

SDS-Polyacrylamide Gel Electrophoresis

BCH 462 [practical]

Objectives:

- Separation of protein fractions 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 be disrupted and the proteins acquire a negative net charge.

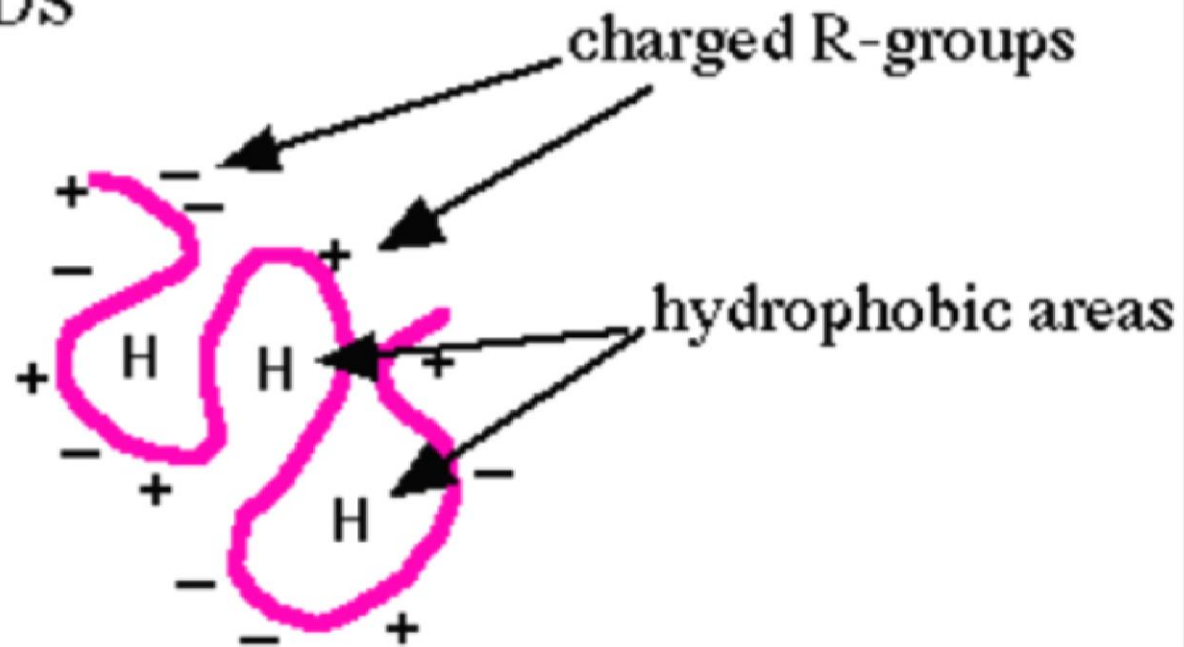
A Concurrent treatment with a disulfide reducing agent such as β -mercaptoethanol or DTT (dithiothreitol) further breaks down the macromolecules into their subunits.

-So, the protein samples are having uniform structure and charge \rightarrow the separation will depend on their molecular weight only.

-Small proteins migrate faster through the gel under the influence of the applied electric field.

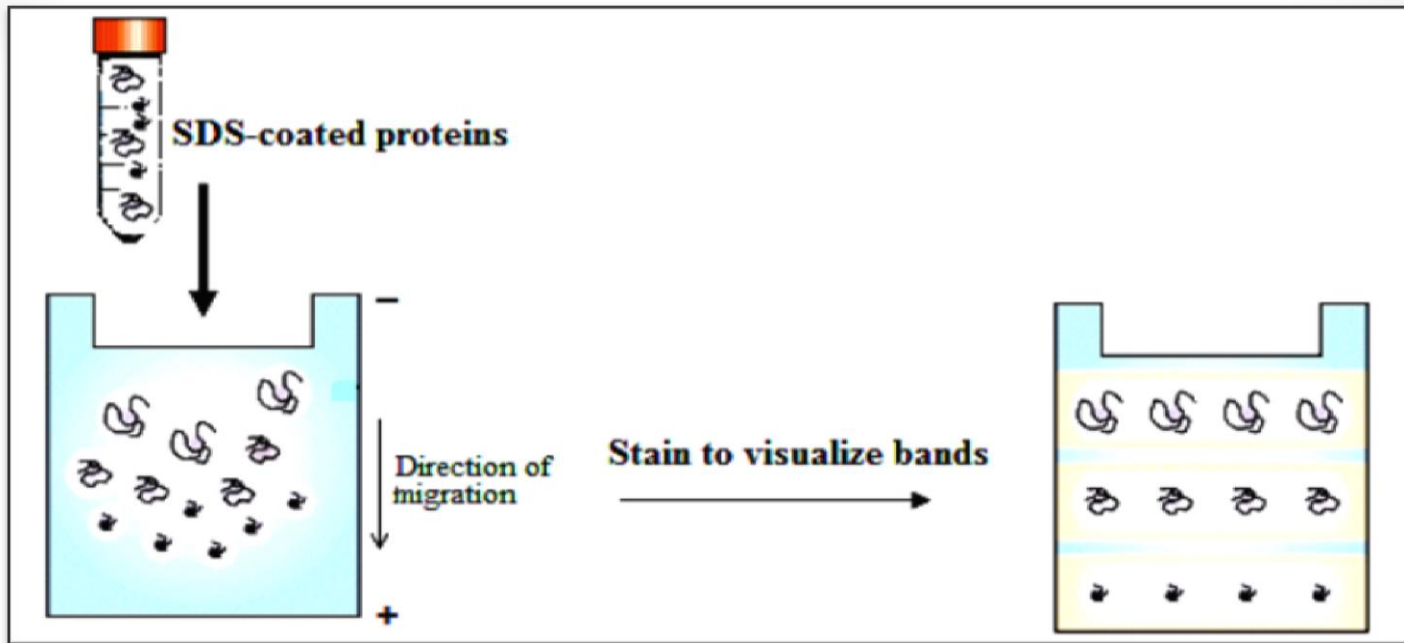
The number of SDS molecules that bind is proportional to the size of the protein, thereby in the electrical field, protein molecules move towards the anode (+) and are separated only according to their molecular weight.

BEFORE SDS



AFTER SDS





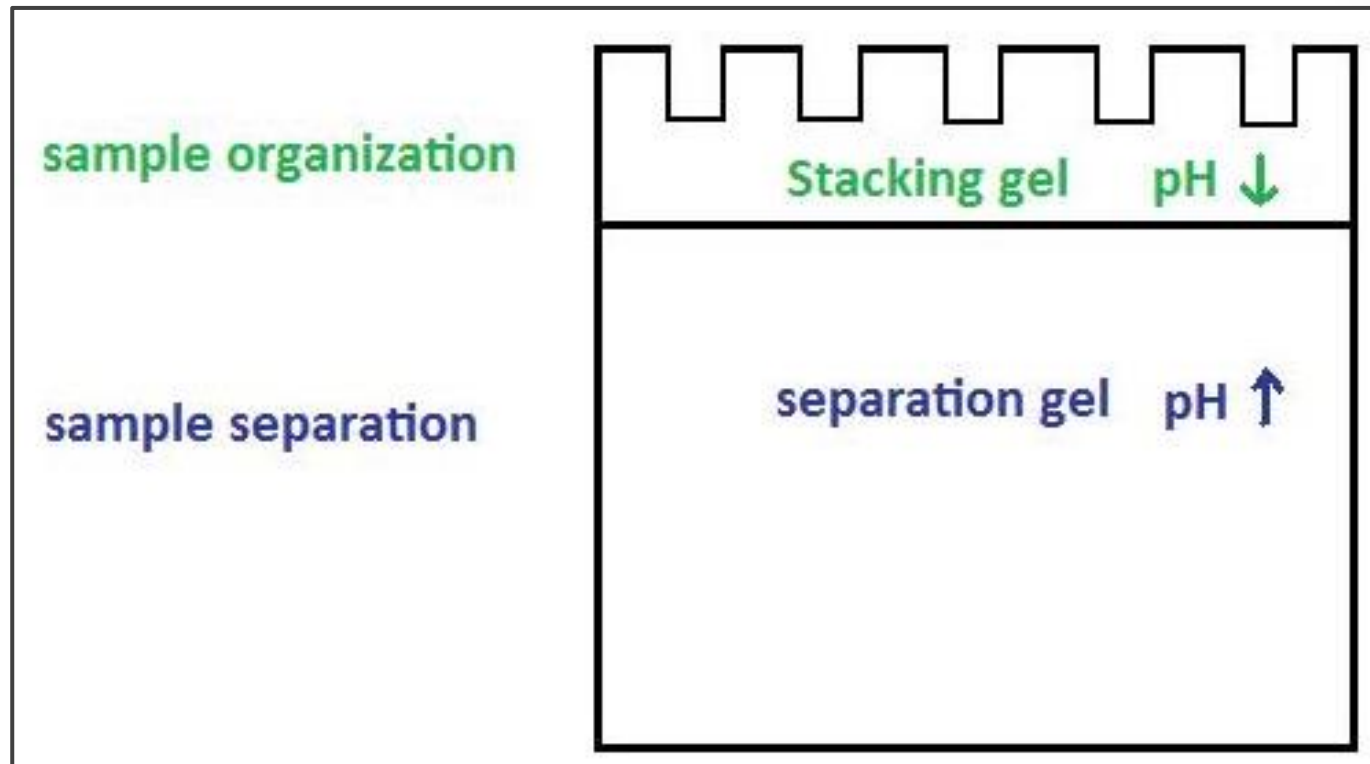
-the proteins samples are having uniformed structure and charge → the separation will depend on their molecular weight only.

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

-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.

polyacrylamide gel:

- 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, consisting of ammonium persulfate (initiator) and tetramethylene ethylene diamin (TEMED) is added[catalyst].



SDS-Polyacrylamide Gel Electrophoresis

SDS-Polyacrylamide Gel Electrophoresis Buffers and solutions:

1- SDS-PAGE, Running buffer (5x) pH 8.4:[?]

Tris-HCl
Glycine
SDS

2-SDS-PAGE, disruption buffer:[?]

10% (w/v) SDS	[?]
1M Tris/HCl, pH 6.8	
Glycerol	[?]
β-Mercaptoethanol	[?]
Bromophenol blue	[?]

3-SDS-PAGE, Stain: [?]

Glacial acetic acid

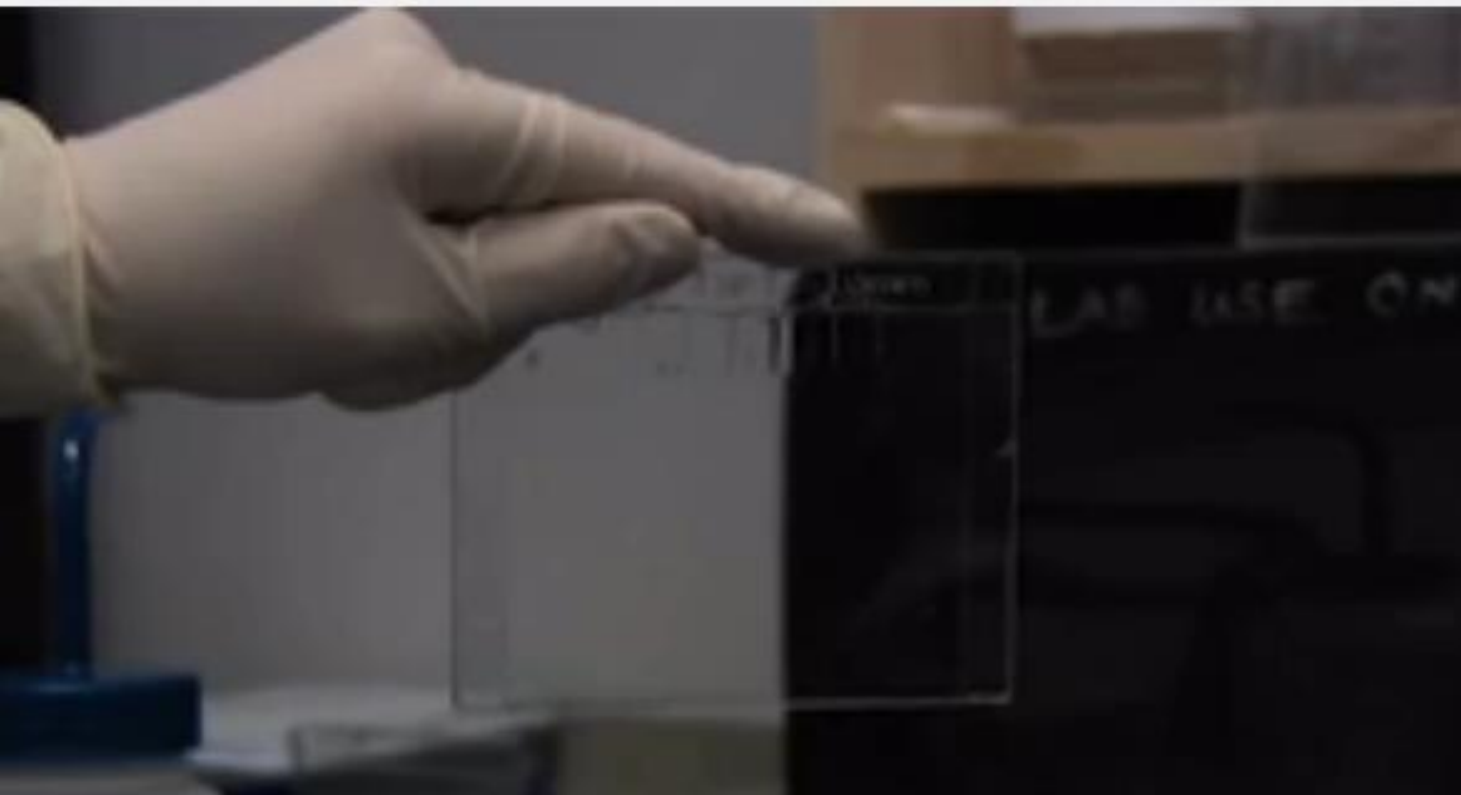
Methanol

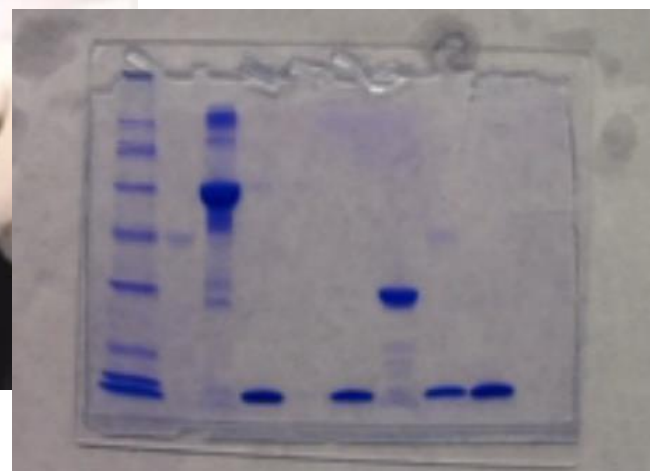
Coomassie brilliant blue R[?]

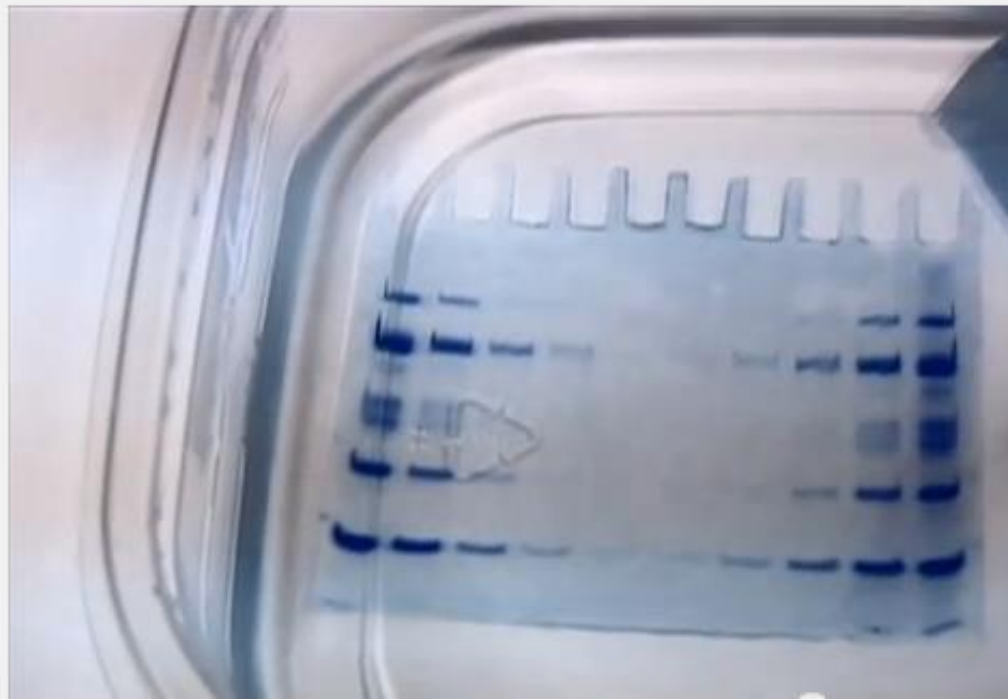
4-SDS-PAGE, de-stain: [?]

Glacial acetic acid

Methanol







<https://www.youtube.com/watch?v=ViJYNOSm6Ww>

5-Sample preparation:

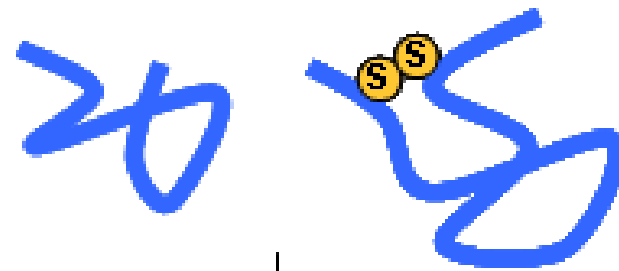
40µl of protein sample + 10 µl of disruption buffer → boil the mixture 3minets at 99°C .

6-Separation gel [?] contents:

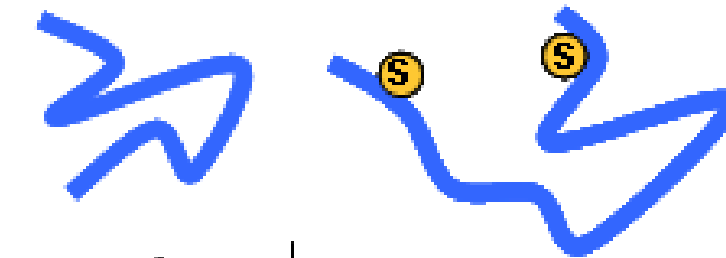
Stock solutions	Volume of stock solution required to make 12% polyacrylamide gel
1.5 M Tris/HCl, <u>pH 8.8</u>	2.0 ml
<u>Acrylamide stock</u>	<u>3.2 ml</u>
Water	2.8 ml
10% SDS	80 µl
<u>10% Ammonium persulphate (fresh)</u>	100 µl
<u>TEMED</u>	20 µl

7-Stacking gel [?] contents:

Stock solutions	Volume of stock solution required to make 12% polyacrylamide gel
0.5M Tris/HCl, pH6.8	1.0 ml
<u>Acrylamide stock</u>	<u>1.0 ml</u>
Water	3.0 ml
10% SDS	80 µl
<u>10% Ammonium persulphate (fresh)</u>	100 µl
<u>TEMED</u>	20 µl



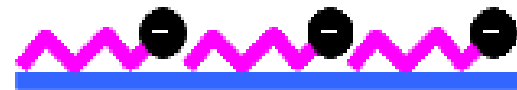
Mercaptoethanol
denatures protein



SDS binds protein
stoichiometrically



Small molecule moves
more quickly through gel



Large molecule moves more
slowly through gel

Applications:

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

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

