SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis):

Aim:

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) is one of the common methods used in the molecular biology to separate protein molecules or fragments in a mixture. SDS is a denaturing agent used to break weak bonds within protein structure and is also responsible for conferring a negative charge to the protein. Thus, the method is based on the separation of the proteins according to their molecular mass whose all polypeptides are made negatively charged with the use of SDS. Because proteins are separated according to their molecular mass, the composition of the polyacrylamide gel is an important consideration to determine the size of pores in which degraded proteins pass through under electric current during electrophoresis.

SDS-PAGE can also be used for identifying a protein of interest, establishing sample purity, peptide mapping and blotting applications such as Western Blotting.

The aim of this experiment is to run SDS-PAGE by using Bovine Serum Albumin (BSA) whose molecular weight is 66 kilo Dalton (kDa).

Process:

- To carry out SDS-PAGE, running buffer, loading buffer, separation gel solution and stacking gel solution were prepared, respectively.

9.1. 1 Liter of 4X Running Buffer:

0.1% (w/v) SDS

$$0.1 = \frac{m_{SDS}}{1000\ ml} \times 100 \quad m_{SDS} = 4\ g$$

100 mM Tris base

$$0.1\ M = \frac{m_{Tris}}{121.14\ g/mol \times 1\ L} \quad m_{Tris} = 12.12\ g$$

800 mM Glycine

$$0.8\ M = \frac{m_{Glycine}}{75.07\ g/mol \times 1\ L} \qquad m_{Glycine} = 60.056\ g$$

- Required amounts of SDS, tris base and glycine were weighed by using analytical balance.
- All reagents were mixed with 1 liter of Type 1 water and were dissolved with the help of magnetic stirrer.

9.2. 10 ml 4X Loading Buffer:

Table 4. Reagents and their required amounts to prepare 4X loading buffer.

Reagent	**Required amount**
SDS	0.8 g
Bromophenol blue	0.04 g
β-Mercaptoethanol	800 µl
Glycerol	4 ml
0.5 M Tris, pH 6.8	5 ml

- SDS and bromophenol blue is weighed and put into a 15 ml falcon tube.
- Tris, glycerol, and β-Mercaptoethanol are pipetted into the tube containing weighed reagents and solution is mixed.
- 4X loading buffer is portioned into aliquots and eppendorf tubes are stored at -20^{o}C.

9.3. Separation and Stacking Gel

Table 5. Reagents and their required amounts for the preparation of separation and stacking gel.

	Concentration	**d-H_2O**	**1M Tris, pH 8.9**	**30% AK/BİS**	**10% SDS**	**10% APS**	**TEMED**
Separation Gel	10%	3 ml	2.1 ml	2.8 ml	80 µl	56 µl	6 µl
Stacking Gel	6%	2 ml	400 µl	600 µl	36 µl	24 µl	4 µl

- For separation gel, the first five reagents are mixed in a 15 ml falcon tube.
- TEMED is added and the solution is mixed just before pouring the gel solution.
- By using Pasteur pipette, glass plates are slowly filled with separation gel solution until 1 cm below of the comb teeth has been reached and without causing any bubble formation.
- The gel solution is carefully overlaid with 1 ml of isopropanol.
- The time required for the gel polymerization is approximately 15 minutes.
- After the separation gel polymerized, isopropanol in the glass plates is removed by inverting the plates and using filter paper.
- Stacking gel solution is then prepared as separation gel, the first five reagents are mixed in a separate tube.
- TEMED is added and the solution is mixed just before pouring the gel solution.
- Glass plates are filled with stacking gel solution up to the top with a pasteur pipette.
- The comb is then inserted into glass plates and the gel is allowed to polymerize for about 30 minutes.
- After polymerization, the comb is carefully removed without damaging the wells.
- 250 µl of the protein sample is mixed with 91.3 µl of 4X loading buffer in an eppendorf tube.
- The sample solution is kept at 90^{o}C for 5 min.
- Inner gel module containing glass plates is transferred into the main tank.
- The tank is filled with 1200 ml of the 1X running buffer (300 ml 4X running buffer mixed with 900 ml type 1 water).
- Wells are washed by slowly pipetting buffer into them.
- 33 µl of the denaturated sample is loaded into the wells gently.
- The lid is then fitted and power supply is connected.
- Desired voltage and mA values are programmed and run is started.
- When the samples reached the bottom of the gel, the power supply is turned off.
- Gel running module is removed and glass plates are taken out.
- Glass plates are separated from each other with the help of the CSL-key which is a plastic apparatus.

- The gel is carefully removed without damaging and stored for further analyses.

Result:

SDS-PAGE is a molecular biology technique which is used in many areas. It is based on the migration of the denaturated protein samples through the pores of the polyacrylamide gel under proper voltage and current. Formation of the polyacrylamide gel results from the interaction between acrylamide and bis-acrylamide. For the polymerization to take places, there should be free radicals in the reaction solution. Therefore APS (ammonium persulfate) which forms free radicals, SO_4^- ions, are used. The role of the TEMED (Tetramethylethylenediamine) in this case is to catalyze the formation of the free radicals from APS. The free radicals formed by APS interact with molecules of the acrylamide and this reaction results in the chemical polymerization of the acrylamide in the form of a linear molecule. After linear polymerization of the acrylamide, bis-acrylamide, which is composed of two acrylamide monomers, becomes a part of the polymerization reaction. Bis-acrylamide forms random cross-links with linear chain composed of acrylamide. These cross-links formed randomly result in the formation of the pores in the polymerized gel.

According to the information given above, separation gel is prepared firstly. Separation gel is the lower gel whose function is the separation of the proteins according to their molecular size. Therefore, TEMED is the last reagent which is pipetted in the reaction solution. Because it results in the rapid formation of the free radicals, therefore it accelerates the polymerization. During polyacrylamide gel preparation, it is observed that after a short time TEMED has been added, the solution starts being viscous which is an indication of the polymerization. The glass plates are then filled with the separation gel. Because there are free radicals in the polymer solution, isopropanol is used to prevent the interaction of the oxygen in the air with the free radicals. The reason is that oxygen inhibits the polymerization of the acrylamide by trapping free radicals. After polymerization has completed, isopropanol is removed. Stacking gel solution which has larger pore size than that of separating gel is then prepared. The remaining part between the glass plates is filled with the stacking solution. Usage of stacking solution results in the movement of the proteins through separation gel which enables resolution of the molecules.

After polymerization, loading buffer including the protein sample is loaded into the wells formed with the help of gel comb. Loading buffer is composed of SDS (sodium dodecyl sulfate), bromophenol blue, β-Mercaptoethanol, glycerol, and tris. SDS is a denaturing agent used to break the bonds resulting in the formation of the secondary, tertiary and quarternary structure of the proteins. It also confers a negative charge to the proteins, thereby electrophoresis is carried out according to only one parameter, which is molecular mass. But it cannot break the disulfide bonds between cysteine residues, therefore, β-Mercaptoethanol which is a reducing agent is used for this purpose. Bromophenol blue is a dye used to track the protein samples during electrophoresis. Glycerol is used to precipitation of the samples into the wells due to its viscous property. By mixing the samples with the loading buffer and heating this mixture, it is expected to have negatively charged linear protein samples. After heating, samples are loaded into the wells as shown in Figure 7a.

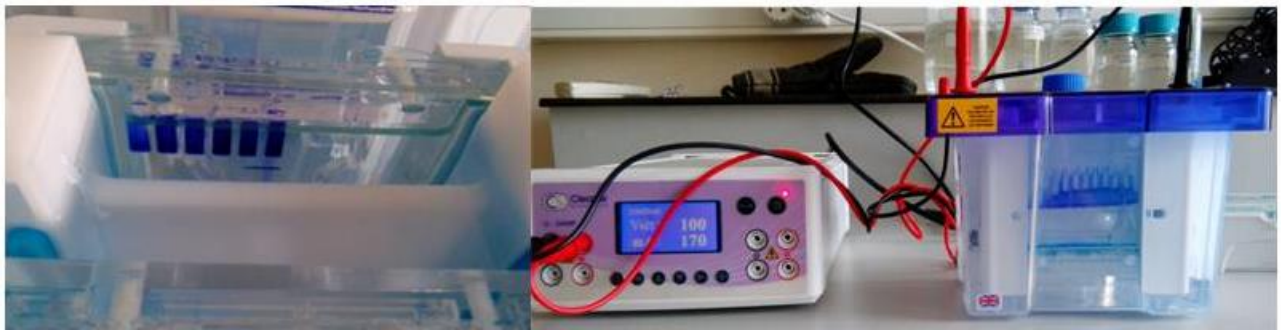

Figure 7. a) Protein samples loaded into the wells. ***b)*** Electrophoresis equipment during the process.

Under the appropriate voltage and current, negatively charged samples run from the negatively charged cathode to the positively charged anode as shown in Figure 7b. Because of the pores in the polyacrylamide gel, low-molecular weight fragments migrate faster than high-molecular weight fragments. After electrophoresis, protein size is compared relative to marker after visualizing the bands with Coomassie brilliant blue or gel is used for western blot (Figure 8).

Figure 8. Polyacrylamide gel containing separated protein fragments before visualizing the bands with Coomassie brilliant blue.

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