جامعة الملك سعود
King Saud University

BCH 462

Sodium Dodecyl Sulfate -PolyAcryl amide Gel Electrophoresis

[SDS-PAGE]

Objectives:

-Separation of bacterial proteins using SDS-PAGE.

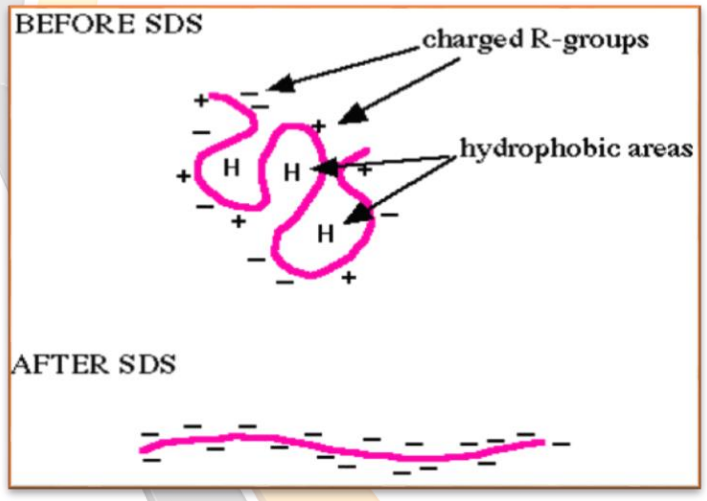
SDS-Polyacrylamide Gel Electrophoresis

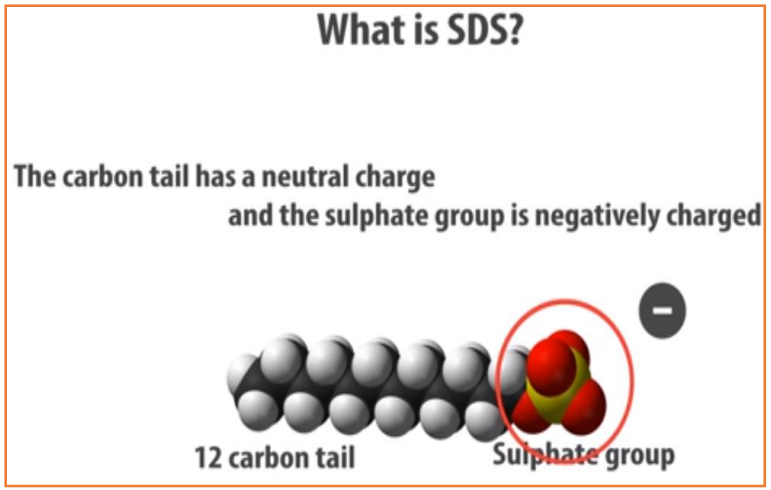
-Sodium Dodecyl Sulfate-Polyacrylamide gel Electrophoresis (SDS-PAGE), is a technique widely used in biochemistry ,forensics, genetics and molecular biology to separate and identify proteins according to their molecular weight.

-This method separates proteins based primarily on their molecular weights.

Principle:

-Sodium Dodecyl Sulfate [SDS]: is a detergent which denature proteins by binding to the hydrophobic regions, **all non-covalent bonds will disrupted** and the **proteins acquire a negative net charge**.

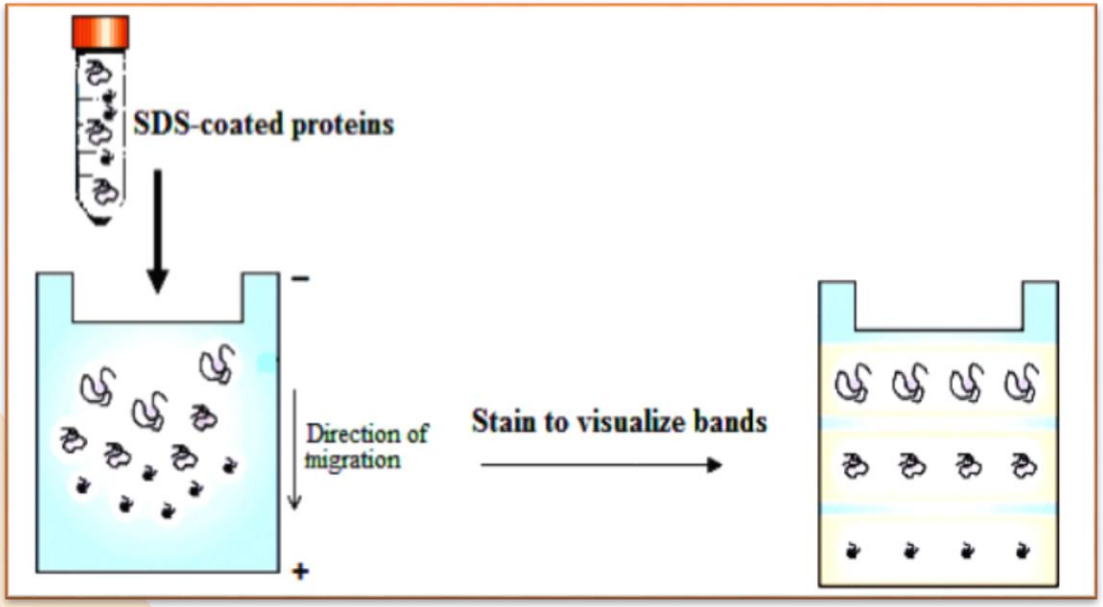




-Treatment with a disulfide reducing agent such as β-mercaptoethanol or DTT (dithiothreitol), which further denatures the proteins **by reducing disulfide linkages**, thus overcoming some forms of tertiary protein folding, and breaking up quaternary protein structure

So, the proteins samples are having uniformed structure and charge(-ve) → the separation will depend on their molecular weight only.

-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



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

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 ".

-To polymerize the gel a system, ammonium persilfate **(initiator)** and tetramethylene ethylene diamin (TEMED) **[catalyst],** are added.

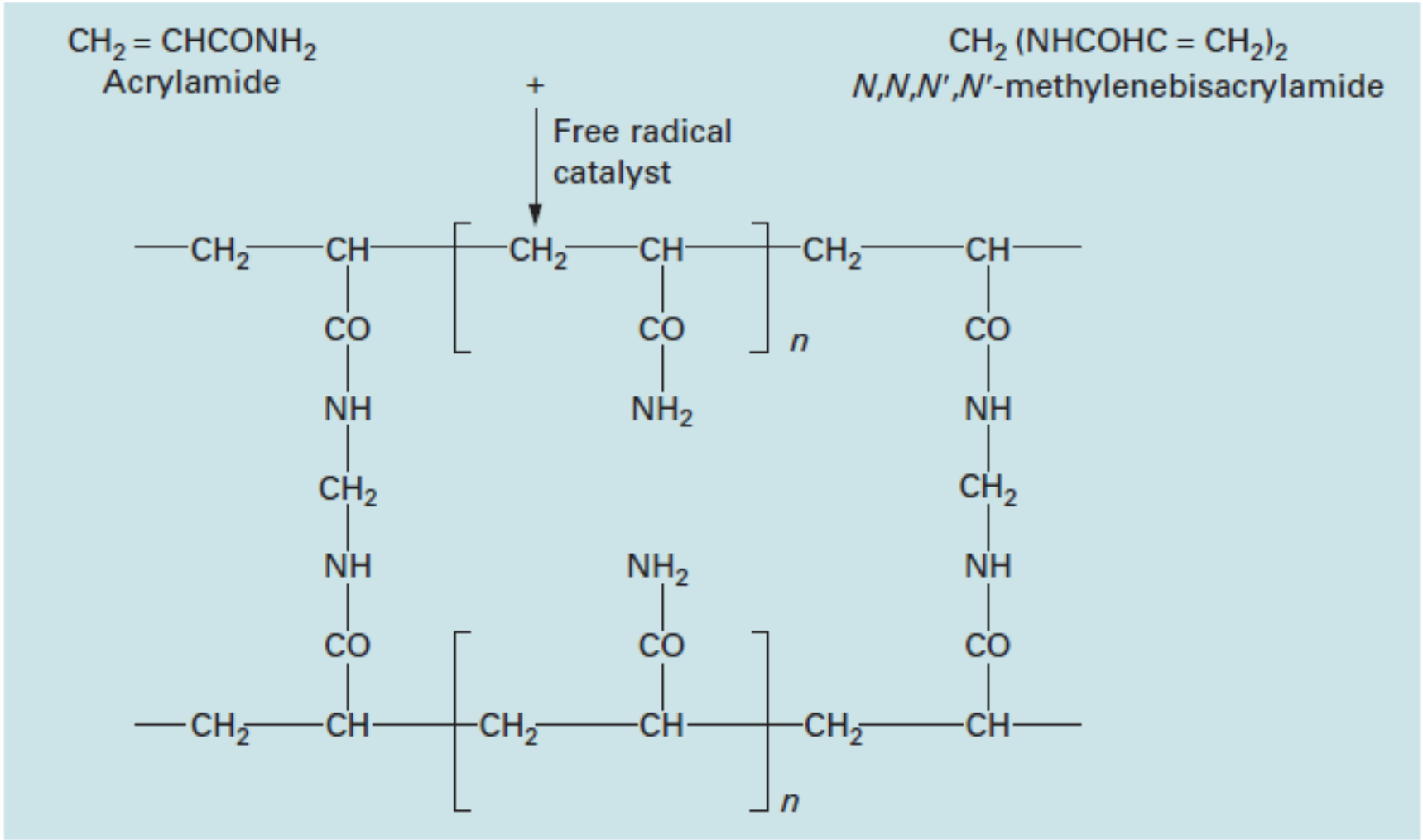
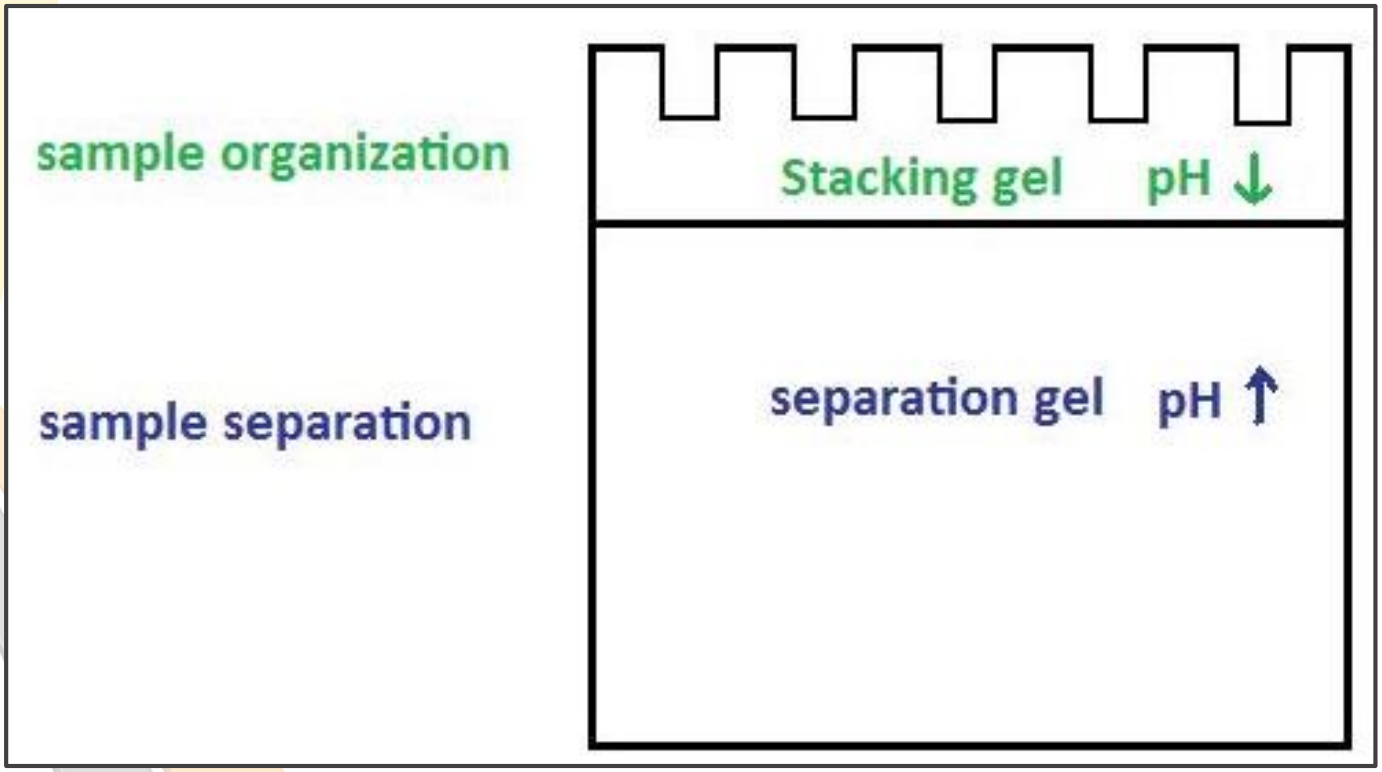


Fig. 10.5 The formation of a polyacrylamide gel from acrylamide and bis-acrylamide.



SDS-Polyacrylamide Gel Electrophoresis

SDS-Polyacrylamide Gel Electrophoresis preparations:

1-Sample Preparation:

-40µl of protein sample + 10 µl of disruption buffer→ boil the mixture 3minets at 99℃.

-Disruption buffer [loading buffer] contain:

-10% (w/v) SDS [?]

-1M Tris/HCl, pH 6.8

-Glycerol [?]

-β-Mercaptoethanol [?]

-Bromophenol blue [?]

2- Polyacrylamide Gel Preparation :

Acrylamide stock should be prepared first :

-Cross-linked polyacrylamide gels are formed from the polymerisation of acrylamide monomer in the presence of smaller amounts of N,N'-methylene-bisacrylamide (some time referred to as 'bis'-acrylamide).

A-Separation gel preparation:

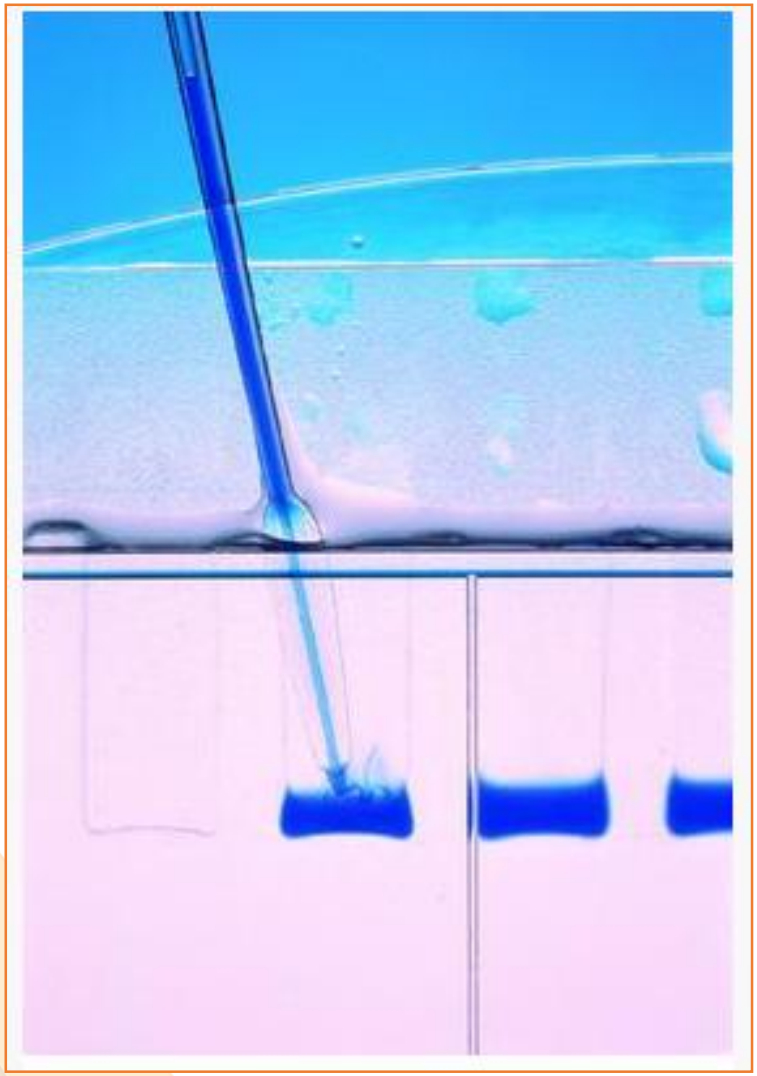
Stock solutions	Volume of stock solution required to make 12% polyacrylamide gel
1.5 M Tris/HCl, pH 8.8	2.0 ml
Acrylamide stock	3.2 ml
Water	2.8 ml
10% SDS	80 µl
10% Ammonium persulphate (fresh)	100 µl
TEMED	10 µl

B-Stacking gel preparation:

Stock solutions	Volume of stock solution required to make 7% polyacrylamide gel
0.5M Tris/HCl, pH6.8	1.0 ml
Acrylamide stock	1.0 ml
Water	3.0 ml
10% SDS	80 µl
10% Ammonium persulphate (fresh)	50 µl
TEMED	5 µl

http://www.youtube.com/watch?v=EDi_n_0NiF4

3-Loading the samples:



4-Running the gel using , Running buffer 1x pH 8.3:

It is contain:

-Tris-HCl .

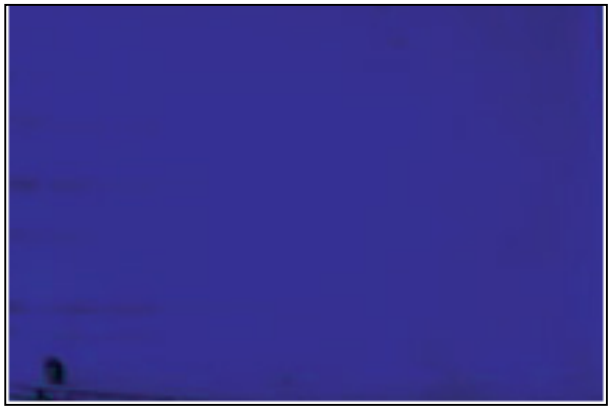
-Glycine.

-SDS.

5- Stain the gel using staining buffer :

It is contain:

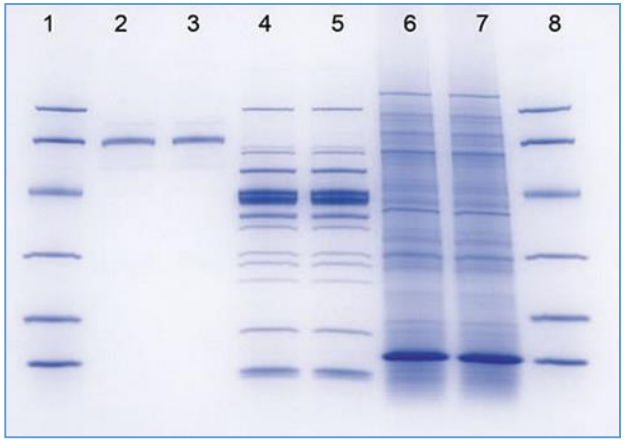
-Glacial acetic acid
-Methanol
-Coomassie brilliant blue 250-R



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

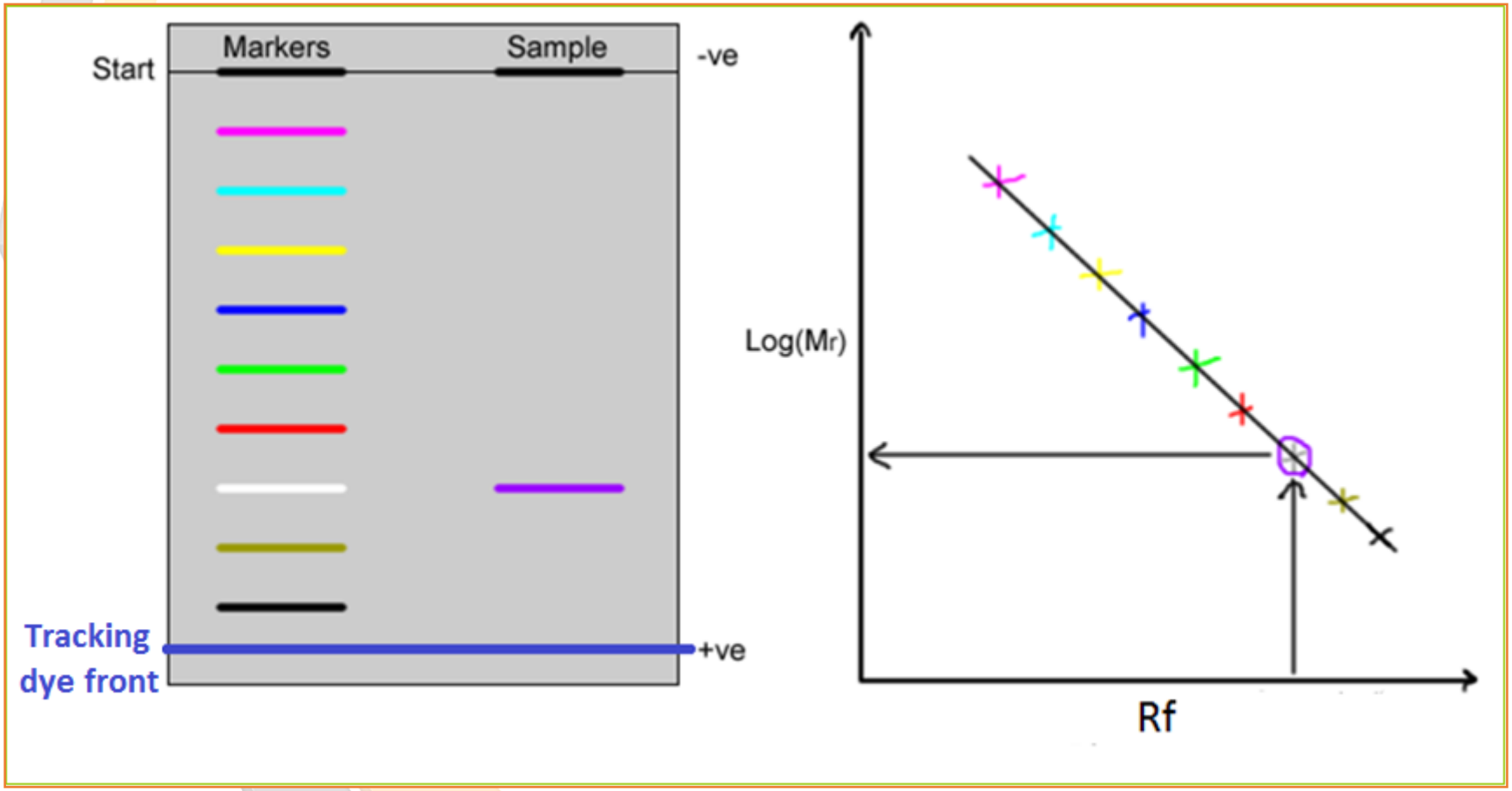
It is contain:

-Glacial acetic acid
-Methanol

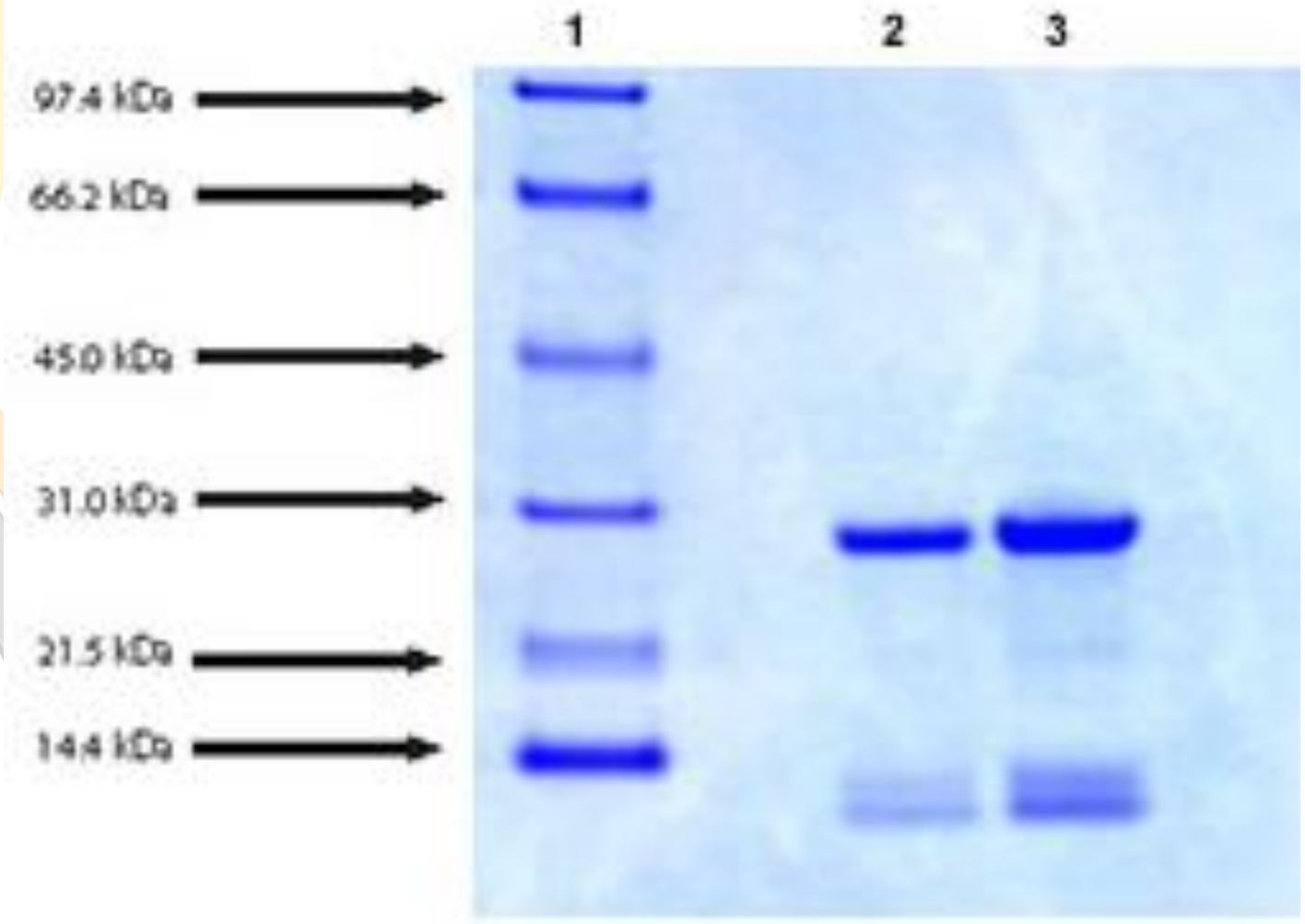


7-Analysis:

For Molecular weight Determination.



- Rf = Distance of migration of sample
  Distance moved by tracking dye


1
2
3
97.4 kDa
66.2 kDa
45.0 kDa
31.0 kDa
21.5 kDa
14.4 kDa

Applications:

1. To detect the purity of the protein.
2. Determine of protein molecular weight.
3. Determine the presence of certain protein.