SDS-PAGE Gel Electrophoresis

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1 Introduction

Gel Electrophoresis is the study of the mobility of molecule in an electric field. As a medium acrylamide and agarose are generally used for proteins and DNA studies, respectively. We are focusing on protein electrophoresis.

A protein will move with a net charge in an electric field (native charged or SDS-charged). The velocity of migration ($\nu$) of a protein depends on the electric field strength (E), the net charge of the protein (z) and the frictional coefficient (f)

$$\nu = \frac{Ez}{f}$$

The electric force $Ez$ driving the charged molecule towards the oppositely charged electrode is opposed to the viscous drag $fv$ arising from friction between the moving molecule and the medium. The frictional coefficient $f$ depends on both the mass and the shape of the migrating molecule and the viscosity of the medium $\eta$. For a sphere of radius $r$,

$$f = 6\pi\eta r$$

Thus on a gel electrophoresis proteins will migrate as function of

1. Charged
2. Size (molecular weight)
3. Conformation

Molecule small compared to the pore of the gel matrix (medium) will move through the gel whereas larger molecule will be almost immobile. Intermediate size molecules will migrate with more or less facility.

Gel Electrophoresis is used to (i) check purity of protein sample, (ii) calculate the protein size (SDS-PAGE), (iii) check protein conformation (native gel).

The gel medium for protein gel is a mixture of acrylamide and Bisacrylamide. Variation of the percentage of this mixture affects the size of the pore of the matrix and therefore the migration of protein samples. For small protein, a better resolution is obtained for high percentage of acrylamide / Bisacrylamide because it makes the pore tighter whereas for big proteins, low percentage of acrylamide / Bisacrylamide gives rise to a better resolution because the matrix is loose and allow migration of big molecule. To further change the matrix set up, it is also possible to change the ratio acrylamide/Bisacrylamide, but that will have to be tested case by case. The polymerization of the matrix is catalyzed by the addition of ammonium persulfate and TEMED (N, N, N’ N’ tetramethylethylemne diamine (Fig. 1).

**TEMED IS NEUROTOXIC PLEASE BE CAREFULL WHEN MANIPULATING** (do not inhale and wear gloves).

![Polymerization reaction of acrylamide bis acrylamide catalysed by TEMED and Ammonium Persulfate](image_url)
Native or non denaturating gel electrophoresis.
Here the protein is migrating under native conditions which means that its functional structure is maintained and it will migrate according to size (molecular weight), charged and conformation. Under such conditions it is not possible to measure the protein molecular weight (size). No protein reference can be used because there are three parameters (size, charged and conformation) fixing the protein migration pattern. However, this technique is able to show difference in charged or conformation after for example, chemical modification or genetic mutation of the protein. This technique can also be used to see oligomeric proteins. To perform native gel electrophoresis, one need to know the pI (isoelectric point) of the protein since that will determine the net charged of the protein and tell you whether the protein will migrate towards anode or cathode.

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide gel Electrophoresis) or denaturating gel electrophoresis.
In order to use electrophoresis for protein molecular weight determination, it is necessary to eliminate at least two of the three parameters affecting the migration pattern. For this purpose, the protein sample is treated to have a uniform charge and its electrophoretic mobility become size dependent only. This is possible by treating the protein under denaturating conditions, meaning that secondary, tertiary and quaternary structures (see biocomputer 1 and 2 and Fluorescence) are disrupted to produce a linear polypeptide chain coated with negatively charged SDS molecules. Then the protein migrates only depending on its size (molecular weight) because the number of SDS molecule bound to a protein is proportional to the number of amino acids. 1.4 g of SDS binds to 1 g of protein which means that there is one SDS molecule every 2 amino acids. The molecular weight of a protein is equal to the sum of the molecular weight of each of its amino acids. Each amino acid has a molecular weight of approximately 100 Dalton (Da). The molecular weight is given on Da or more often in kDa. One Da is one grammol⁻¹. Because SDS is negatively charged, protein will migrate towards the positive electrode.

2 Experiments

Acrylamide-Bis acrylamide, TEMED are neurotoxic compounds. Gloves should be wear at all time during the experiments when manipulating both solution and gel. Once polymerized (i.e. solid) however, the toxicity is far less but because there is always residual non polymerized solution, wearing gloves is also compulsory when manipulating the gel.
Fig. 2. indicates the equipment required for a gel electrophoresis experiment. Check that all is on the bench.

2.1 Assemble the plate

After cleaning the plate with ethanol 70%, assemble them according to Fig. 3 and adjust them in the plate holder. Check with water if there is any leaking.

2.2 Casting the gel

We are doing a SDS-PAGE in which proteins are separated according to their molecular weight only and a Native gel in which proteins migrate according to molecular weight, charged and conformation. Both the gels are cast in the plate holder. There is a stacking gel in which proteins are concentrated and a separating gel where the proteins are separated.
Separating Gel:

The gel separating is the lower gel (Table 1) and is the medium in which the proteins are going to be separated according to their size (molecular weight). Prepare a 12% acrylamide-Bis acrylamide gel (Table 1).

<table>
<thead>
<tr>
<th>Percentage of acrylamide/Bis acrylamide</th>
<th>7</th>
<th>10</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acry/Bis acry (ml)</td>
<td>1.9</td>
<td>2.7</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td>Lower gel buffer 1 (ml)</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>SDS (10%) (µl)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>4.2</td>
<td>3.4</td>
<td>2.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Add 50 µl of Ammonium persulphate 5 ml of gel and 5 µl of TEMED per 5 ml of gel.*

- Put the comb between the two glasses and put a mark on the glass with marker at about 1-1.5 cm below the bottom of the wells.
- Mix solution gently and add in between the two glasses to just above the mark (2-3 mm above) with Pasteur pipette.
- Gently add water on top of it with Pasteur pipette to prevent shrinking upon polymerization.
- Wait about 15-30 min for polymerization of the gel.
- When the gel polymerized, clean with several wash of DI water and dry with whatman paper.

Stacking gel:

The stacking gel is the upper gel and is for proteins to concentrate before entering the separating gel. If proteins are not concentrated at the interface between separating and stacking gel, they will enter at different time into the separating gel and their migration pattern will not be comparable.

- Clean comb with ethanol 70% and place it between the two glasses. Mark with marker each well.
- Follow recipe indicated in Table 2 to prepare stacking gel, note that buffer for stacking is different from buffer for separating. Prepare a 3% acrylamide-bis acrylamide gel.
- Mix solution gently and add in between the two glasses.
- Wait about 15-30 min for polymerization of the gel.

<table>
<thead>
<tr>
<th>Percentage of acrylamide/Bis acrylamide</th>
<th>5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acry/Bis acry (ml)</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Upper gel buffer 1 (ml)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>SDS (10%) (µl)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>3.4</td>
<td>3.84</td>
</tr>
</tbody>
</table>

*Add 50 µl of ammonium persulphate 5 ml of gel and 5 µl of TEMED per 5 ml of gel.*
Do not discard non-polymerized solution in sink **THEY ARE TOXIC.** Put the plastic tube when solutions have polymerized in the yellow trash bag **ONLY.**

When the gel is polymerized, remove comb slowly and clean with water. Remove excess of acrylamide with tissue paper (ask demo). Set up the gel in chamber according to Fig. 4.

**Prepare running buffer:**

A 10× concentrated running buffer is given to you. You must dilute it with DI water 10 times for use. One liter is needed for running the gel. Add running buffer in chamber to cover the wells properly.

<table>
<thead>
<tr>
<th>Table 3. Recipe for SDS-PAGE</th>
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</table>

**Running buffer 1 (1L)**
- Tris base: 150 g
- Glycine: 72 g
- SDS: 50 ml @ 10%
  - Adjust pH with formic acid. Dilute running buffer 5× before use.

**Lower Buffer 1 (1 L)**
- Tris base: 181.69 g
- SDS: 4 g of SDS/40 ml @ 10%
  - Adjust pH at 8.0 with HCl

**Upper gel Buffer 1 (1 L)**
- Tris base: 60.55 g
- SDS: 4 g of SDS/40 ml @ 10%
  - Adjust pH at 6.0 with HCl

**Sample buffer 1 (10 ml)**
- 5 M Tris-HCl pH 6.8: 1.2 ml
- SDS (10%): 2 ml
- Glycerol: 1 ml
- DTT (100 mM): 1 ml
- Bromophenol Blue (0.1% w/v): 0.5 ml
- WATER: 4.5 ml
  - **SB1 contains DTT**
  - **SB2 as SB1 but without DTT**

**Staining solution**
- 0.15% (w/v) bromophenol blue in destaining solution

**Destaining (5 L)**
- Acetic acid: 500 ml
- Ethanol: 2000 ml
- H2O: 2000 ml
2.3 Loading the sample

A standard ladder and an unknown protein sample will be given to you.

- Clean Hamilton syringe several times with running buffer between each load.
- The standard ladder will be loaded by demo to show you.
- You can train to load samples with the Hamilton syringe using SB as a sample and load on well 9 and 10.
- Slowly Load 20µl unknown sample using the Hamilton syringe in the appropriate well to the top of the well.

2.4 Run SDS-Gel

- Check that there are enough buffers in upper and lower chamber (with demo). Be careful when adding more running buffer in upper chamber not to empty the wells.
- Place the lid on top of the chamber. Make sure connections are correct (red with red and black with black) otherwise the sample will run wrong direction.
- Plug lid to power supply again check the proper polarity of the connections.
- Run the gel at 40 mA for 45-50 mins.
- When the migration front (blue line) is almost out of the glasses (becomes yellow), stop migration by switching power supply off.
- Remove gel from buffer chamber. Remove glass plates from holder.
- Using the green spatula, separate the two glass plates gently.

2.5 Staining gel

There are two main staining procedures, either comassie blue (CB) staining or silver staining. Comassie blue staining is routinely used but requires more sample than silver stain. CB can detect up to 1 µg (10^-6 g) of protein whereas silver stain can detect up to 1 ng (10^-9 g). Silver stain is more costly and is used only either when only tiny amount of protein is available or when not much sample is expected to be present.

In this experiment, comassie blue is used.

- Take gel and put in a box containing staining solution (see Table 3).
- Leave it for 15 min.
  During this staining procedure, please wash and clean the plates, combs, spacers and gel chambers using detergent then rinse with water. Plates, comb and spacers must also be dried and clean with ethanol before putting back in their box.
- Remove staining solution and replace by destaining solution. Put the Staining buffer back to its bottle.
- Add a tissue paper in one corner of the box to destain faster.
- Wait until protein band appear. When protein appear discard destaining buffer in sink (rinse sink with water to avoid stain mark on the sink) and add distilled water. Leave overnight.

3 Results and questions

Calculate the molecular weight of the unknown sample:
Data Sheets:

Distance of dye front = _______________ cm

Protein Ladder:

<table>
<thead>
<tr>
<th>Molecular weight (Da)</th>
<th>Log (MW)</th>
<th>Distance traveled (mm)</th>
<th>Rf</th>
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<tbody>
<tr>
<td>250,000</td>
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<td>130,000</td>
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<td>100,000</td>
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<td>70,000</td>
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<td>35,000</td>
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<td>15,000</td>
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<td>10,000</td>
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Unknown Samples:

<table>
<thead>
<tr>
<th>Protein</th>
<th>Distance traveled (mm)</th>
<th>Rf</th>
<th>Log (MW)</th>
<th>Molecular weight (Da)</th>
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Problems:

1. Calculate the relative mobility (Rf) of each band in the standards and your samples by measuring the distance each band traveled from the top of the separating gel, and then dividing this distance by the distance traveled by the dye front. Enter your answers in the data tables.

2. Using the molecular weight protein standards, plot log MW (y-axis) versus relative mobility (x-axis). Add a linear trendline; be sure to print the equation of the line and the correlation coefficient on the plot.

3. Using the standard curve, calculate the molecular size of the unknown protein sample, and decide which protein(s) is/are inside the unknown sample. Enter the answers in your data table.
The molecular weights of some proteins are:

Albumin, egg: 45,000 Da
Bovine Serum Albumin: 66 000 Da
Carbonic Anhydrase, bovine: 29,000 Da
Glyceraldehyde-3-phosphate dehydrogenase, rabbit muscle: 36,000 Da
Lysozyme: 14,300 Da
Trypsin Inhibitor, soybean: 20,100
Trypsinogen, bovine pancreas: 24,000 Da