Lecture 3

GEL

Gel is a solid, jelly-like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute crosslinked system, which exhibits no flow when in the steady-state.[1] By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional crosslinked network within the liquid. It is the crosslinks within the fluid that give a gel its structure (hardness) and contribute to stickiness (tack).

Gel electrophoresis is commonly carried out on a slab of agarose. The dry substance is allowed to swell in hot buffer solution, and cast into a mould, which leaves small "wells" in the gel, into which the samples are applied. Other media that are used as support are: paper, cellulose acetate, starch, or polyacrylamide. For the latter, small slabs are prepared and run vertically in specially designed apparatus, to be described later.

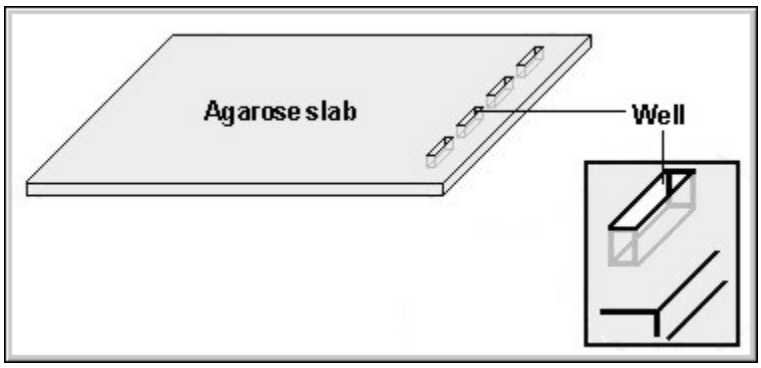
The velocity, **v**, will also depend on the resistance to the movement of the molecules provided by the matrix through which the molecules are moving. Thus, the type of support that is used is very important. Agarose gels of various concentrations may be prepared by altering the ratio of dry agarose to the buffer. Typically, agarose gels are used in a concentration varying between 0.5% and 2%. Since molecular sieving takes place to varying extents, the more concentrated the gel, the slower the mobility of the molecules in the same buffer and applied potential difference.

Agarose gels are normally used to separate native proteins, that is, proteins that have retained higher orders of structure. One frequently refers to such gels as "native gels". Separation on native gels takes place by both charge AND size. Polyacrylamide gels are normally used in conjunction with sodium docecyl sulphate.

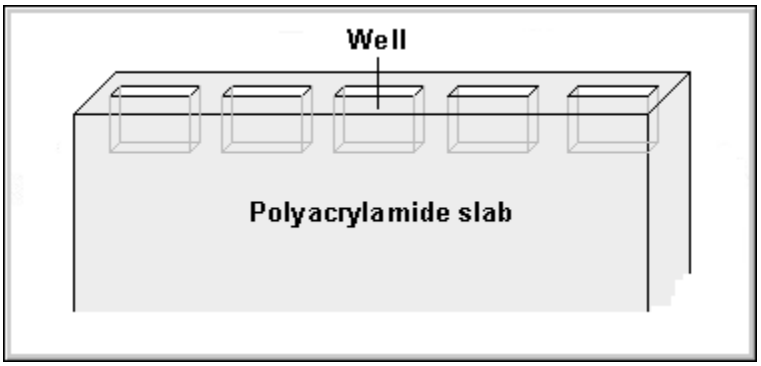
Two major materials used in making gels are-:

1. **Agarose –** One of the materials used in electrophoresis. It is extracted in the form of agar from several species of red marine algae, or seaweed. It is highly fragile and can easily destroyed by handling. Agarose gels have a very large pore size and are used primarily to separate molecules (large molecular mass). It can be processed faster than polyacrylamides gels but their resolution is

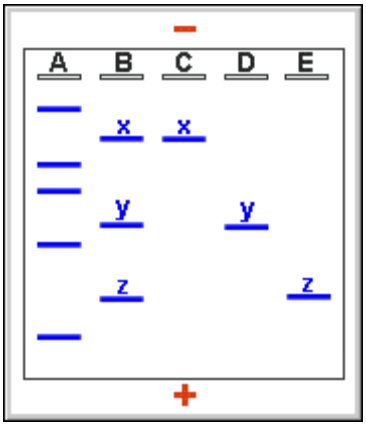
inferior. The band formed are usually far apart. Agarose is a linear polysaccharide made up of basic agarobiose unit which comprise of alternate unit of galactose and anhydrogalactose. It is usually used between 1% and 3%.



2. **Polyacrylamide –** Polyacrylamide gels can be used to provide varieties of electrophoretic condition. It is currently most often used in the field of immunology and protein analysis, often used to separate different proteins or isoforms of the same protein into separate bands. The pore size can be varied so as to produce different molecular sieving for protein of different sizes. Polyacrylamide gels offer greater flexibility and more sharply defined banding than agarose gel.



Polyacrylamide gels are made by polymerising acrylamide monomer ( ) with ammonium persulphate ( ) in the presence of N,N'-methylene-bisacrylamide ( ) ("bis crosslinker").



The resulting gel consists of minute "tunnels" of various diameters, which can selectively accomodate the passage of molecules, based on their sizes. There exists a linear relationship between the distance travelled in a given time under defined conditions and the logarithm of the molar mass of the proteins (diagram above, left). This is exploited in determining the molar mass of samples. The migration distance of unknown proteins is simply related to that of "markers" of known molecular masses. A schematic representation of this is shown in the diagram above on the right, where protein samples are electrophoresed in five "lanes" **A-E**, all migrating towards the positive pole of the electrophoretic cell:

Lane **A** consists of protein markers. Lane **B** consists of a mixture three proteins: **x** being the largest, and **z** the smallest, with **y** having a size intermediate between the two. Lane **C**, has pure protein **x**, lane **D** has pure **y** and lane **E** pure **z**.