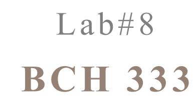
SODIUM DODECYL SULPHATE -POLYACRYLAMIDE
GEL ELECTROPHORESIS
[SDS-PAGE]


Lab#8
BCH 333

SDS-PAGE:

- ***SDS-PAGE:*** Sodium Dodecyl Sulfate-PolyacrylAmide gel Electrophoresis.
- The separation of macromolecules in an electric field is called ***electrophoresis.***
- For separating proteins by electrophoresis uses a discontinuous polyacrylamide gel.
- SDS-PAGE aim to separate and identify proteins according to their molecular weight.

- ***Applications:***

➢ Quantifying.

➢ Comparing (relative abundance).

➢ Characterizing proteins (e.g. determining MW of proteins).

➢ Checking purity of protein samples.

Principle of SDS-PAGE:

- In general, fractionation by gel electrophoresis is based on differences in size, shape and net charge of macromolecules (in molecules native condition using native gel electrophoresis).
- Systems where you separate proteins under native conditions cannot distinguish between these effects and therefore proteins of different sizes may have the same mobility in native gels.

➔In SDS-PAGE, this problem is overcome by:

1. Using an anionic detergent Sodium Dodecyl Sulfate (SDS), which binds strongly to most proteins.
2. Using disulfide reducing agent such as β-mercaptoethanol or DTT (dithiothreitol).

Principle of SDS-PAGE:

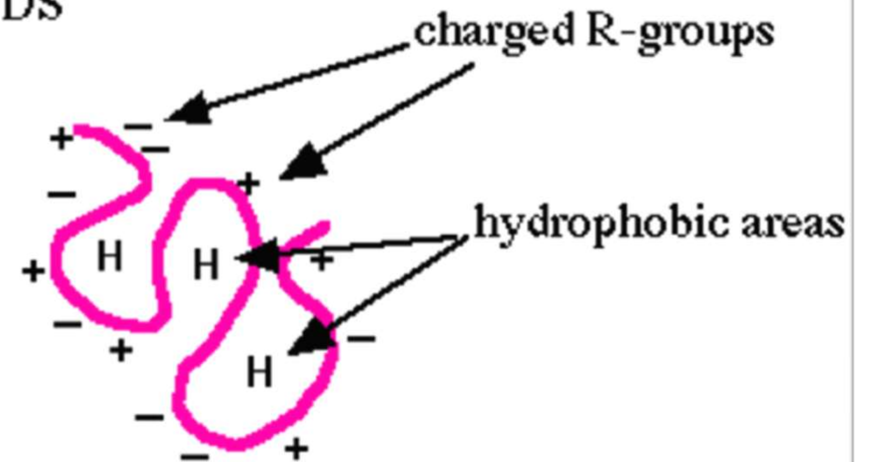
- Sodium Dodecyl Sulfate (SDS) is a detergent which ***denature proteins*** by binding to the hydrophobic regions, all non-covalent bonds will disrupt, and the proteins acquire a ***negative net charge.***
- A Concurrent treatment with a disulfide reducing agent such as β-mercaptoethanol or DTT (dithiothreitol) further breaks down the strong bound disulfide.

➔ ***So, the proteins samples are having uniformed structure and charge (-ve).***

Effect of SDS

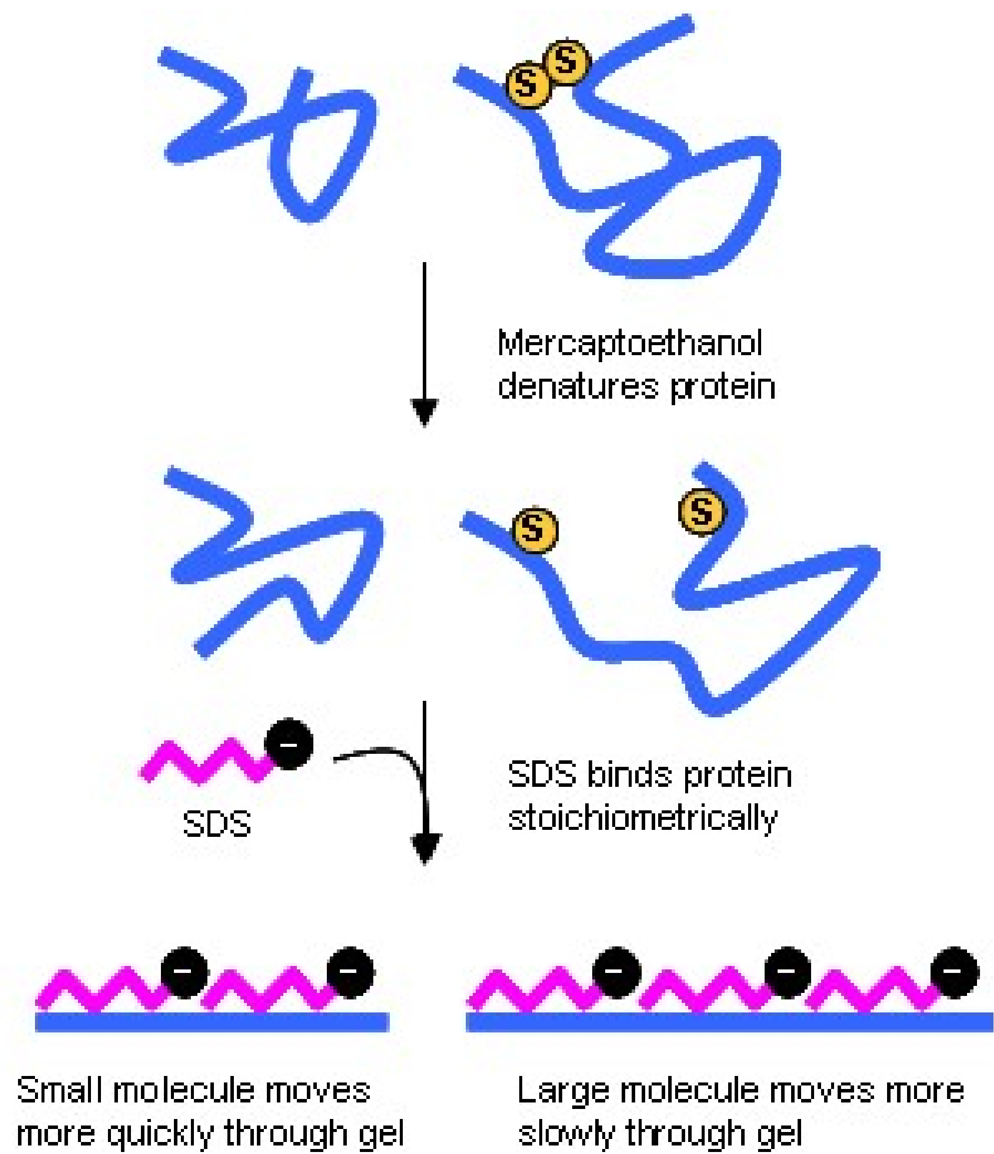
What is SDS?

The carbon tail has a neutral charge
and the sulphate group is negatively charged



AFTER SDS

Effect of β-mercaptoethanol



Principle of SDS-PAGE:

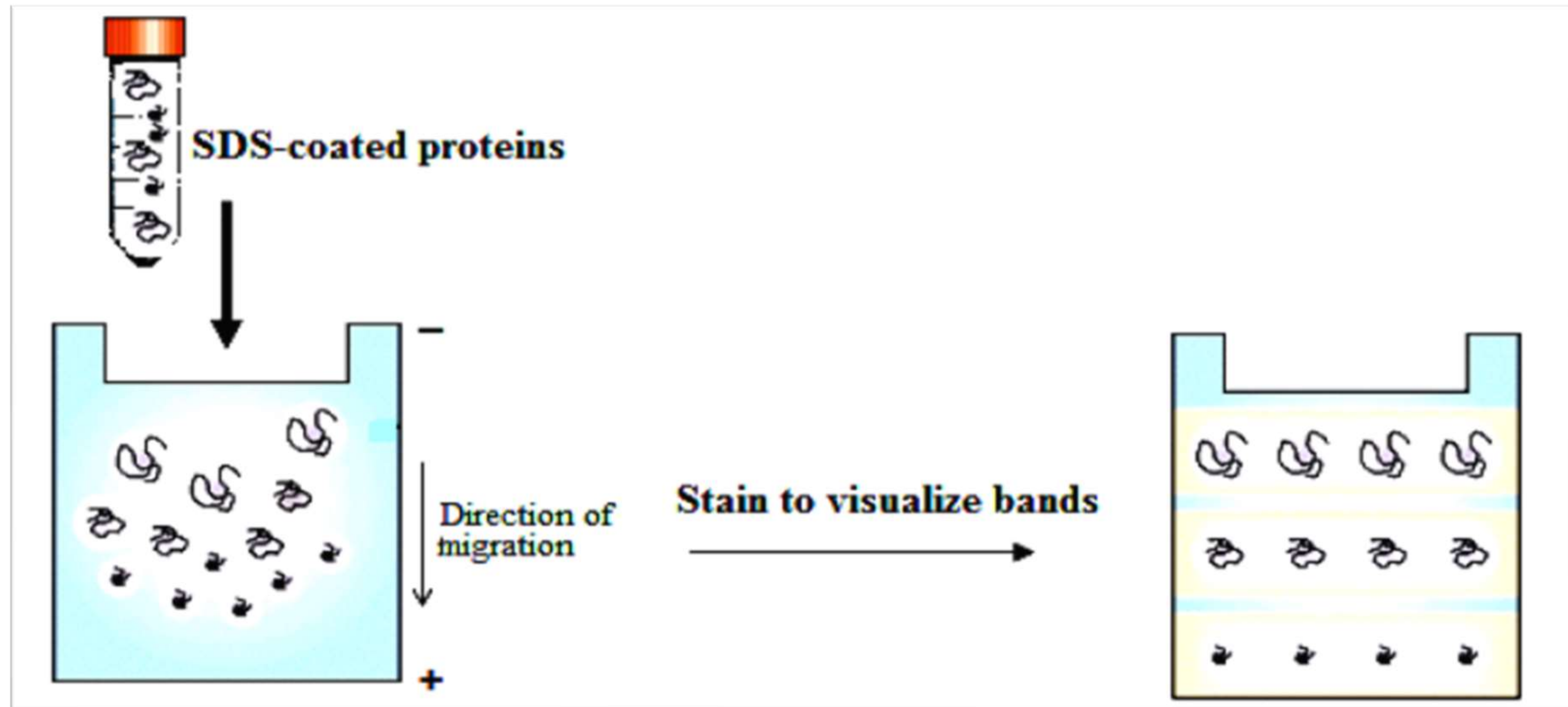
- Because the ***charge-to-mass ratio is nearly the same*** among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides (separation will depend on their molecular weight only).

➔i.e The electrophoretic mobility of the molecules is now considered to be a function of their size (the protein samples are having uniformed structure and charge → the separation will depend on their molecular weight only).

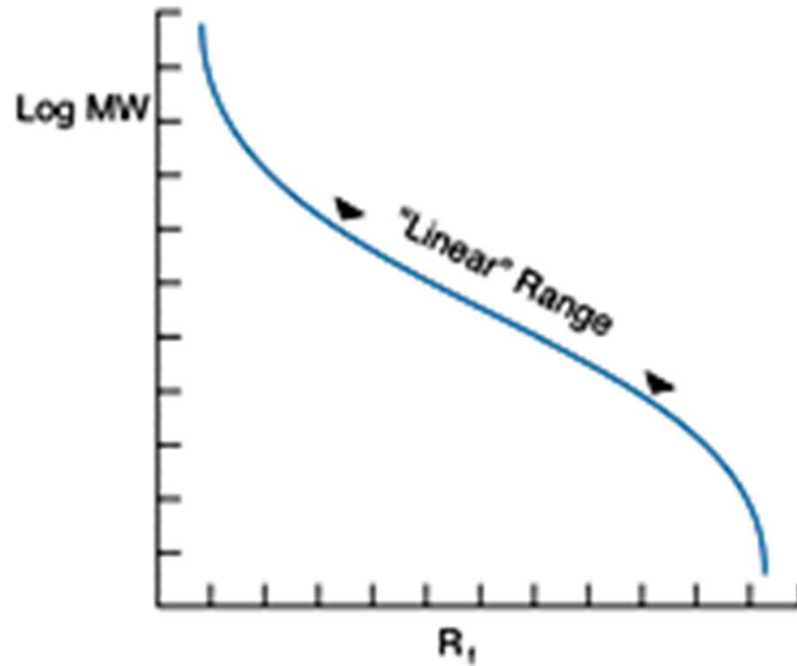
- Polyacrylamide gels ***restrain larger*** molecules from migrating ***as fast as smaller molecules.***
- Thus, small proteins migrate faster through the gel under the influence of the applied electric field, whereas large proteins are successively retarded, due to the sieving effect of the gels.

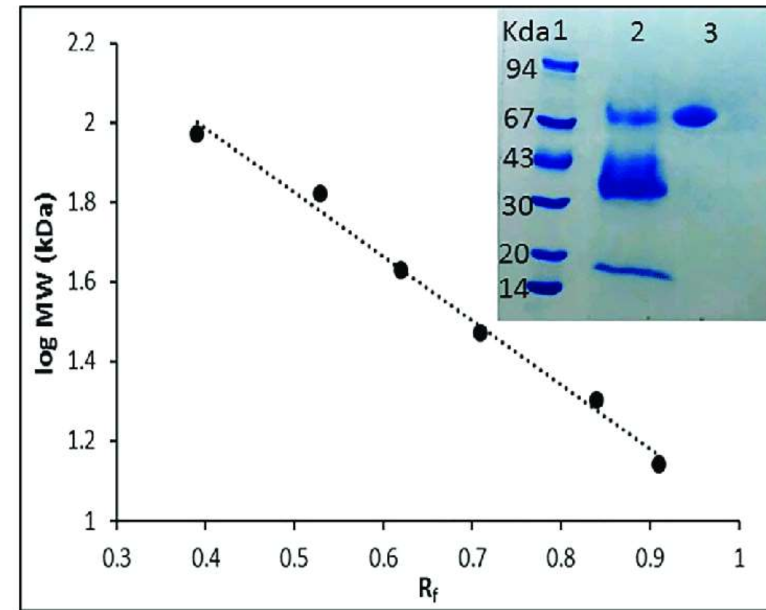
➔i.e the migration of the (SDS-treated proteins), towards the anode (+) is inversely proportional to the logarithms of their molecular weights, or more simply expressed:

Small proteins migrate faster through the gel



SDS-treated proteins have very similar charge-to-mass ratios, and similar shapes. During SDS-PAGE, the rate of migration of SDS-treated proteins is effectively determined by molecular weight.





Graph of log MW vs. R_f is sigmoidal, it is nearly linear for a range of molecular weights excluding very small and very large MW.

Practical part

OBJECTIVES:

- To separate and calculate the molecular size of proteins by comparing the separated bands with known standard molecular weight marker.

SDS-Polyacrylamide Gel Electrophoresis
preparation

1. Sample Preparation:

- 40 μl of protein sample + 10 μl of disruption buffer ➔ boil the mixture 3 min at 99 ̊C.

- ***Disruption buffer [loading buffer] contain:***

➢ 10% (w/v) SDS [?]

➢ 1M Tris/HCl, pH 6.8

➢ Glycerol [?]

➢ β-Mercaptoethanol [?]

➢ Bromophenol blue [?]

2.Polyacrylamide Gel (Acrylamide stock):

- The polyacrylamide gel is formed by co-polymerization of acrylamide and a ***cross-linking*** by N,N'-methylene-bis-acrylamide "bis-acrylamide ".
- Polyacrylamide= acrylamide + bis-acrylamide.

- ***To polymerize the gel a system:***
- 1- Ammonium PerSilfate (APS) [initiator].
- 2-TetraMethylene Ethylene Diamin (TEMED) [catalyst].

$CH_2 = CHCONH_2$
Acrylamide

+

$CH_2(NHCOHC = CH_2)_2$
N,N,N',N'-methylenebisacrylamide

Free radical catalyst

$$-CH_2-CH-[-CH_2-CH-]_n-CH_2-CH-$$

(side chains: CO–NH–CH_2–NH–CO cross-links; $CONH_2$ on repeating units)

$$-CH_2-CH-[-CH_2-CH-]_n-CH_2-CH-$$

Fig. 10.5 The formation of a polyacrylamide gel from acrylamide and bis-acrylamide.

2.Polyacrylamide Gel:

- It is composed of ***two*** gels layers:

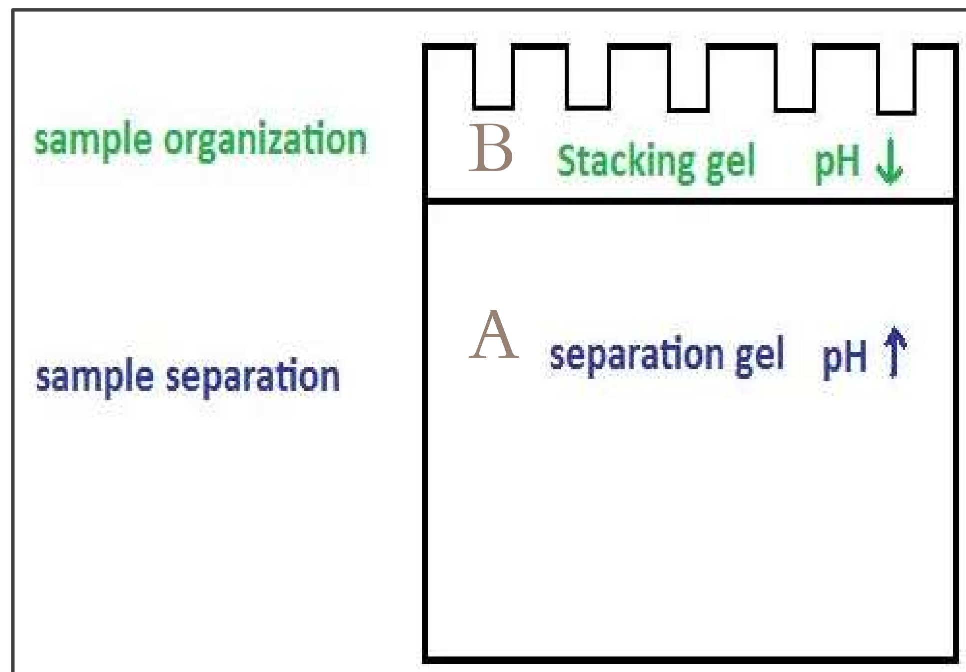
A. Separation gel:

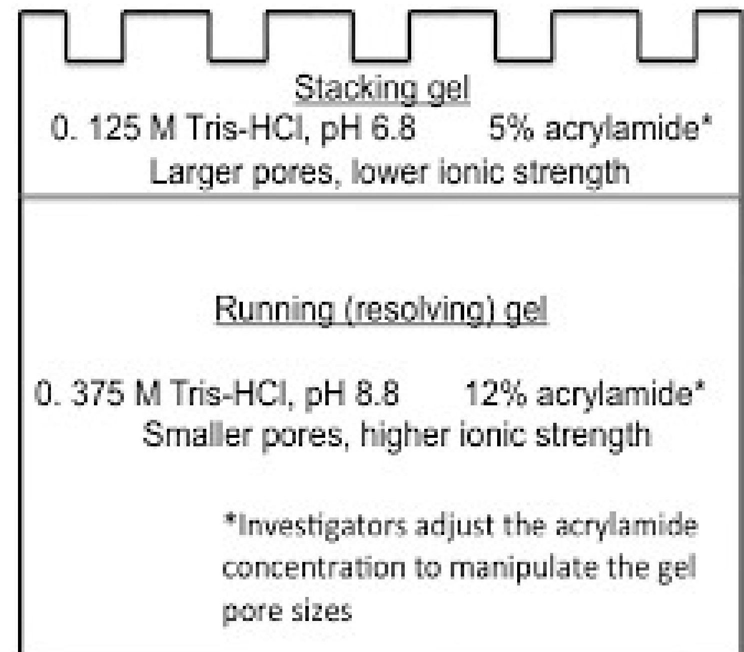
- HIGH concentration of the acrylamide stock and pH value.

B. Stacking gel:

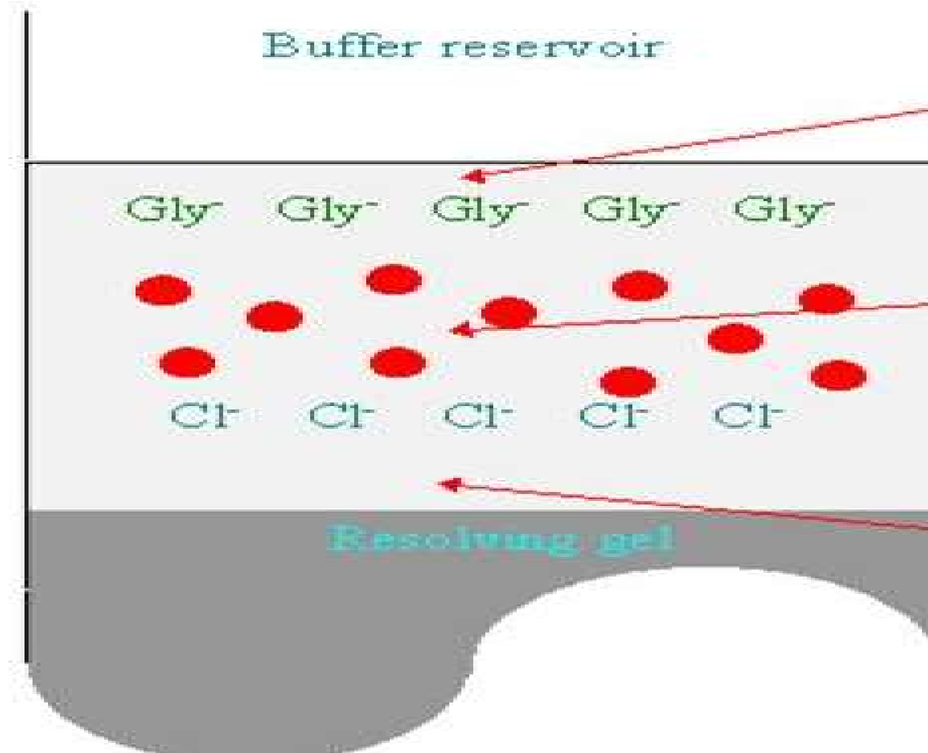
- LOW concentration of the acrylamide stock and pH value.

The gel's two layers:





Proteins are sandwiched between the Cl^- ions and the glycine molecules.



A. Separation Gel Contents:

- For 10 ml of separation gel:

Acrylamide%	*6%*	*8%*	*10%*	*12%*	*15%*
H_2O	5.2 ml	4.6 ml	3.8 ml	3.2 ml	2.2 ml
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	2 ml	2.6 ml	3.4 ml	4 ml	5 ml
1.5 M Tris (pH=8.8)	2.6 ml	2.6 ml	2.6 ml	2.6 ml	2.6 ml
10% w/v SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
10% w/v Ammonium persulfate (APS)	100 µl	100 µl	100 µl	100 µl	100 µl
TEMED	10 µl	10 µl	10 µl	10 µl	10 µl

*Note: APS and TEMED must be added right before each use.

- The acrylamide percentage in SDS PAGE gel depends on the size of the target protein in the sample.

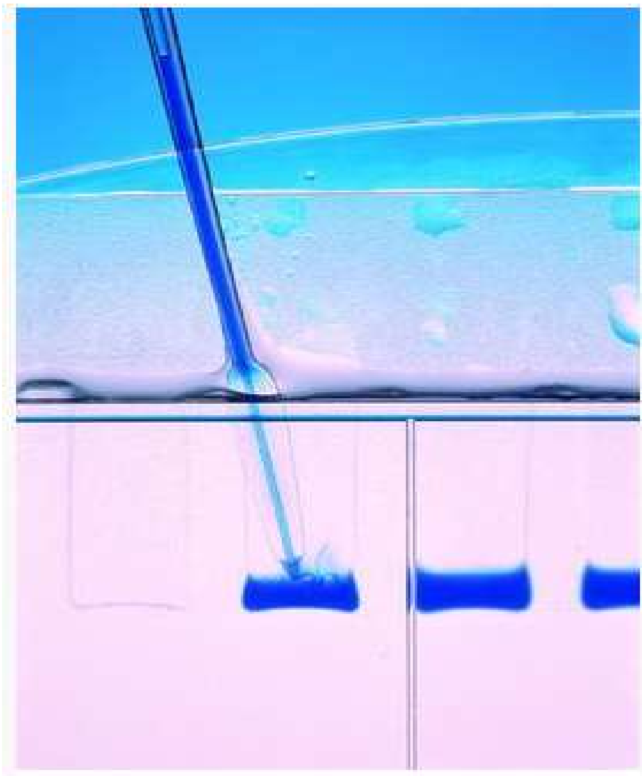
Acrylamide %	M.W. Range
7%	50 kDa - 500 kDa
10%	20 kDa - 300 kDa
12%	10 kDa - 200 kDa
15%	3 kDa - 100 kDa

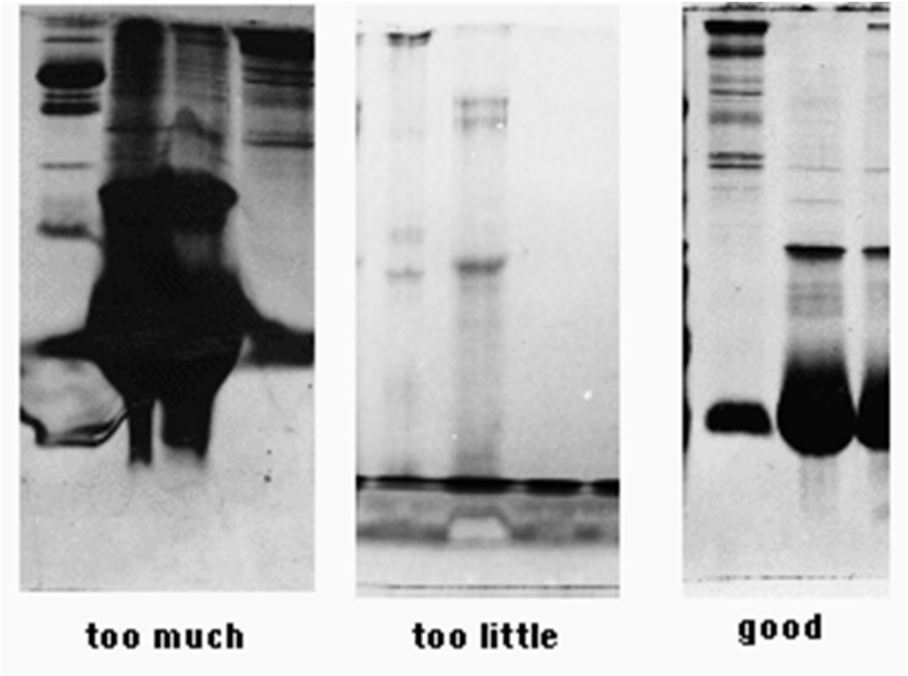
B. Stacking Gel Contents:

- For 5 ml of stacking gel:

	Volume
H_2O	2.975 ml
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	0.67 ml
0.5 M Tris-HCl (pH=6.8)	1.25 ml
10% w/v SDS	0.05 ml
10% w/v Ammonium persulfate (APS)	0.05 ml
TEMED	0.005 ml

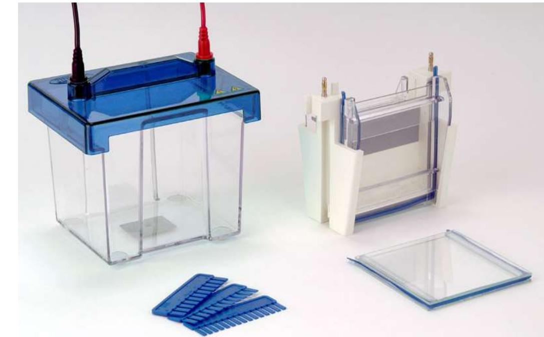
3. Sample Loading:





4. Running the gel using, Running buffer (pH 8.3):

- It helps is deliver the electric current through the gel.
- It contains:
  - ➢ Tris-HCl.
  - ➢ Glycine.
  - ➢ SDS.

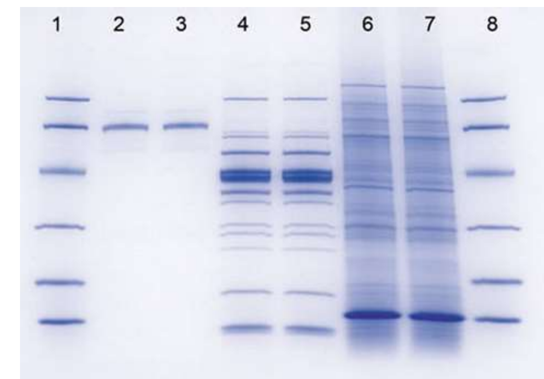


5. Stain the gel using staining buffer :

- It contains:
  - ➢ Glacial acetic acid
  - ➢ Methanol
  - ➢ ***Coomassie brilliant blue 250-R.***

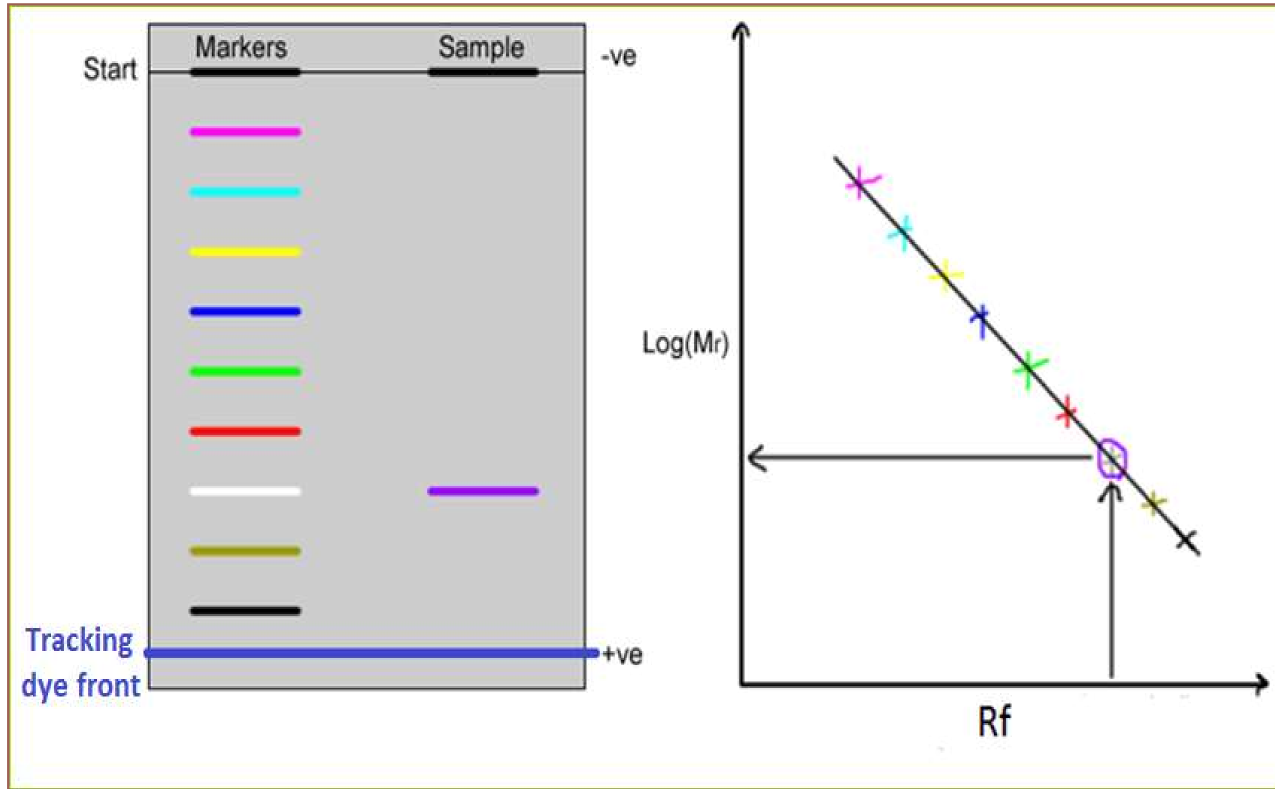
6. De-stain the gel using De-staining buffer:

- It contains:
  - ➢ Glacial acetic acid
  - ➢ Methanol



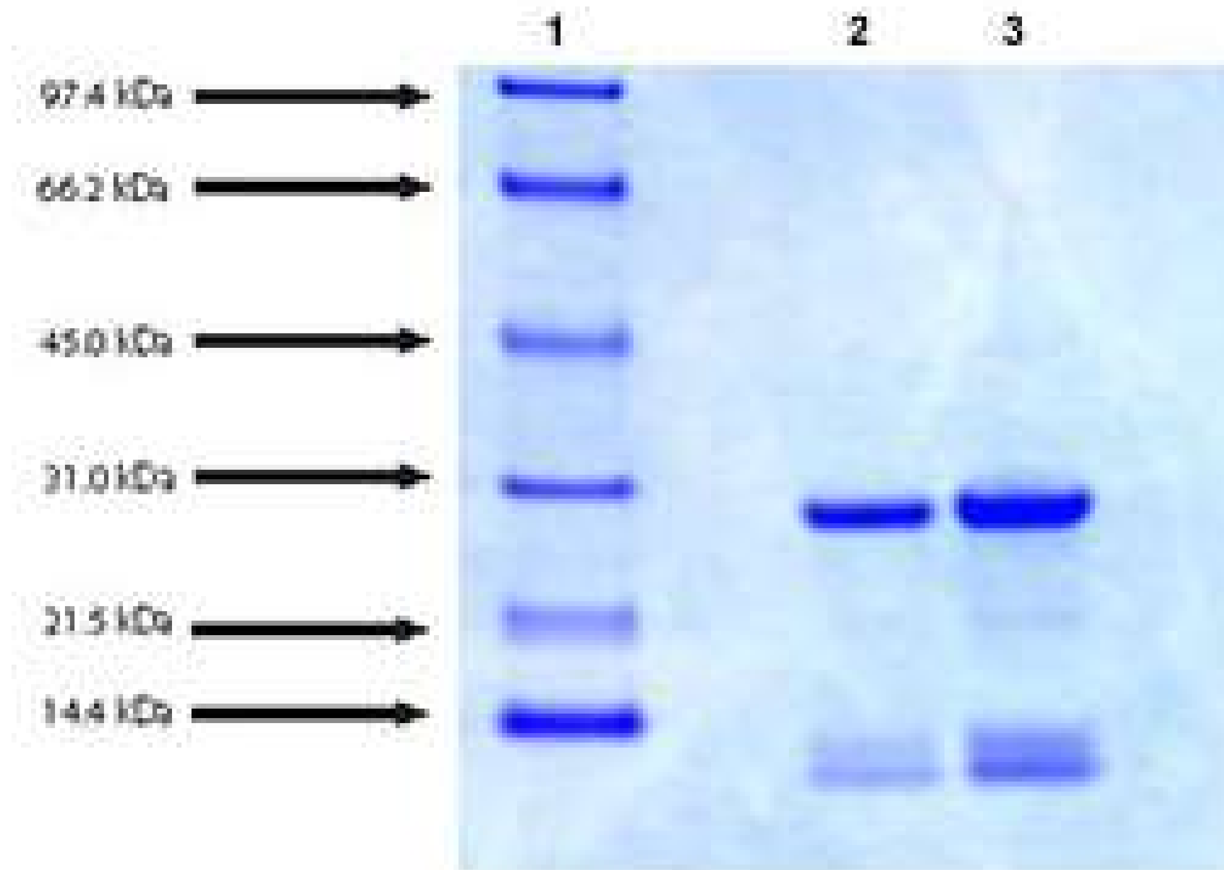
Data Analysis

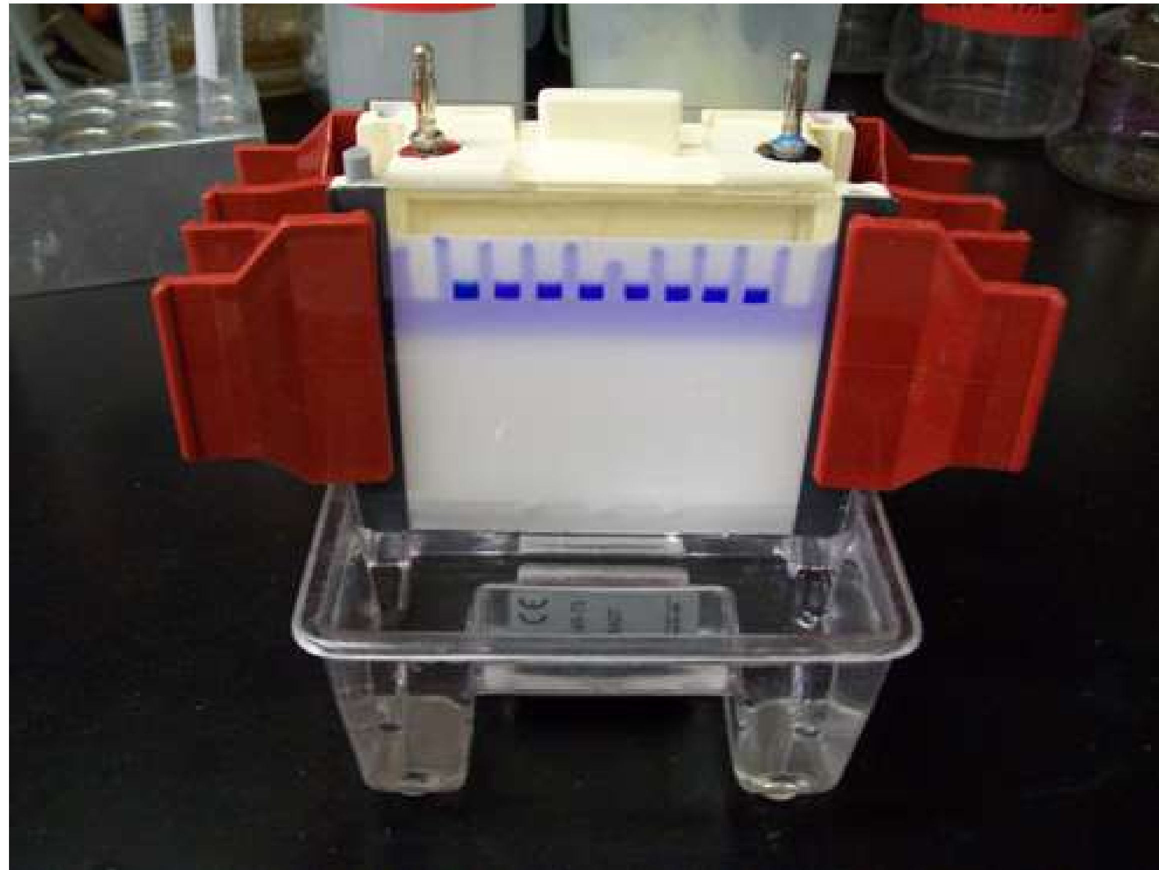
For Molecular Weight Determination:



$$- R_f = \frac{\text{Distance of migration of sample}}{\text{Distance moved by tracking dye}}$$

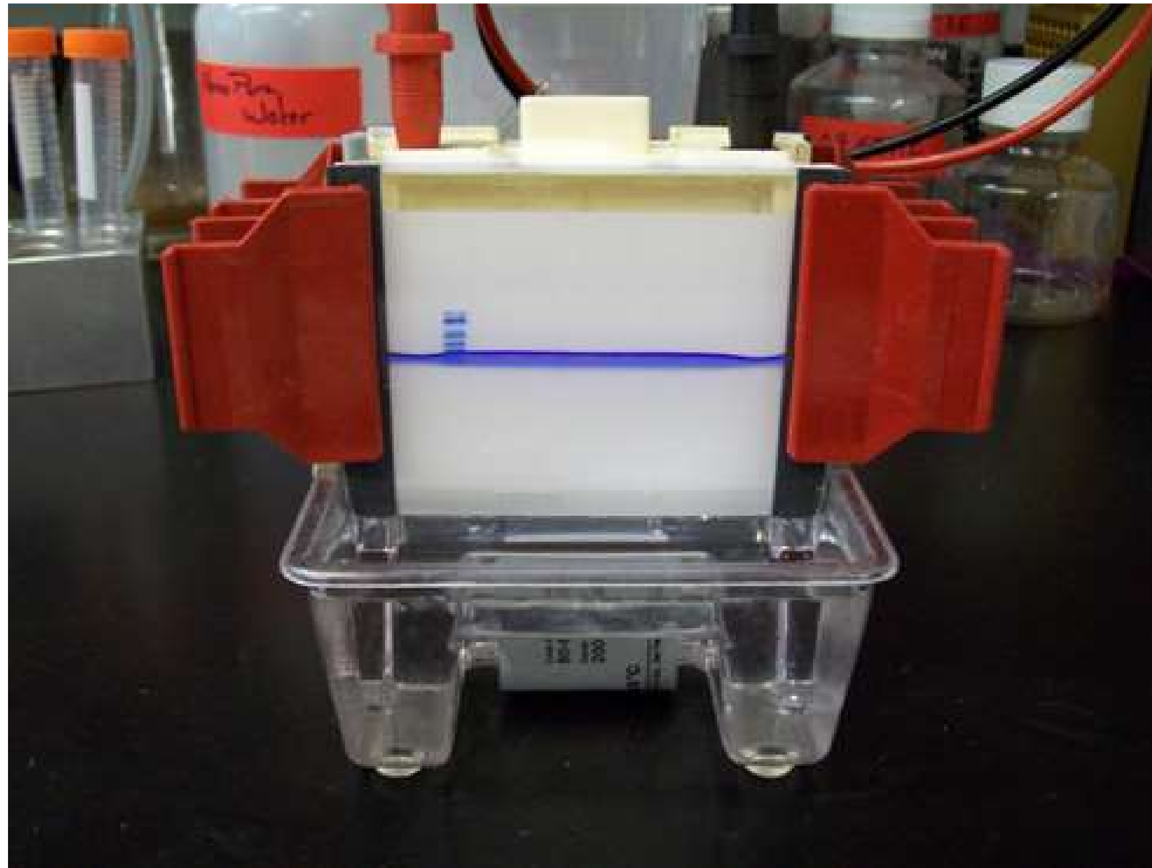
For Molecular Weight Determination cont':



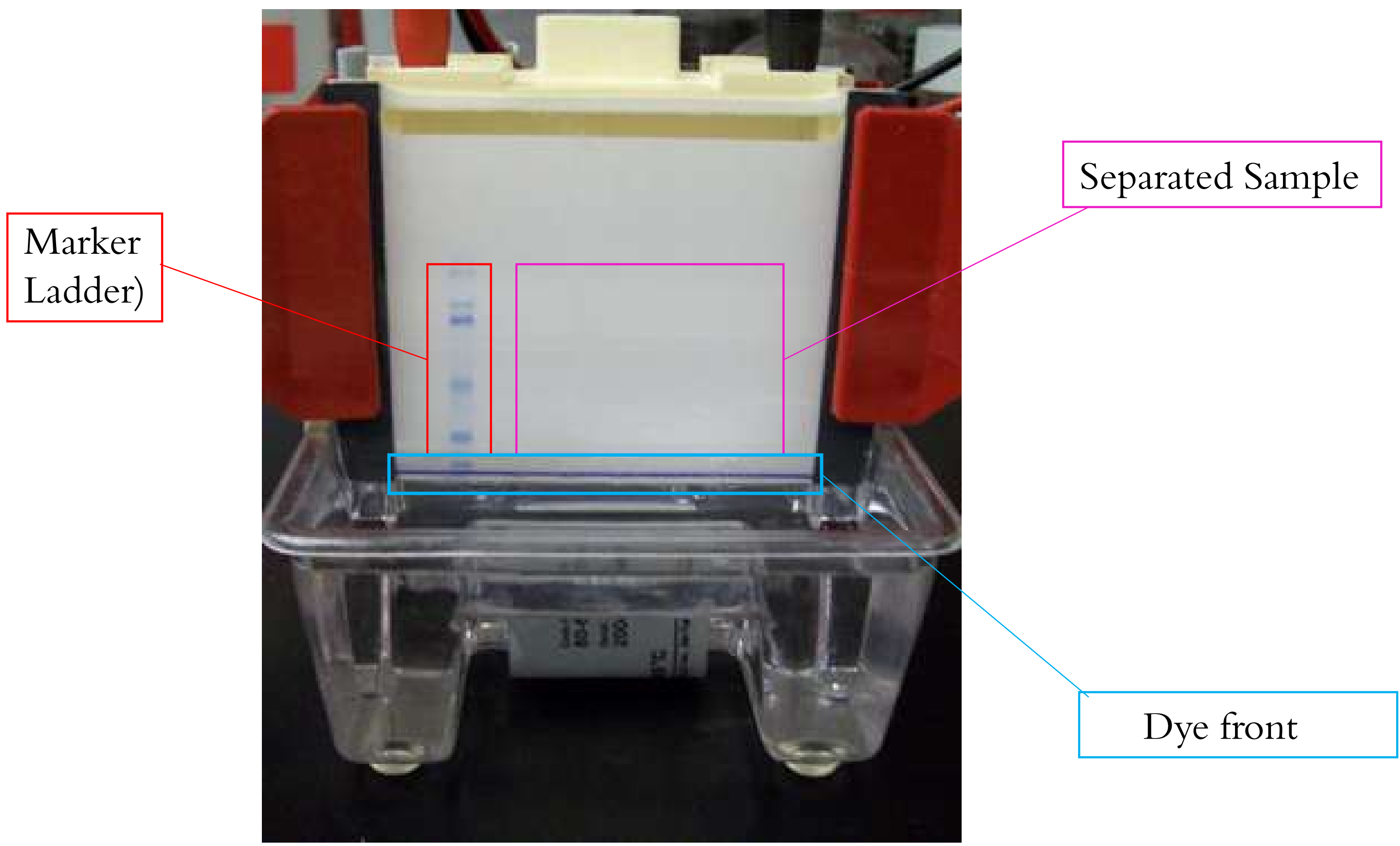


At beginning

After a while

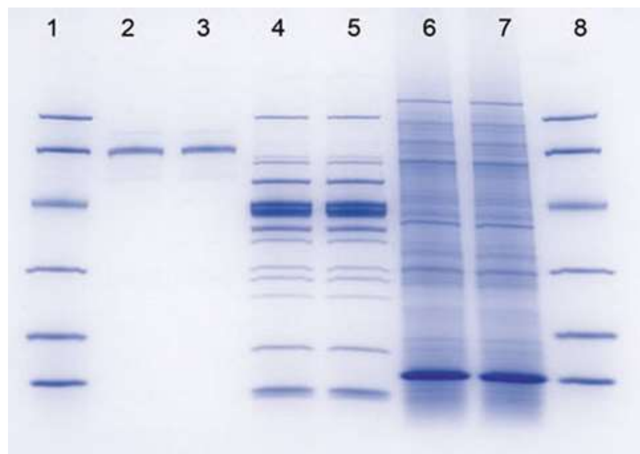


After more mins.

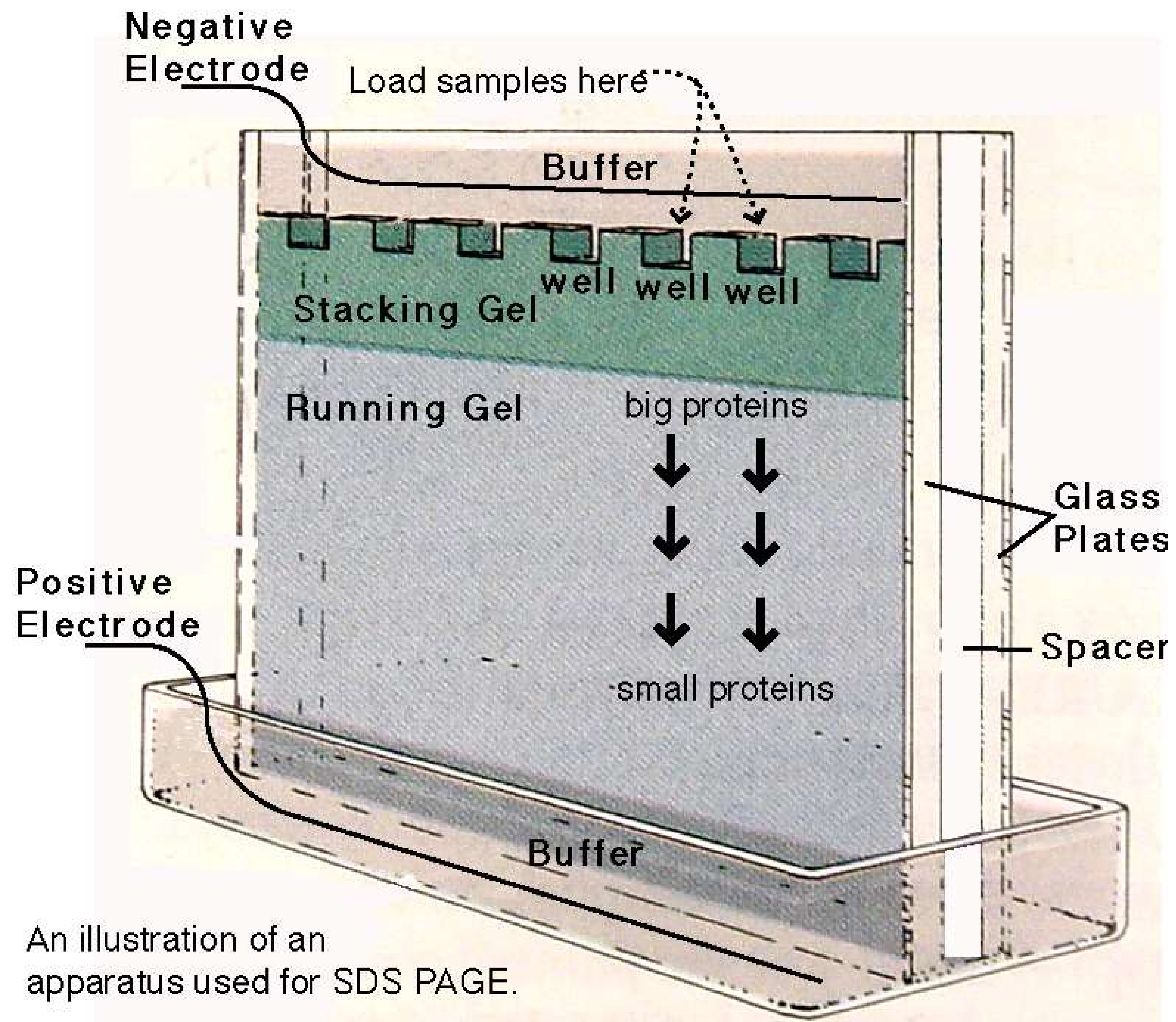


At the end

Staining



De Staining



An illustration of an
apparatus used for SDS PAGE.

Supporting Materials:

- ***SDS PAGE principles – simple animated tutorial:***

https://www.youtube.com/watch?v=3CrzY7jb9fQ

https://www.youtube.com/watch?v=i_6y6Z5UvwE