Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_29

Chapter 29

SDS-PAGE Analysis of proteins: Principles, methodology, and applications in proteomics

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1. Introduction:

Electrophoresis, driven by an electric field, guides charged molecules' movement, influenced by factors like charge, size, shape, and medium resistance (Hames, and Rickwood, 1990). In protein electrophoresis, molecules exhibit varying charges due to differing pH conditions, except at their isoelectric point (pI), directing migration towards oppositely charged electrodes (Laemmli, 1970; Yadav et al., 2019). Polyacrylamide gel electrophoresis (PAGE), pioneered by Raymond & Weintraub in 1959, utilizes acrylamide gels known for chemical inertness and stability. A clear gel matrix, minimally affected by endosmosis, forms through chemical copolymerization of acrylamide monomers and N-N'-methylene bisacrylamide cross-linker. Gel pore size, determined by acrylamide (%T) and bisacrylamide crosslinker (%C) concentrations, governs protein mobility. In native electrophoresis, proteins maintain structure and activity without SDS, allowing enzymatic assessments post-electrophoresis (Weber and Osborn, 1969; Yadav et al., 2021).

SDS-PAGE, a common protein analysis method, unfolds proteins with SDS, facilitating linear migration based on size. SDS disrupts disulfide bonds, while β-mercaptoethanol aids denaturation. SDS-protein complexes migrate towards the anode by charge, separated by size (Srivastava et al., 2022). Gels with optimized Bisacrylamide to Acrylamide ratios effectively resolve polypeptides, with stacking gels concentrating proteins for enhanced resolution. Isotachophoresis, exploiting pH and ionic strength differences, concentrates proteins in the stacking gel, ensuring precise separation. Electrophoresis buffers maintain pH and provide ions for optimal migration (Laemmli, 2019; Yadav et al., 2016).

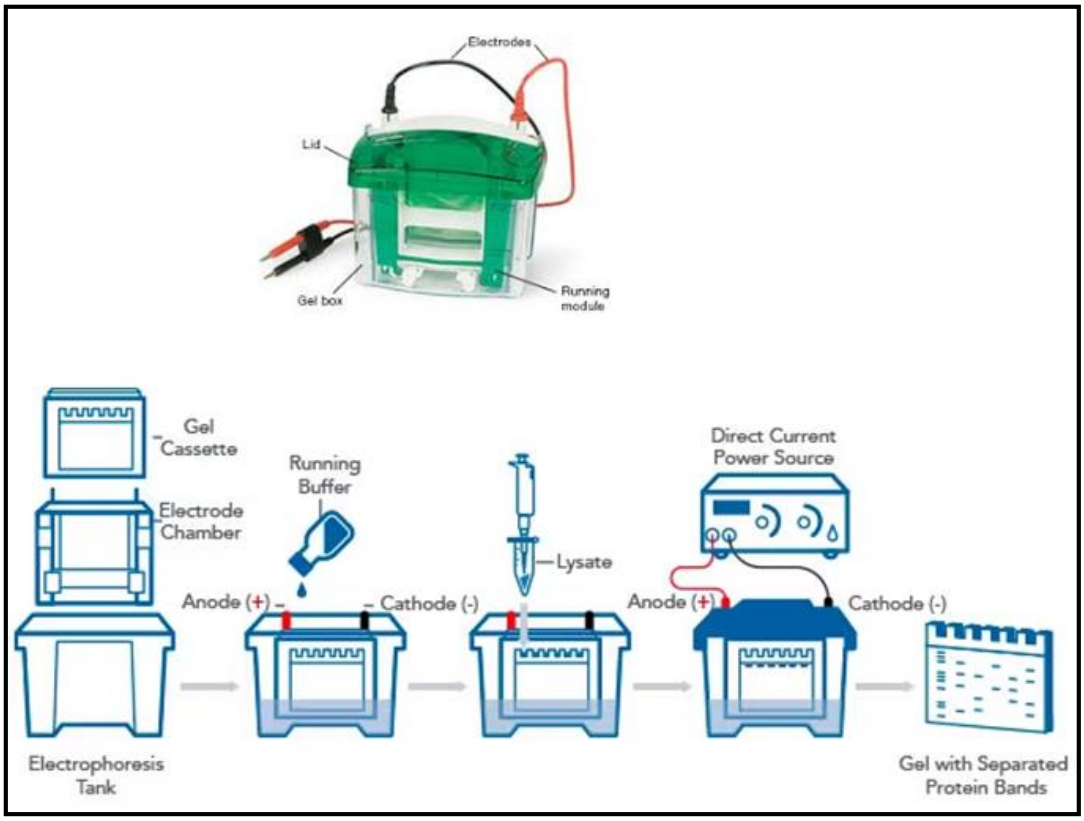

Figure 1: Schematic representation of SDS PAGE electrophoresis

2. Materials Required:

1. Power supply.
2. Micropipette for loading samples
3. Conical flask
4. Beaker
5. Graduated cylinder

2.1. Stock acrylamide solution:

a. Measure out 30 grams of acrylamide along with 0.8 grams of bisacrylamide.
b. Dissolve the measured compounds in distilled water to create a solution, ensuring a maximum volume of 100ml. Subsequently, meticulously filter the solution using a Whatman No1 filter.
c. Safely transfer the filtered solution into an amber bottle and store it at a controlled temperature of 4°C. (WARNING: Acrylamide monomer poses a neurotoxic risk. Exercise utmost caution by wearing protective gloves and ensuring adequate ventilation to prevent inhalation.)

2.2. Buffers:

Table 1: Instructions for preparation of buffers

Steps	Preparation	Instructions
a)	Separating Gel Buffer	Prepare a solution of 1.875M Tris-Cl adjusted to pH 8.8.
b)	Stacking Gel Buffer	Prepare a solution of 0.6M Tris-Cl adjusted to pH 6.8.
c)	Initiating Agents	Make a fresh solution containing 10% w/v Ammonium Persulfate. Store it at 4°C.
d)	Sodium Dodecyl Sulfate (SDS)	Prepare a solution of 10% w/v Sodium Dodecyl Sulfate.
e).	N,N,N',N'tetramethylethylenediamine	Prepare the solution of N,N,N',N'tetramethylethylenediamine (TEMED).

Table 2: Composition of Sample buffer

S.No	Chemicals	Amount (in 50ml)
1.	0.6M Tris-HCl, pH 6.8	5.0 ml
2.	10% SDS	0.5g
3.	Sucrose	5.0g
4.	β-mercaptoethanol	0.25ml
5.	Bromophenol blue (0.5% stock)	5.0ml

Makeup the volume up to 50ml using double distilled or distilled water.

Table 2: Composition of Electrophoresis Buffer

S.No	Chemical required	Amount (in g)
1.	Tris	12g
2.	Glycine	57.6 g
3.	SDS	2.0 g

Prepare the electrophoresis buffer by combining according to the table above. Make up the volume to 2 liters with water. No pH adjustment is required.

2.3. Protein Staining and Destaining solution:

Table 3: Composition of Protein Staining and Destaining solution

Name of the solution	S. No	Chemical required	Amount (in %)
Staining solution	**1.**	Comassive Brilliant Blue	0.1%
	2.	Methanol	50%
	3.	Glacial acetic acid.	10%
De-staining solution	**1.**	Methanol	10%
	2.	Glacial acetic acid.	7%

Prepare a staining solution as per the table above.

a. Begin by dissolving the dye in a methanol-water mixture.
b. Add the acetic acid.
c. Filter the solution through a Whatman filter paper.

2.4. Protein Molecular Weight Markers (standard).

2.5. Protein Sample.

3. Procedure:

1. Start by using methylated spirits to thoroughly clean the gel plates' interior surfaces.
2. As soon as they are dry, put the gel plates together to form the cassette and firmly fasten it vertically.
3. To make the separating gel, combine the materials in an Erlenmeyer flask or a throwaway plastic tube.

Table 4: Composition for 15% Gel preperation

Chemical	Amount
1.8 M Tris-Hcl, pH8.8	8.0ml
Stock acrylamide	20ml
Water	11.5ml
10% SDS	0.4ml
Ammonium persulfate (10%)	0.2 ml

4. Allow the solution to degas for about 30 seconds to remove any trapped air bubbles.
5. Add 14µl of tetramethylethylenediamine (TEMED) into the flask and mix gently to make sure uniform distribution.

6. Transfer the separating gel mixture to the gel cassette using a Pasteur pipette, pouring it along one edge. Ensure the solution is about 1cm away from the bottom of the comb to create loading wells. Precision is vital for optimal gel formation.
7. Using a Pasteur pipette, carefully run distilled water down one edge of the cassette to create a flat surface.
8. To make the 4% stacking gel solution, mix the materials listed in a 100ml Erlenmeyer flask or disposable plastic tube while the separating gel is being made.

Table 5: Composition for 4% Gel preperation

Chemicals	**Amount**
0.6M Tris-Hcl, pH6.8	1.0ml
Stock acrylamide	1.35ml
Water	7.5ml
10%SDS	0.1ml
Ammonium persulfate (10%)	0.05ml

9. Use a vacuum to degas the solution for about 30 seconds to eliminate any trapped air bubbles.
10. Gently wipe the top surface gel for removal of any extra water left on the surface, after the separating gel has set.
11. Stir 14 µl of TEMED into the stacking gel solution, then carefully spread it over the polymerized resolving gel.
12. Making sure no air bubbles are caught, insert a clean Teflon comb into the stacking gel solution.
13. Stand the gel vertically, then give it twenty minutes to set at room temperature.

Sample Preparation and Gel Electrophoresis:

a. Preparing Samples:

1. Using a vortex, combine around 10µl of the protein sample with 5µl of sample buffer.
2. To denaturate the proteins, heat the sample for five minutes at 95–100°C and then refrigerate.

b. Gel Assembly and Loading:

1. Carefully remove the Teflon comb after polymerization, then use electrophoresis buffer to rinse the wells of any leftover acrylamide solution.
2. Without first running the gel, assemble the cassette in the
    i. electrophoresis tank.

c. Buffer Preparation:

1. Fill the top and bottom reservoirs of the tank with Tris-glycine electrophoresis buffer, avoiding pre-running the gel to maintain buffer system integrity.

d. Loading Samples:

1. Load approximately 5-10µl of both unknown and standard samples into the wells following a predetermined order.

e. Electrophoresis Setup:

1. With the positive electrode attached to the bottom buffer reservoir, correctly attach the electrophoresis apparatus to the power pack.
2. Using suitable settings run a current of 30 mA for large format gels or 200V for minigels.

f. Running the Gel:

1. Run the gel for 2.5–3.0 hours for large format gels and 40 minutes for minigels, or until the bromophenol blue reaches the bottom of the resolving gel.
2. For safety, never remove the lid until you have turned off and disconnected the power source.
3. After Electrophoresis open the gel plates, extract the gel, discard the stacking gel, and immerse the separating gel in stain solution.

14. **Preserving and Disposing:** Gently swirl the gel for at least two hours at room temperature.
15. To remove extra stain, leave the gel in a methanol:acetic acid solution on a gently moving platform for 4–8 hours.
16. **Storage:** Once destaining is done, keep the gels in a 20% glycerol solution in water.
17. Immunoblotting: The protein sample may now be analysed by immunoblotting the gel.

4. Data Analysis:

a. Lane Labeling:

Assign distinct labels to each lane on the gel photograph.

b. Molecular Weight Determination:

Run calibration proteins of known molecular weight alongside the unknown protein to determine its molecular weight.

c. Standard Curve Creation:

Create a standard curve illustrating the relationship between relative mobility (Rf) and Log molecular weight.

Rf = (Migration distance of protein/Migration distance of tracking dye)

5. Precautions:

1. Wear gloves and handle SDS-PAGE gels and staining solutions carefully to avoid skin contact.
2. Prepare SDS-PAGE gels with appropriate acrylamide concentration and running buffer according to the size range of proteins.
3. Mix protein samples with SDS-sample buffer and β-mercaptoethanol thoroughly but gently to denature proteins and reduce disulfide bonds.
4. Heat protein samples to ensure complete denaturation and breakage of secondary and tertiary structures.
5. Load protein samples into wells carefully to prevent spillage and distortion of gel wells.
6. Run SDS-PAGE at a constant voltage to ensure uniform migration of proteins based on their molecular weight.
7. Monitor the electrophoresis process closely to prevent overheating and potential gel damage.
8. Handle stained gels with care during staining and destaining steps to avoid damaging or tearing the gel.
9. Use appropriate molecular weight markers to estimate the size of protein bands accurately.
10. Dispose of used acrylamide solutions, staining solutions, and contaminated materials according to laboratory waste disposal guidelines.

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