels:
  - Large pore stacking gel (upper)
  - Small pore resolving gel (lower)
- Buffer used to prepare these two gels are of different ionic strength and pH.
- Relatively large volume of diluted protein sample can be applied and yet a good resolution of sample componet can be obtained.

ELECTROPHORESIS OF PROTEINS

SDS PAGE (nonreducing)

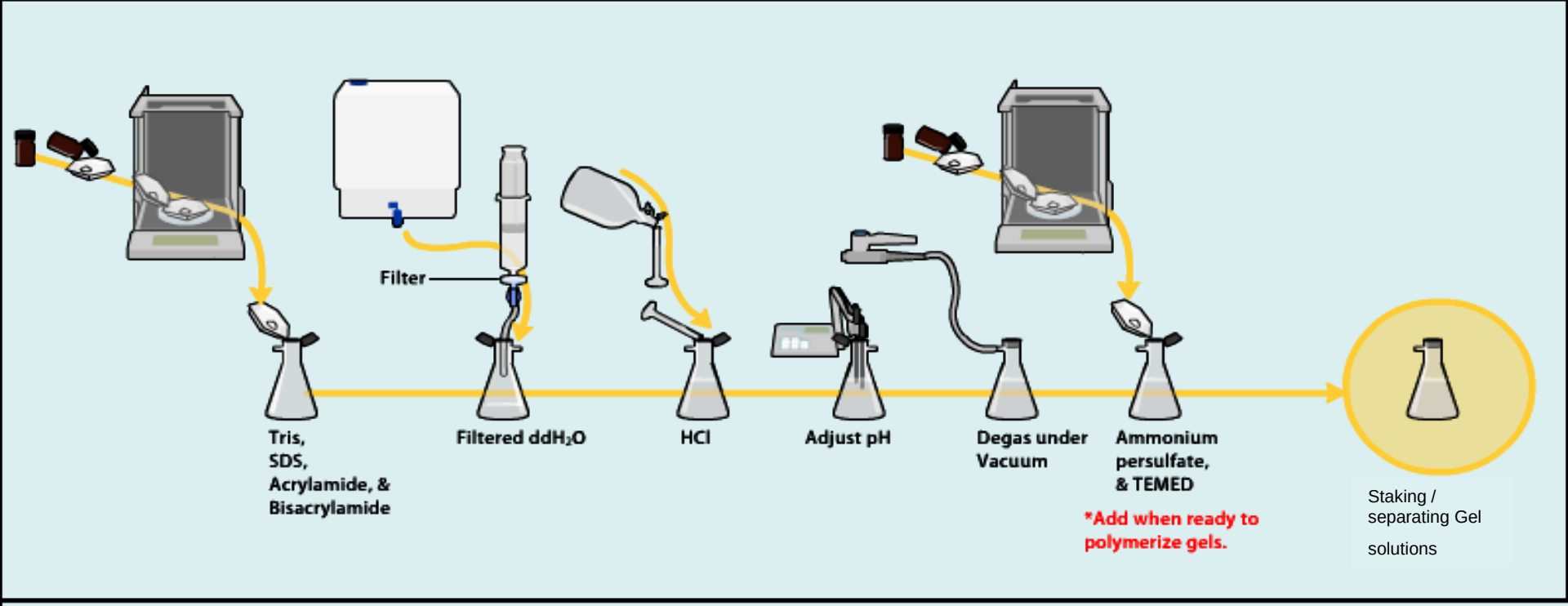
- Sodium dodecyl sulfate (SDS)- anaionic detergent
- Boiling sample protein in presence of excess SDS causes denaturation of protein.
- Proteins are separated only by molecular mass (but not by differences in charge).
- Allows estimation of the size of polypeptide chains
- Molecular weight can also determined by using molecular weight marker.

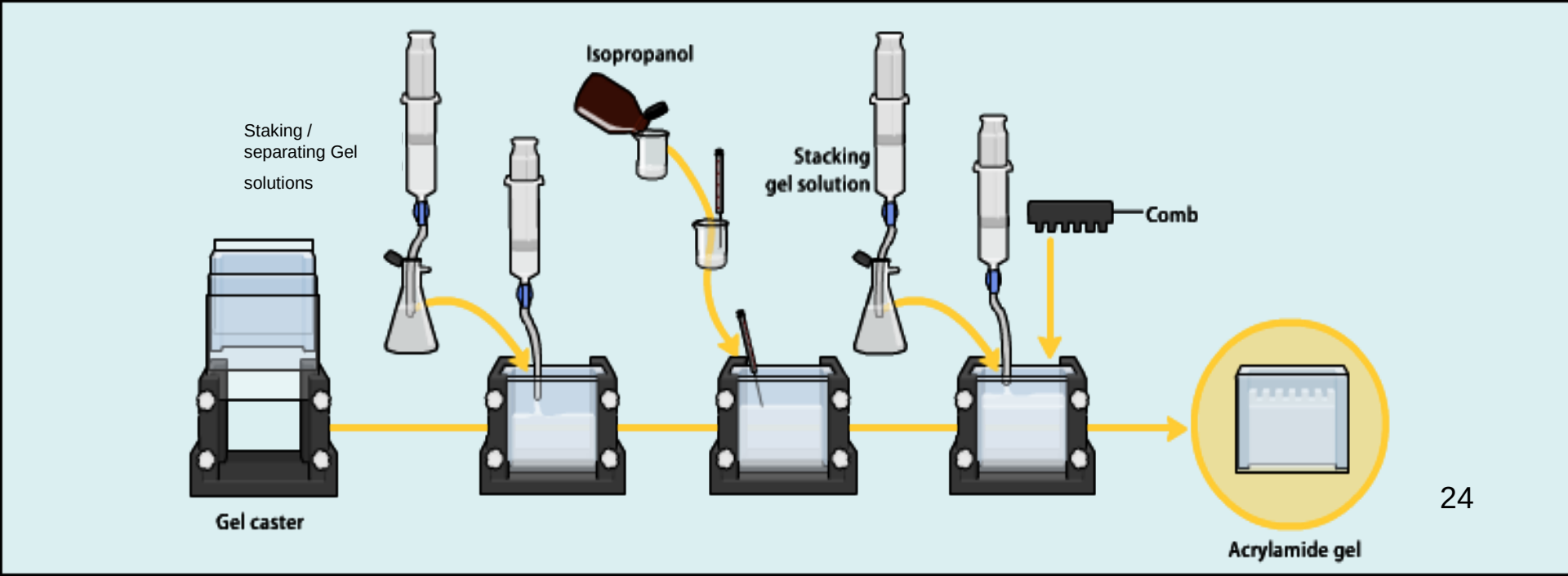


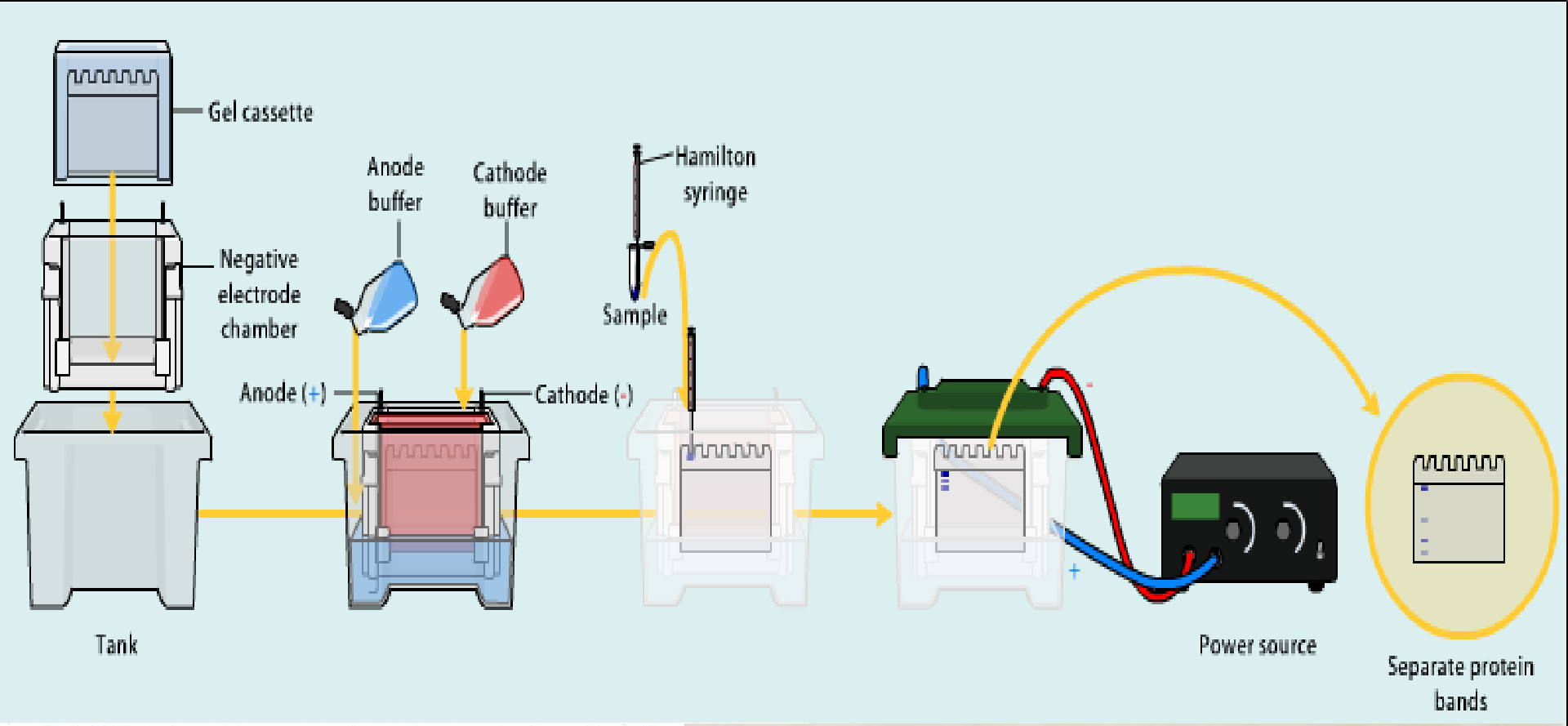
ELECTROPHORESIS OF PROTEINS

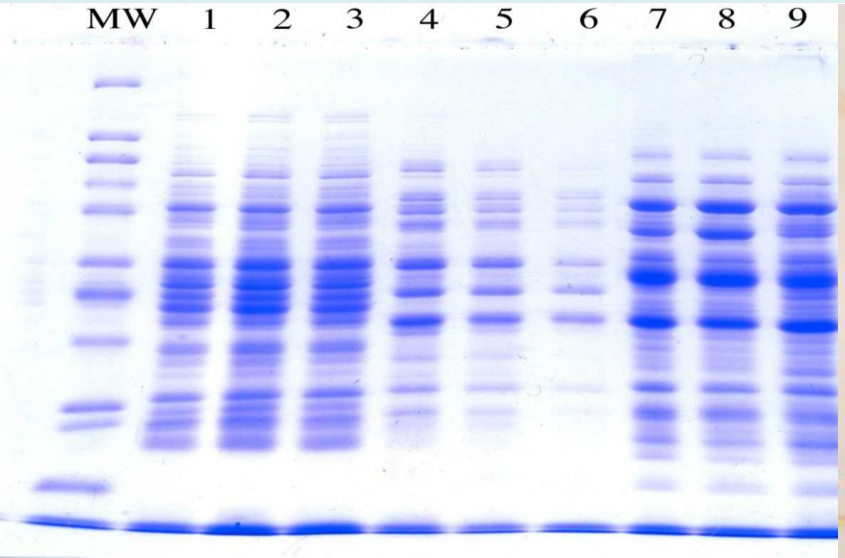
SDS PAGE (reducing)

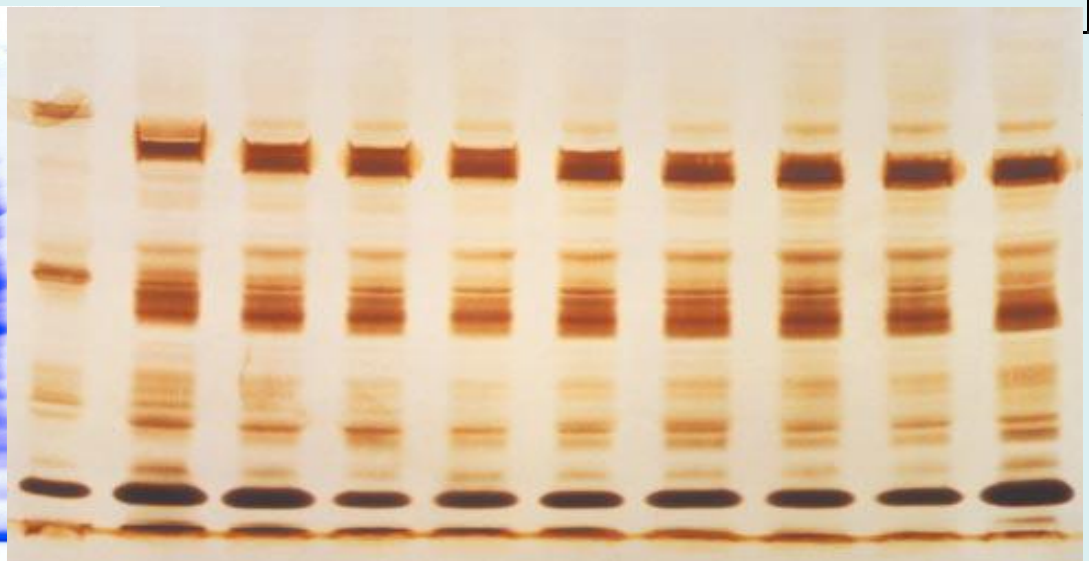
- Similar to SDS PAGE (non-reducing)
- In addition to SDS a thiol reducing agent (β-mercaptoethanol) is included in the sample mixture which cleaves the disulphide bond.
- Polypeptide in the native protein still remain associated in the presence of SDS get separated.


Filter
Tris,
SDS,
Acrylamide, &
Bisacrylamide
Filtered ddH2O
HCl
Adjust pH
Degas under
Vacuum
Ammonium
persulfate,
& TEMED
*Add when ready to
polymerize gels.
Staking /
separating Gel
solutions


Staking /
separating Gel
solutions
Isopropanol
Stacking
gel solution
Comb
Gel caster
Acrylamide gel

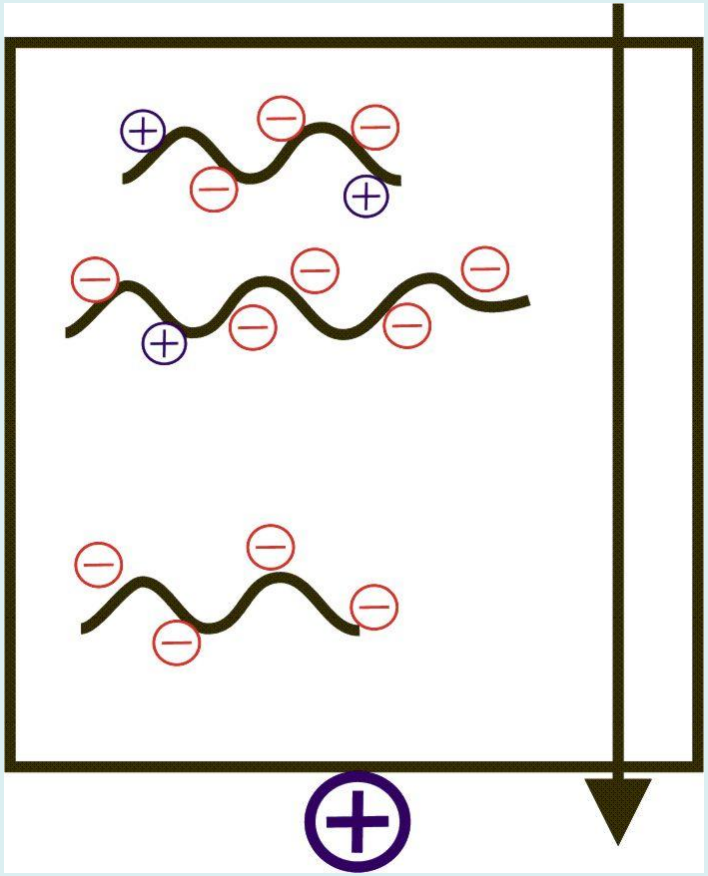

Gel cassette
Negative electrode chamber
Anode buffer
Cathode buffer
Hamilton syringe
Sample
Anode (+)
Cathode (-)
Tank
Power source
Separate protein bands


MW 1 2 3 4 5 6 7 8 9



ELECTROPHORESIS OF PROTEINS

Urea PAGE

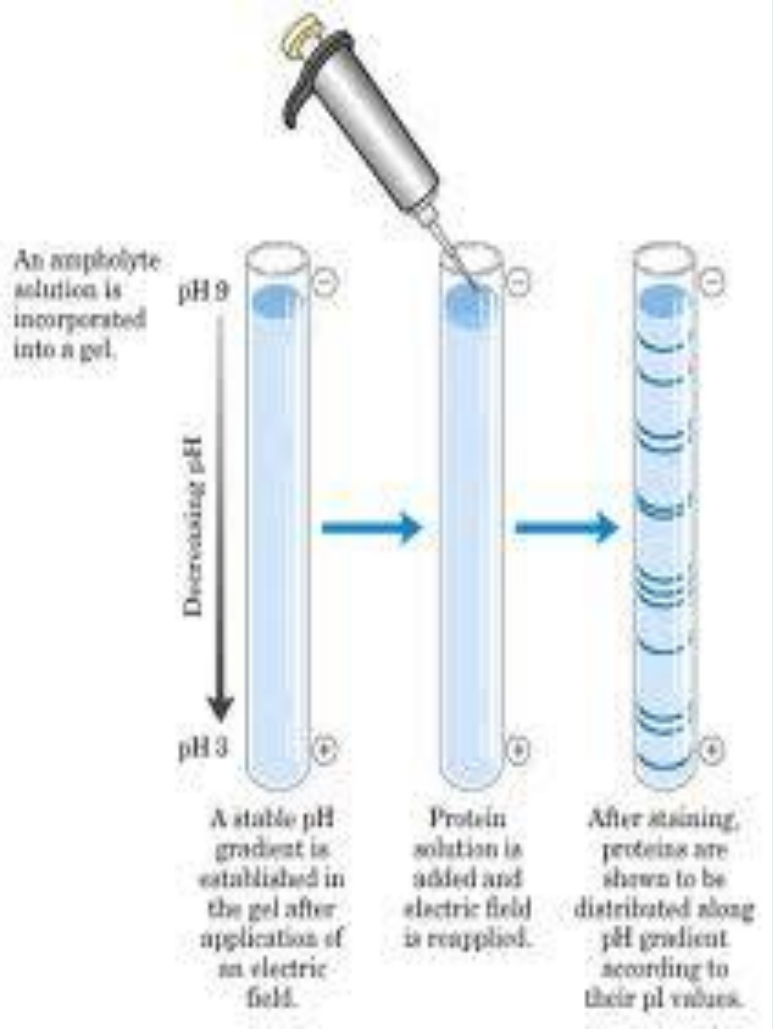


Separates denatured proteins by size/charge

Typically 6-8 M urea is added into the gel

ELECTROPHORESIS OF PROTEINS

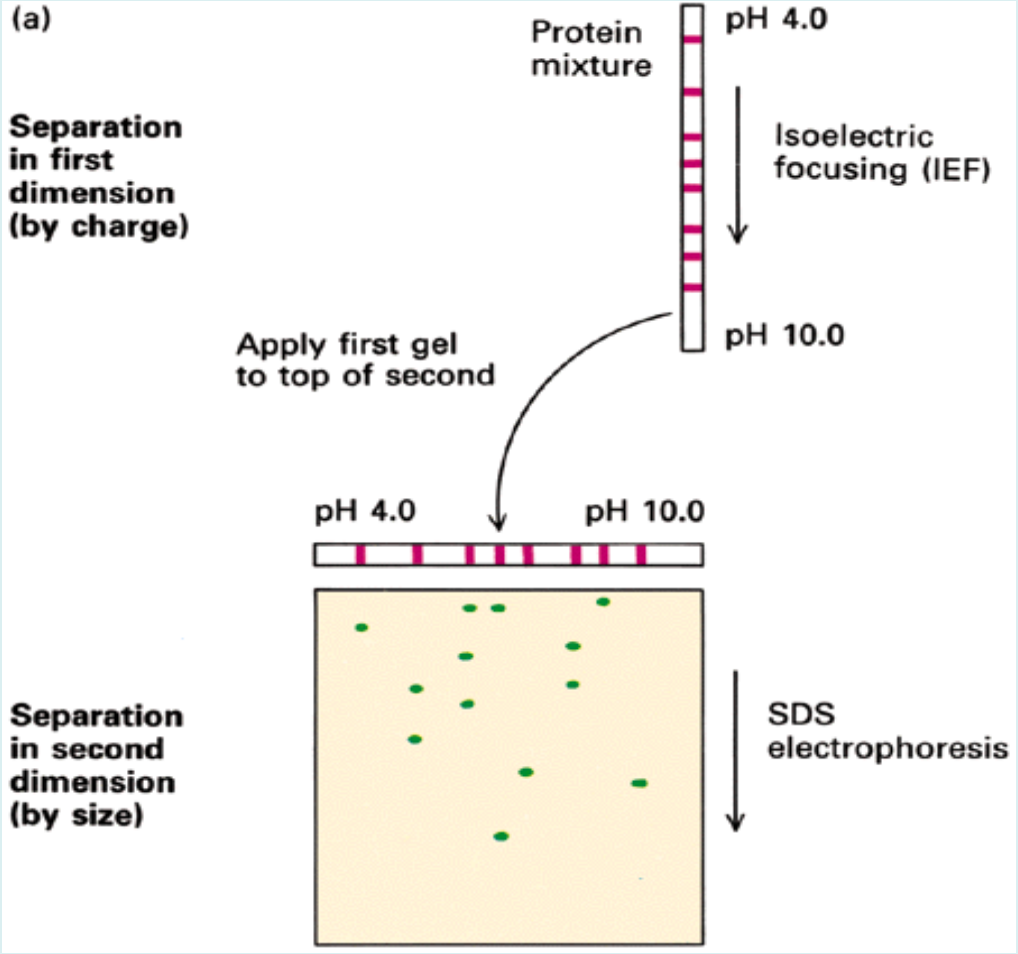
ISOELECTROFOCUSSING



- Separates proteins by their isoelectric points (pI)
- Each protein has own pI = pH at which the protein has equal amount of positive and negative charges (the net charge is zero)
- Mixtures of ampholytes, small amphoteric molecules with high buffering capacity near their pI, are used to generate the pH gradient.
- Positively and negatively charged proteins move to – and +, respectively, until they reach pI.
- pI of proteins can be theoretically predicted. Therefore, IEF can also be used for protein identification.

ELECTROPHORESIS OF PROTEINS

2 DIMENSIONAL PAGE

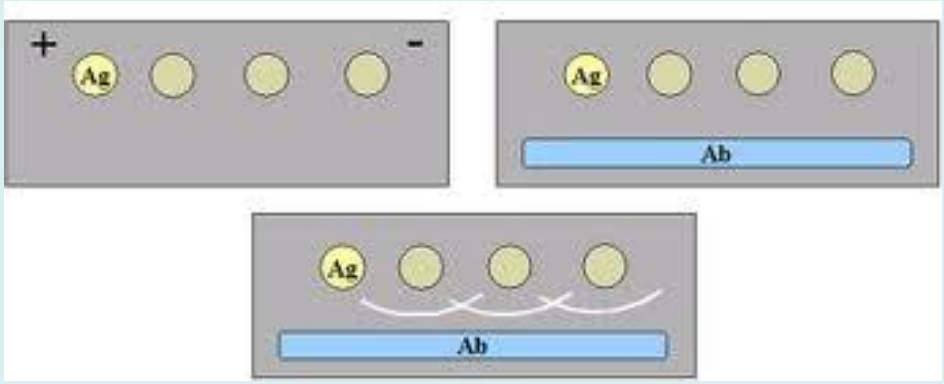


- Used to analyze proteins
- First separation based on the isoelectric point
- Second separation is based on the molecular weight

ELECTROPHORESIS OF PROTEINS

Immunoelectrophoresis

- First reported by Graber and Williams in 1953.
- Basic principle is interaction between antigen and antibody.
- Protein mixture is separated by standard electrophoresis in an agarose gel, followed by exposing the separated proteins to a specific antibody preparation.
- If the antibody has a specific affinity for one of the proteins, a visible precipitate arc is formed.
- It is useful for the analysis of protein purity, composition and antigenic propertis.



References

Boyer, R. (2006) Characterization of proteins and nucleic acids by electrophoresis. **In**: Modern experimental biochemistry. Boyer, R. (3rd eds.). Dorling kindersley (India) pvt. Ltd. New Delhi, India. pp: 129-158.

Kastenholz, B. (2006) Important contributions of a new quantitative preparative native continuous polyacrylamide gel electrophoresis procedure for elucidating metal cofactor metabolisms in protein misfolding diseases- a theory. *Protein Pept. Lett.* **13**: 503–508.

Laemmli, U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage T4". *Nature* **227**: 680–685.

Rabilloud, T. (1974) Silver-staining of proteins in polyacrylamide gels: a general overview. *Cell. Mol. Biol*., **40**: 57-75.

References

Shapiro, A. L., Vinuela, E. and Maizel, J. V. (1967) Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Commun.,* **28**: 815–820.

Switzer, R. C. (1979) A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem*., **98** : 231-237.

Walker, J. M. (2004) Electrophoretic techniques. **In**: Practical Biochemistry principles and techniques. Wilson, K. and Walker, J. (5th eds.). Cambridge University Press, Cambridge, UK. **pp**: 580-618.

Weber, K. and Osborn, M. (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.,* **244**: 4406–4412.

Thank you
for your attention