Poly Acrylamide Gel Electrophoresis (PAGE)

Vertical separation of Molecules

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- Polyacrylamide gel electrophoresis (PAGE) is a technique widely used to separate biological macromolecules, usually proteins
- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N’-methylene-bisacrylamide

Polyacrylamide gel:

- Cross-linked polysaccharide gel are formed from the polymerization of acrylamide monomer in the presence of small amount of N,N’-methylene bis acrylamide (bis-acrylamide)
- Bis-acryl amide is basically two acrylamide molecules linked by a methylene group, and is used as a cross-linking agent
- Acrylamide monomers is polymerized in head to tail fashion into long chain, thus introducing a second site for chain extension
- The polymerization of acrylamide is initiated by the addition of ammonium persulfate and the base N, N, N’, N’- tetra-methylene diamine (TEMED)

- **TEMED catalyses decomposition of the persulphate ion to give free radical**
- **Acrylamide gels are defined in terms of the total percentage of acrylamide present, and the pore size in the gel can be varied by changing the concentration of both acrylamide and bis-acrylamide**
- **Thus, the low percentage gels (e.g., 4%) have large pore size and are used for electrophoresis of protein**

Principle

- SDS-PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separate components of a protein mixture based on their molecular weights
- The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign
- The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size
- To overcome this, the biological samples needs to be treated so that they acquire uniform charge, then the electrophoretic mobility depends primarily on size

- **For this different protein molecules with different shapes and sizes, needs to be denatured (done with the aid of SDS) so that the proteins lose their secondary, tertiary or quaternary structure**
- **The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode (positively charged electrode) are separated by a molecular sieving effect based on size**
- **After the visualization by a staining (protein-specific) technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder (marker)**

Requirements for PAGE

1. Acrylamide solutions (for resolving & stacking gels)
2. Isopropanol / distilled water
3. Gel loading buffer
4. Running buffer
5. Staining, destaining solutions
6. Protein samples
7. Molecular weight markers

The equipment and supplies necessary for conducting SDS-PAGE includes:

1. An electrophoresis chamber and power supply
2. Glass plates (a short and a top plate)
3. Casting frame
4. Casting stand
5. Combs

Steps Involved

1. Sample preparation

- The sample to analyze is optionally mixed with SDS for proteins
- SDS is an anionic detergent that denatures secondary and non–disulfide–linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass
- A tracking dye may be added to the solution which typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run

2. Preparation of Polyacrylamide gel

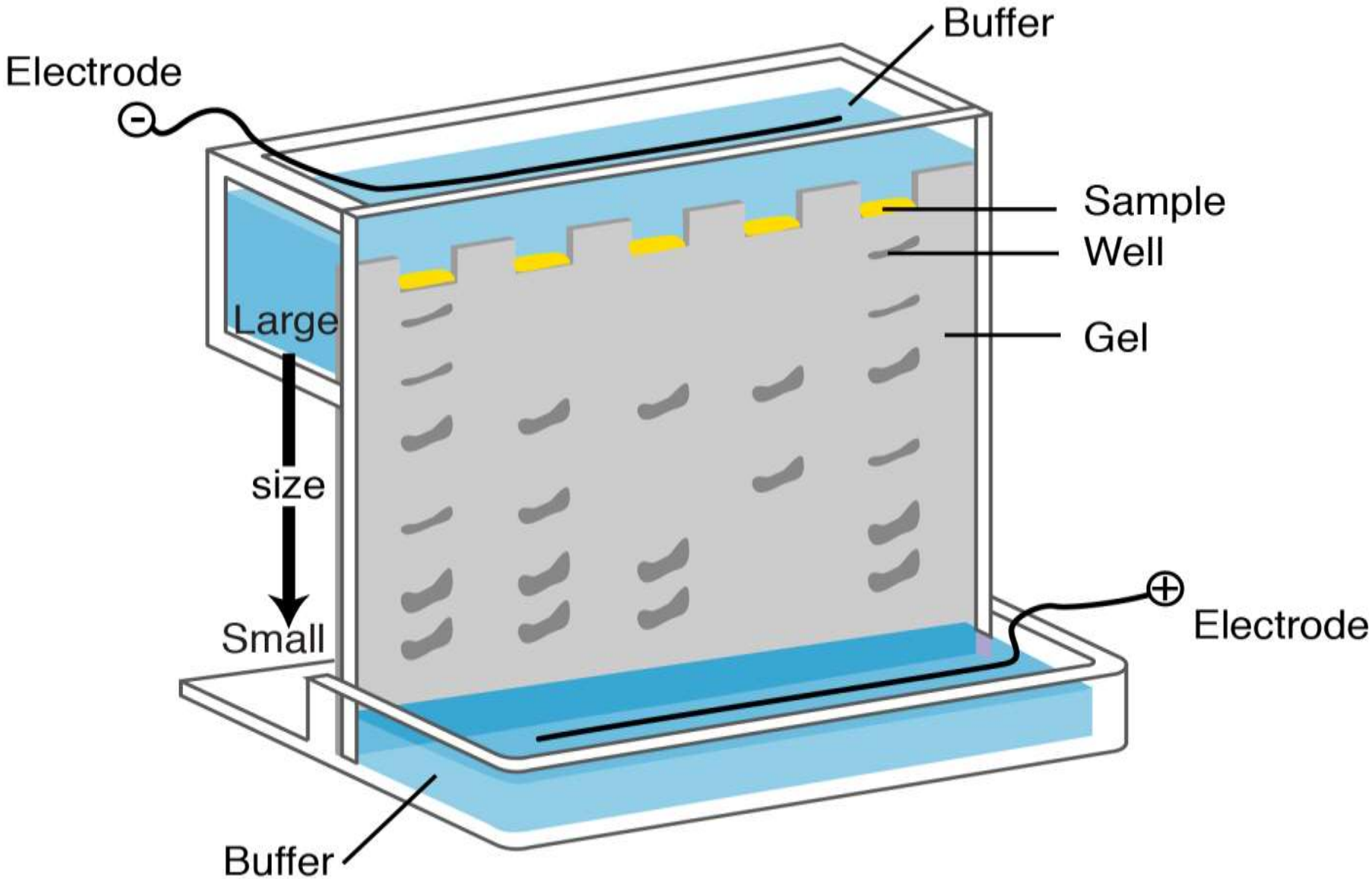
- The gels typically consist of acrylamide, bisacrylamide, and a buffer with an adjusted pH
- Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells
- After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis

3. Electrophoresis

- An electric field is applied across the gel, causing the negatively charged proteins to migrate across the gel away from the negative and towards the positive electrode
- Depending on their size, each biomolecule moves differently through the gel matrix: small molecules more easily fit through the pores in the gel, while larger ones have more difficulty
- The gel is run usually for a few hours, though this depends on the voltage applied across the gel
- After the set amount of time, the biomolecules will have migrated different distances based on their size
- Biomolecules may therefore be separated roughly according to size, which depends mainly on molecular weight under denaturing conditions

4. Detection

- Following electrophoresis, the gel may be stained most commonly with Coomassie Brilliant Blue allowing visualization of the separated proteins
- After staining, different species biomolecules appear as distinct bands within the gel
- It is common to run molecular weight size markers of known molecular weight in a separate lane in the gel to calibrate the gel and determine the approximate molecular mass of unknown biomolecules by comparing the distance traveled relative to the marker


Buffer
Electrode
Sample
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Gel
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Buffer

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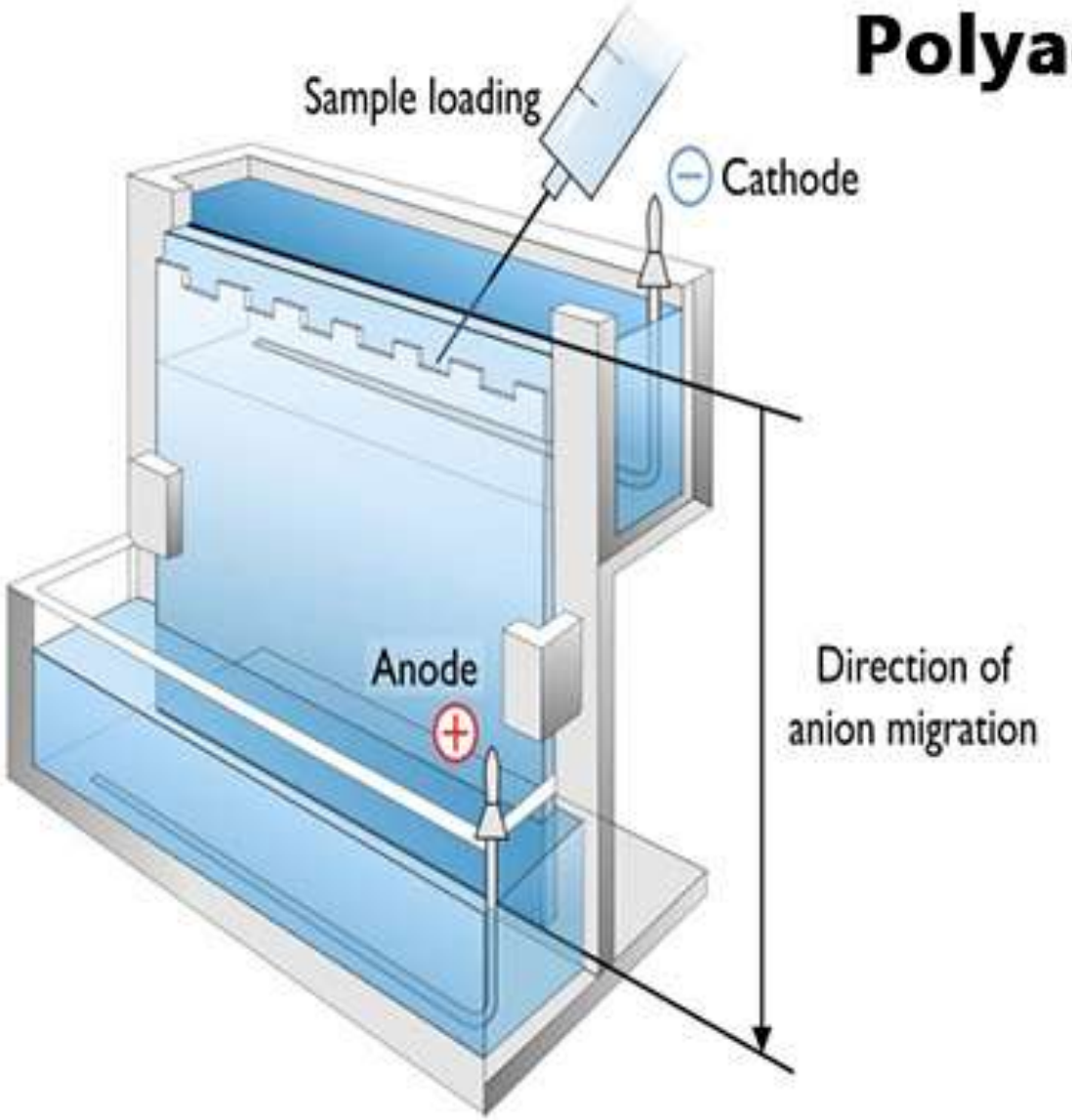
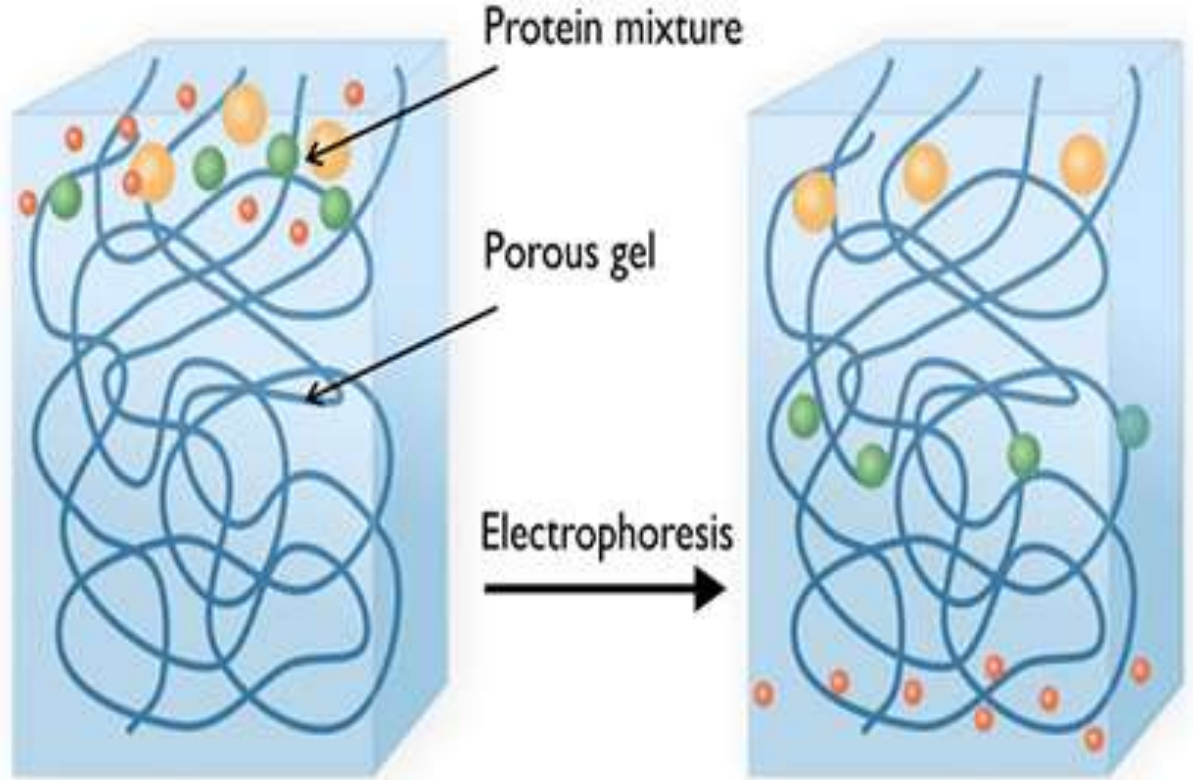
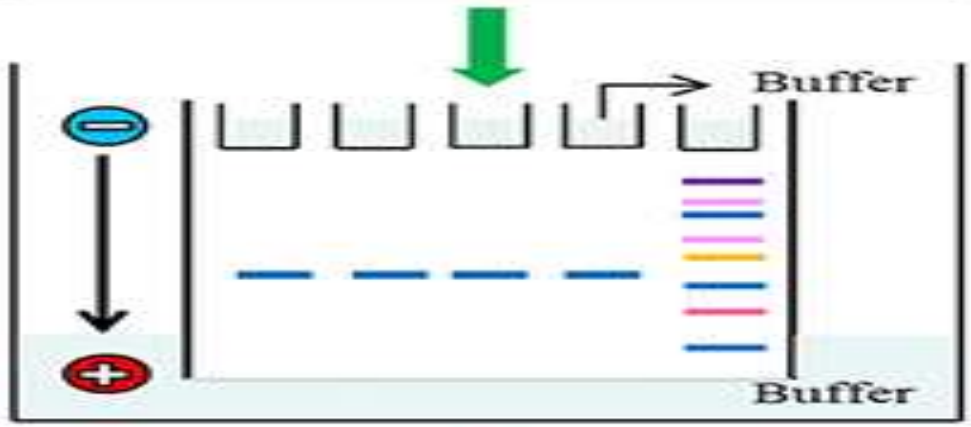
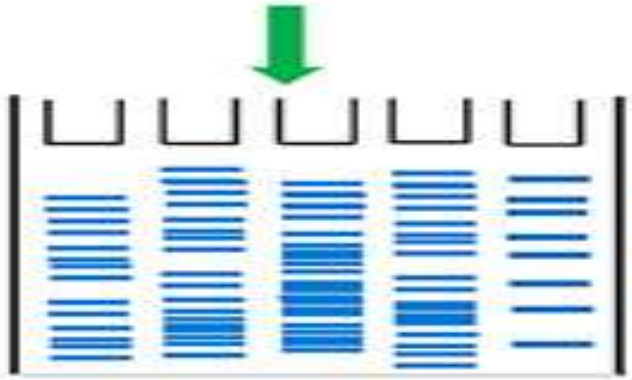




Protein samples and marker loaded in vertical SDS-PAGE system

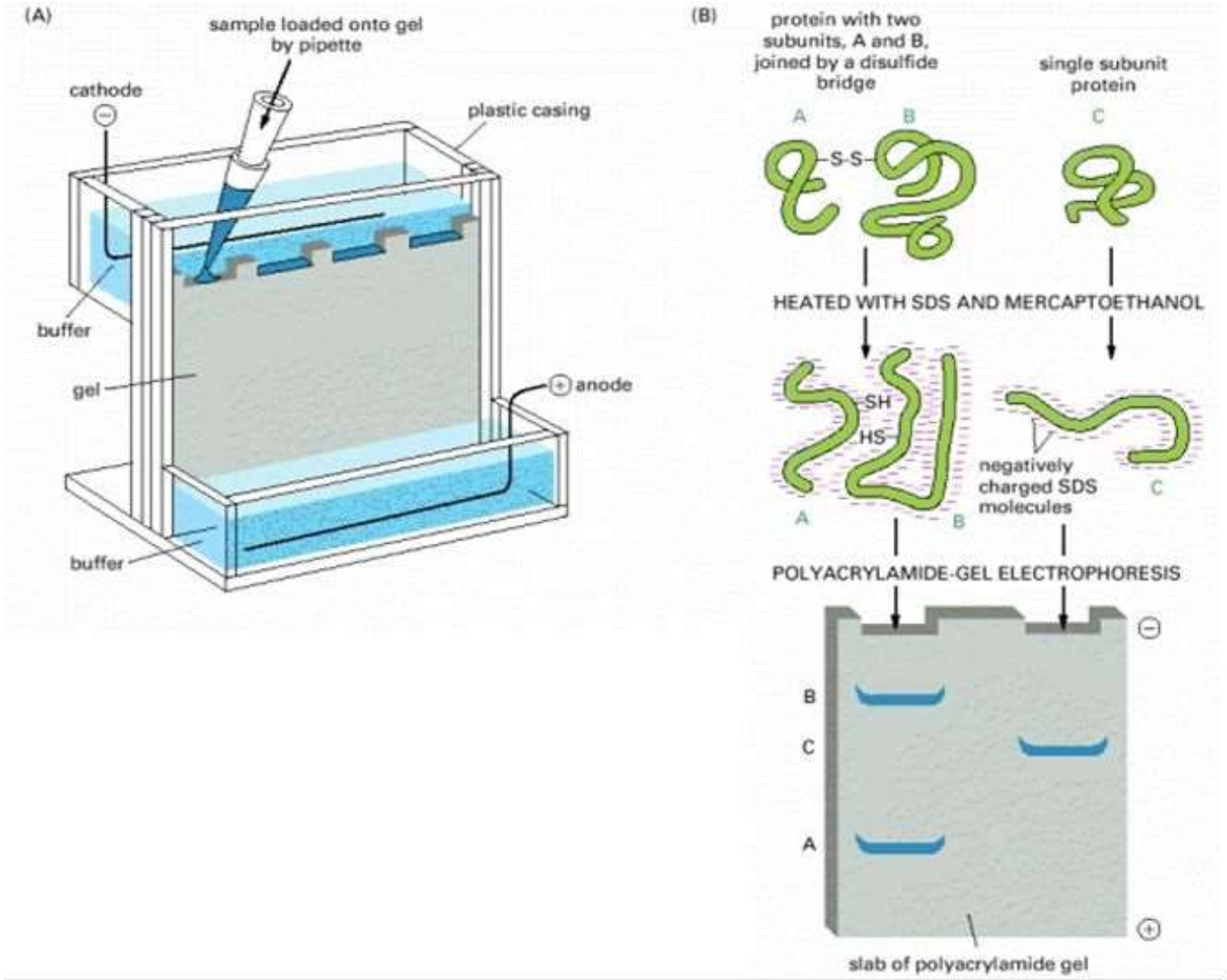


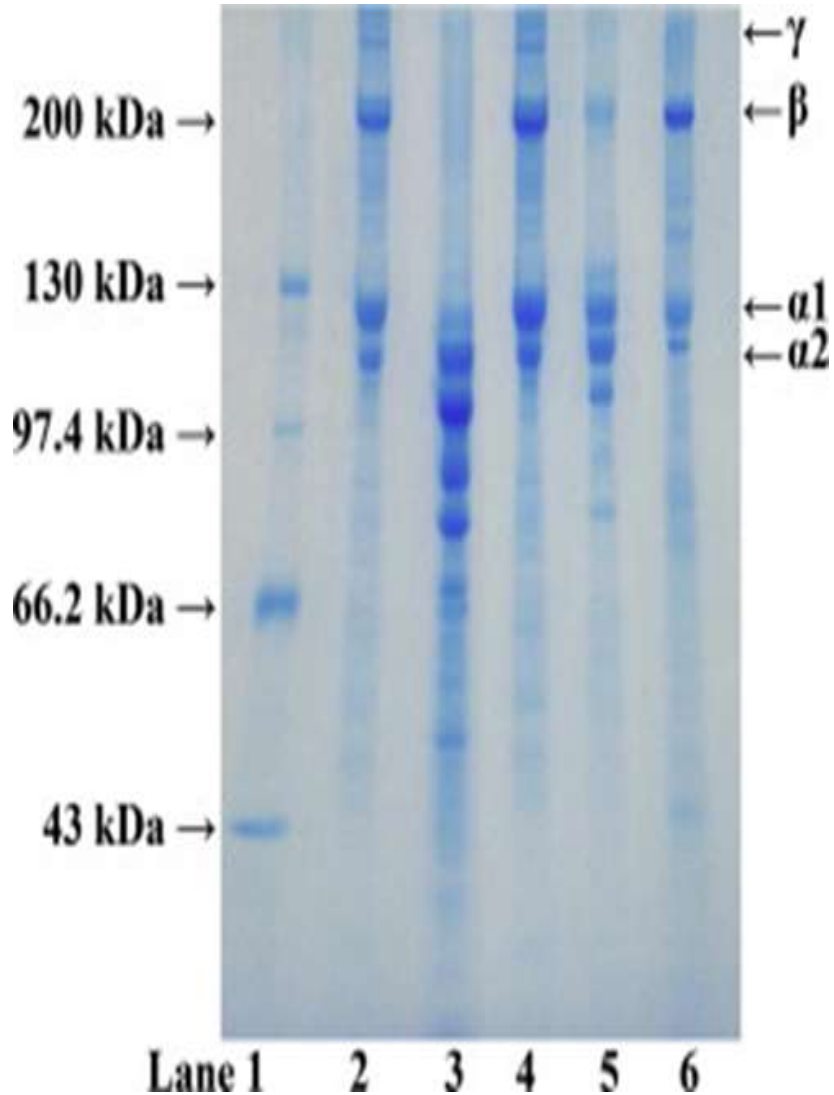
Direction of migration of samples in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie blue staining

SDS Polyacrylamide Electrophoresis





Applications of Polyacrylamide Gel Electrophoresis (PAGE)

- Measuring molecular weight
- Determination of protein subunits or aggregation structures
- Estimation of protein purity
- Protein quantitation
- Monitoring protein integrity
- Analysis of the number and size of polypeptide subunits

Advantages of Polyacrylamide Gel Electrophoresis (PAGE)

- Stable chemically cross-linked gel
- Greater resolving power (Sharp bands)
- Can accommodate larger quantities of proteins without significant loss in resolution
- The pore size of the polyacrylamide gels can be altered in an easy and controllable fashion by changing the concentrations of the two monomers
- Good for separation of low molecular weight fragments

Disadvantages of Polyacrylamide Gel Electrophoresis (PAGE)

- Generally more difficult to prepare and handle, involving a longer time for preparation than agarose gels
- Toxic monomers
- Gels are tedious to prepare and often leak
- Need new gel for each experiment Stable chemically cross-linked gel