Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)  
Theory

Polyacrylamide gel electrophoresis is one of the most frequently employed techniques for separating macromolecules (DNA, RNA, and proteins). It is widely used for displaying proteins, for generating Western blots, and for separating nucleic acids that differ in length by as little as one nucleotide. The exact conditions (percentage of polyacrylamide, ratio of cross-linking, thickness of gel, etc.) pertain to the kind of separation you are trying to achieve. The conditions recommended here are for separating by size the whole spectrum of typical cellular polypeptides, ranging from about 10,000 Da to more than 100,000 Da.

Electrophoresis in general is the process of applying an electric field to move charged molecules through a solution. The essence of the technique is this: The mobility of a charged molecule is directly proportional to its net charge and inversely proportional to the resistance of the solution through which it is moving. The mobility is also proportional to the voltage of the field. In a gel, because all molecules experience the same voltage, mobility is governed by net charge and resistance.

In the case of a matrix, such as polyacrylamide or agarose, it is thought that resistance is the more important of the two parameters. Resistance provided by the matrix is in relation to the molecular size and shape of the molecules moving through it. For a given pore size (size of the holes in the matrix through which the molecules move), bulkier molecules experience greater resistance and therefore move more slowly through the matrix than petite molecules. But we can’t leave out entirely the role of charge. Consider a race pitting a large and highly charged molecule against a small, more neutral molecule. Who will move through the gel more quickly? It’s hard to say.

Let’s deal with this complication of shape by getting rid of it. And while we’re at it let’s make sure that the ratio of charge/mass is constant. If we can do that, molecular size becomes the only relevant factor. This is already the case for linear double-stranded DNA, for instance, which has a constant ratio of charge/mass (think of all those phosphate groups that form the backbone). In that case it’s simple enough: the longer the DNA molecule, the longer the time it takes to slip through the gel. You have probably had experience running DNA fragments on an agarose gel, and remember that larger fragments end up closer to the top of the gel, smaller fragments nearer to the bottom.

How do we get proteins, which are a much more heterogeneous bunch of macromolecules than DNA or RNA, to behave rationally on a gel? The answer is that we need to eliminate differences in charge/mass ratio and differences in shape. How? By mixing the proteins with the ionic detergent sodium dodecyl sulfate (SDS), which binds to protein at a uniform ratio of SDS/protein—approximately one SDS molecule for every two amino acid residues. The result is that, in a manner of speaking, the protein becomes coated with negative charges, unfolding into a rod, a conformation that maximally separates those mutually repelling negative charges. Now the protein looks a bit more like DNA: a rod-like, negatively charge macromolecule, the mobility of which depends exclusively on its length.

A detail – what about disulfide bonds in proteins?  
Along with the addition of SDS to denature (i.e., unfold, destroy native structure of) the cellular proteins, a second reagent is added. This reagent contains a soluble thiol (-SH) group to reduce any inter- or intra-molecular disulfide bonds. This is important because disulfide bonds, which can exist within as well as between polypeptide chains, prevent the polypeptide
from fully unfolding. The horribly stinky β-mercaptoethanol is most commonly used; dithiothreitol (DTT) is another disulfide reducing compound.

**And now, a little more complexity – the stacking gel**

If you have ever watched or run in a marathon, you know that with a large field of competitors, some of the runners are well on their way long before other runners in the back of the pack have even crossed the starting line. The same is true for macromolecules undergoing electrophoresis. When a mixture of proteins is dragged by the electric field into the gel, they begin to separate on the basis of size. But the smallest of the proteins will have started to make their way through the gel even before all the sample has entered the gel. What is desired is a way to condense the entire sample of 10 or 20 µl into the narrowest possible band right at the interface of the gel, lining them up at the starting line. This can be achieved by a neat trick called a stacking gel. To understand this, keep in mind two things: that polyacrylamide gels are run in a vertical orientation, unlike agarose gels, which are horizontal; and that SDS-coated protein are negatively charged, regardless of the pH of the buffers.

Here’s how it works. The polyacrylamide gel is created in two layers, the larger, “resolving” (or “running”) gel on the bottom, and a narrow “stacking” gel on top. The reservoirs on the top and the bottom of the gel contain a buffer made with glycine and adjusted to pH 8.3. The stacking gel has a pH of 6.8, while the resolving gel has a pH of 8.8. After you load the samples into the wells at the top of the gel, you will turn on the voltage, and current will flow through the gel from one reservoir to the other, carried by negatively charged molecules. The current in the top reservoir is carried by some of the glycine molecules; but note that at pH 8.3, only about 10% of the glycine molecules are negatively charged.

Now, address your mind’s eye to the top surface of the stacking gel, where the proteins first enter. Glycine at pH 6.8 has almost no negative charge, so here the current is carried mainly by Cl⁻ in the stacking buffer. Because of their small size the Cl⁻ ions rush ahead of the proteins, which move more slowly. This results in a concentration gradient of Cl⁻ ions, which pile up towards the bottom of the stacking gel. The effect is that within the stacking gel, the SDS-coated protein molecules are sandwiched between glycine molecules above and Cl⁻ below, and are compressed into a band of much smaller volume than was originally loaded.

As the current continues to flow, the proteins move into the resolving gel. But glycine molecules in the resolving gel buffer at pH 8.8 become negatively charged and move quite fast, nearly keeping up with those small, agile Cl⁻ ions, and leaving the proteins trailing behind. It is at this point, when the proteins enter the resolving gel, that they are carrying the current in the trail of the Cl⁻ and glycine anions, and their mobility is a function of molecular size alone.

**Other points to remember**

- You can vary the percentage (w/v) of acrylamide in the final gel. Just as we saw with agarose gels, higher percentage gels are used to separate smaller molecules.
- The ratio of acrylamide/bisacrylamide is usually about 29:1.
- A graph of the log of the MW of proteins vs. their mobility in the gel gives a fairly straight line that can be used to determine the MW of an unknown.

**Pre-cast gels**

We occasionally use commercially prepared pre-cast gels instead of making our own. The advantage is convenience; the disadvantage is cost. These gels do not have a stacking region on top, but rely on a gradient of acrylamide to effect a compression of the sample. Typically the acrylamide concentration varies linearly from 4% at the top to 20% at the bottom.