Aim: Protein Profile Study of given protein samples by SDS PAGE

Requirements: Vertical slab gel or tube gel electrophoresis apparatus, SDS buffer, TEMED, Acrylamide, Sample buffer, ammonium per-sulphat, 2-Mercaptoethanol, glycerol or 10% sucrose solution, bromophenol blue, Coomassie blue stain, methanol, acetic acid and standard protein (marker) and unknown protein samples

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Principle

1. Principle of Electrophoresis:

- The migration and separation of charged particles (ions) under the influence of an electric field
- An electrophoretic system consists of two electrodes of opposite charge (anode, cathode), connected by a conducting medium called an electrolyte
- When charged molecules are placed in a support medium under an electric field, they migrate toward either the positive or negative pole according to their net charges
- The rate and direction of movement in the electric field depends on the molecule's size and net electric charges of the molecule
- Usually electrophoresis is used to separate macromolecules, such as DNA, RNA, and proteins

2. SDS PAGE:

- SDS PAGE is the most widely used method for analysis of any protein mixture, monitoring purity of proteins and to determine their molecular weights
- Sodium Dodecyl Sulphate (CH3 (CH2)10 CH2OSO3- Na+) is an ionic detergent that readily binds to proteins
- At pH 7.0 in the presence of 1 % w/v SDS and 2- Mercapto ethanol, proteins dissociate into their subunits and bind to SDS, which completely masks the natural charge giving a constant negative charge to their mass ratio
- The sieving effect of polyacrylamide (PAGE) is important support medium in this technique which separate a range of molecular weights depends on the pore size of the gel
- By running standard proteins of known molecular weight on the same gel, the molecular weight of unknown protein samples can be determined
- Mobility of protein in SDS gel is expressed as relative mobility (Rf) with respect to the tracking dye bromophenol blue

Procedure

1. Preparation of reagents and buffer:

- **TEMED: 5 % v/v**
- **SDS Buffer: Sodium phosphate buffer pH 7.1 containing 0.2% SDS**
- **Running Buffer: Dissolve 30.0 g of Tris base, 144.0 g of glycine, and 10.0 g of SDS in 1000 ml of H_2O pH 8.3 dilute to 1X before use**
- **Sample buffer: Sodium phosphate pH – 7.1 containing 1 % w/v SDS and 1 % w/v 2- Mercaptoethanol**
- **Acrylamide solution: Dissolve 50 g Acrylamids and 1.36 g Bis-Acrylamids in water to 200 ml**
- **Ammonium Persulphate: 10 % w/v in water (prepare flesh)**
- **Bromophenol blue: 0.25 % w/v in sample buffer**
- **Coomassie blue: 0.2 % w/v in Methanol : Acetic acid (7%w/v): Water 5 : 1 : 5 filter and store in dark battle**

2. Preparation of Sample:

- Dissolve 1 mg of protein sample in 1 ml of sample buffer, heat for 2 min. at $100^{o}C$ if necessary
- Add 10 µl of protein solution with 45 µl of SDS buffer, 10 µl of bromophenol blue and 15 µl 10% sucrose or 15 µl of glycerol
- The 2-Mercaptoethanol maintains a reducing environment, the bromophenol blue is act as tracking dye and glycerol increases the density of mixture
- Treat the marker (Standard) protein similarly

3. Preparation of Gel:

- The electrophoresis gel is divided into two layers
- The lower one is high concentration Polyacrylamide, called separating gel or electrophoresis gel
- The upper one is a macroporous gel with low concentration Polyacrylamide, called stacking gel

A. Preparation of Separating gel:

Stock solution	Volume required for 12% gel
1.5 M Tris HCl Buffer pH 8.8	2.0 ml
Acrylamide stock	3.2 ml
Water	2.8 ml
10% SDS	80 µl
10% APS (Freshly Prepared)	100 µl
TEMED	20 µl

B. Preparation of Staking gel:

Stock solution	Volume required for 12% gel
0.5 M Tris HCl Buffer pH 6.8	1.0 ml
Acrylamide stock	1.0 ml
Water	3.0 ml
10% SDS	80 µl
10% APS (Freshly Prepared)	100 µl
TEMED	20 µl

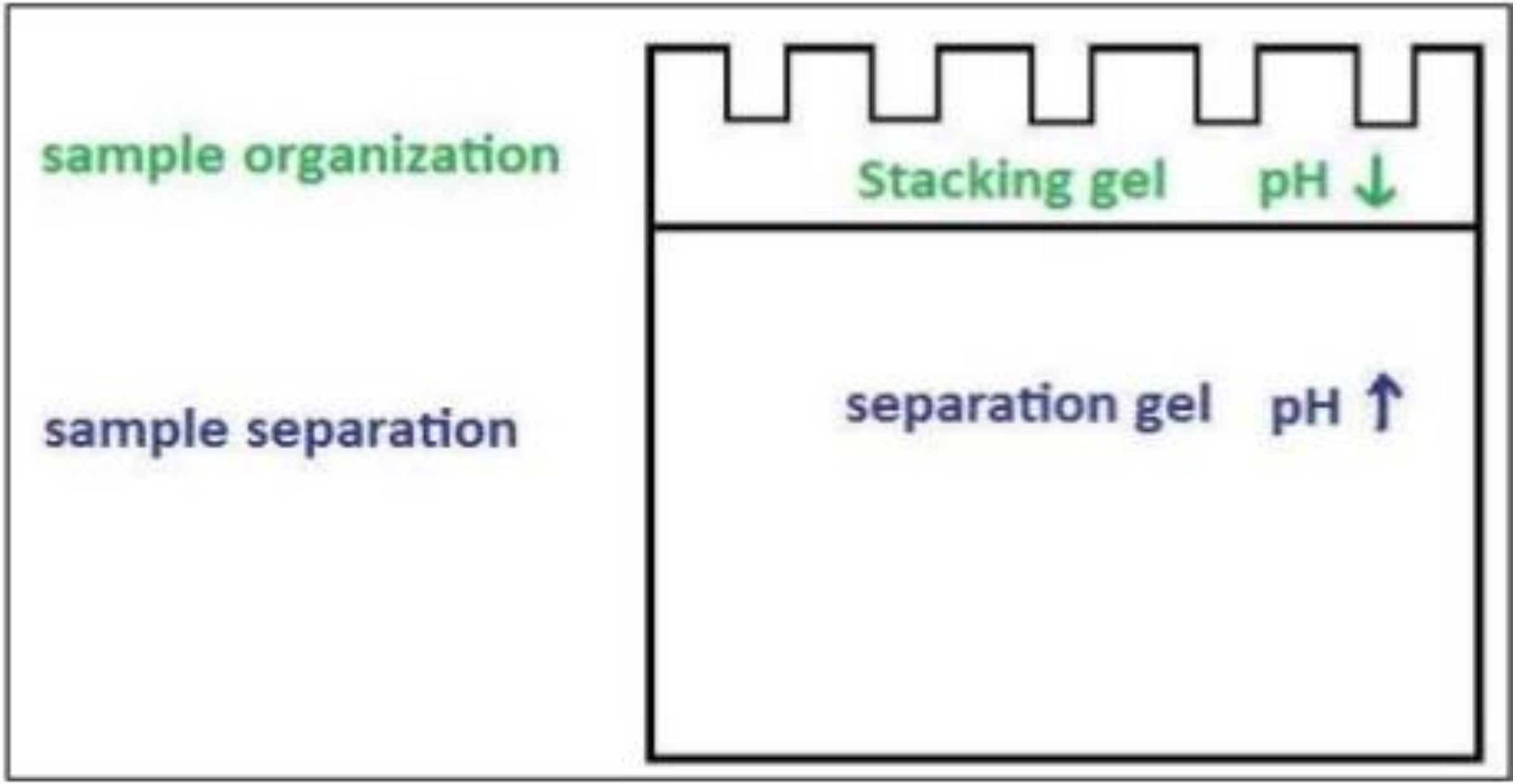
- Check the leakage of glass plates before casting the gel
- Cast the gel carefully between the glass plates provided
- Insert comb in staking gel part before solidification

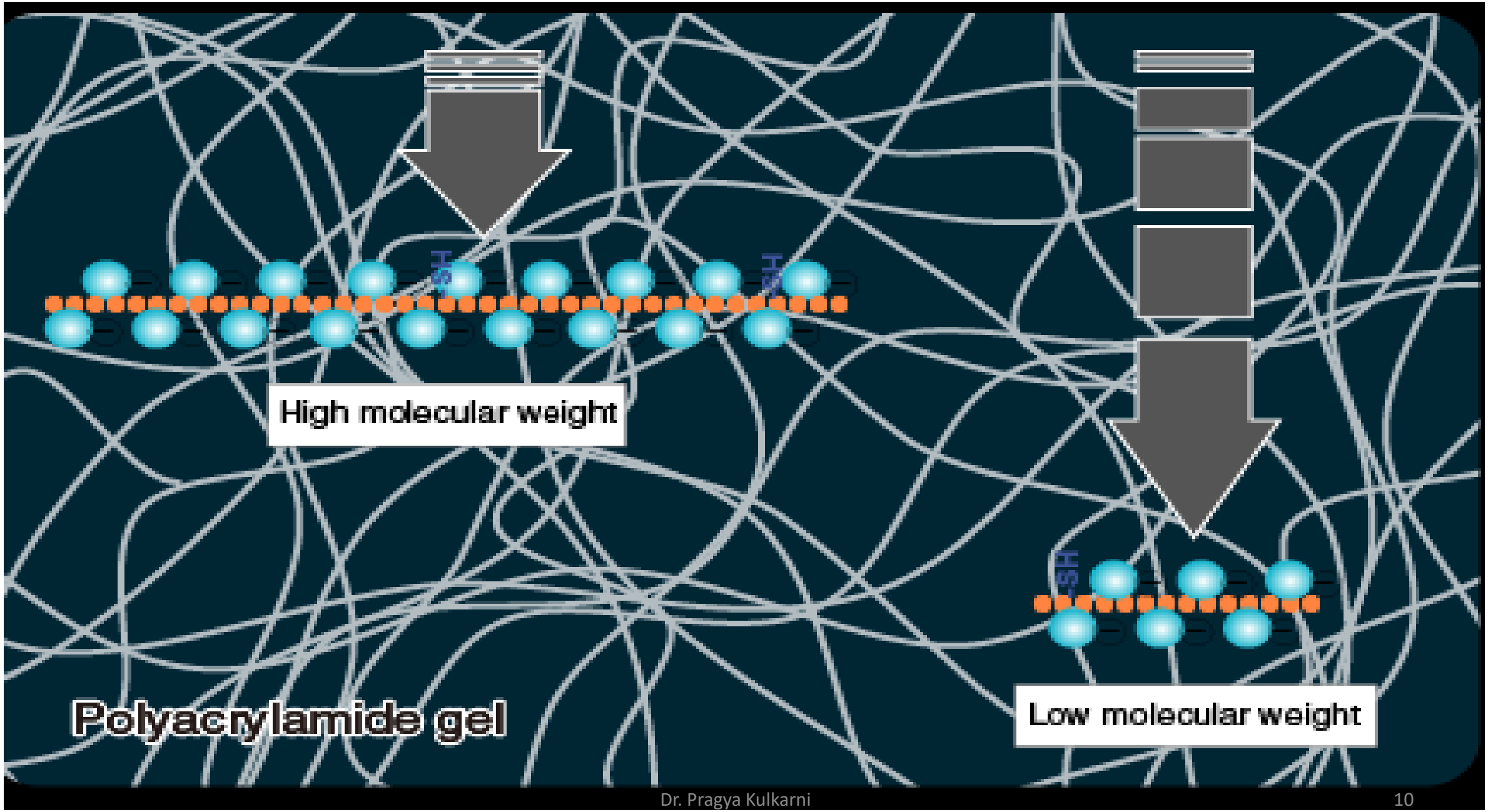
4. Electrophoresis:

- Carefully arrange the gel plate in buffer chamber, add the sample to sample well and fill the Running buffer to over flow the well
- Switch on the power pack at 50 mA / well until the tracking dye is almost at the end of gel

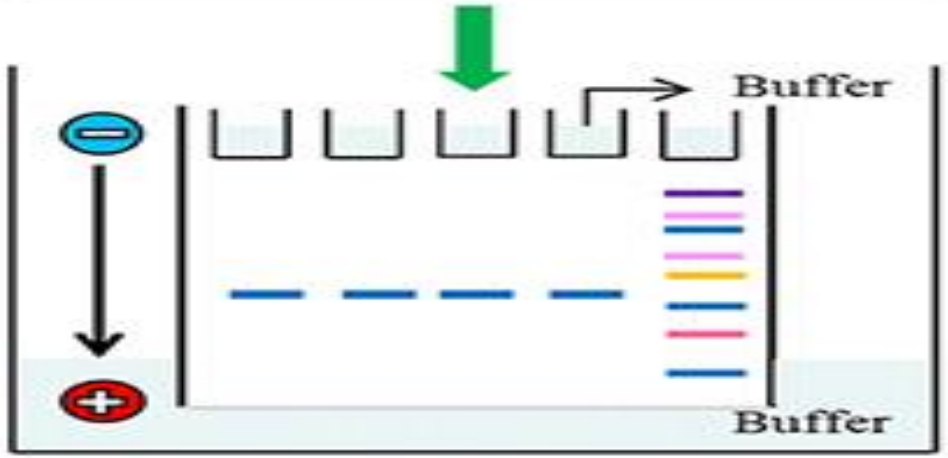
5. Fixing and Staining:

- Carefully remove the gel from gel plates, cut the staking gel portion and wash with water and immerse in solution of Coomassie blue stain for overnight
- Remove the excess staining by washing with water followed by rapid washing with de-staining solution

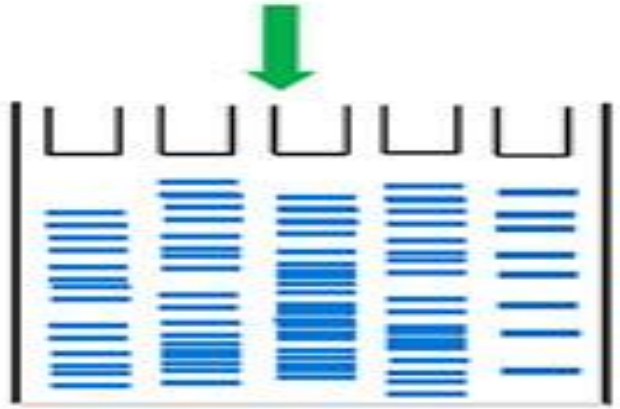

sample organization
Stacking gel
pH ↓
sample separation
separation gel
pH ↑


High molecular weight
Low molecular weight
Polyacrylamide gel

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

6. Mobility determination:

- Measure the distance traveled by each protein and calculates the mobility relative to the tracking dye

$$\text{Relative flow (Rf)} = \frac{\text{Distance travelled by protein (mm)}}{\text{Distance moved by bromophenol blue dye (mm)}}$$

7. Standard curve:

- Prepare standard curve with the values of Rf for the standard protein by using semi log graph paper

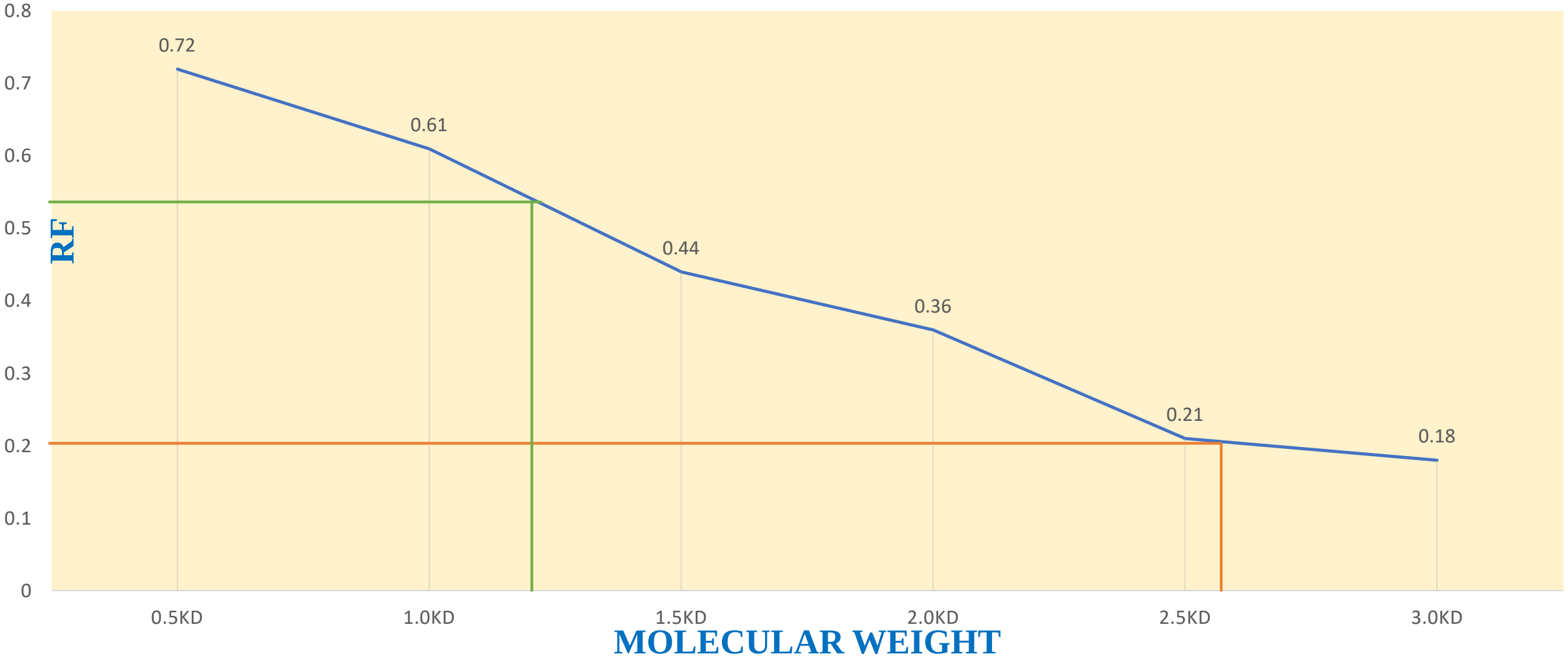
8. Mol. Weight determination:

- Determine the Mol.Wt. of unknown protein samples with the help of standard curve and analyze the results to make a conclusion

Observation Table

S.No.	Standard Protein (Mol.Wt.)	RF
1	0.5 KD	0.72
2	1.0 KD	0.61
3	1.5 KD	0.44
4	2.0 KD	0.36
5	2.5 KD	0.21
6	3.0 KD	0.18
7	Unknown Protein 1	0.20
8	Unknown Protein 2	0.53

Standard Curve



9. Result:

- Calculate the Mol. Wt. of unknown Protein bands with the help of Standard Curve and tabulate the result

10. Interpretation:

- Interpretation is given for the source or origin of polypeptide and their Mol.Wt.
- The complete profile gives the idea of total number of polypeptides, their molecular pattern and can be compared for molecular characterization of any organism
- It can also be used for comparison and to find out genetic relationship between organisms
- Similarity in banding pattern shows genetic relatedness of the source

Precautions

- The acrylamide should be handled carefully because it is a toxic material
- The buffers should be prepared accurately
- There should not be any air bubble in casted gel
- There should not be any voltage fluctuations while running
- Staining and de-staining should be done carefully
- Standard curve must be prepared accurately

Thank You……..