ELECTROPHORESIS

The term "electrophoresis" is applied to the movement of charged ions or molecules through a solution or medium by the application of an outside electrical field. The rate of migration or movement of the molecule will depend on its size, shape, and charge, as well as the overall resistance displayed by the medium.

For example, if we have a mixture of negatively charged proteins in solution and an electrical field is applied, the proteins will migrate toward the positive electrode. The rate of movement, and hence the separation, will depend on the charge to mass ratio. The protein will migrate at a rate proportional to their net charge and inversely proportional to their effective size. Hence, a mixture of proteins will migrate at different rates in solution dependent upon their overall size and charge.
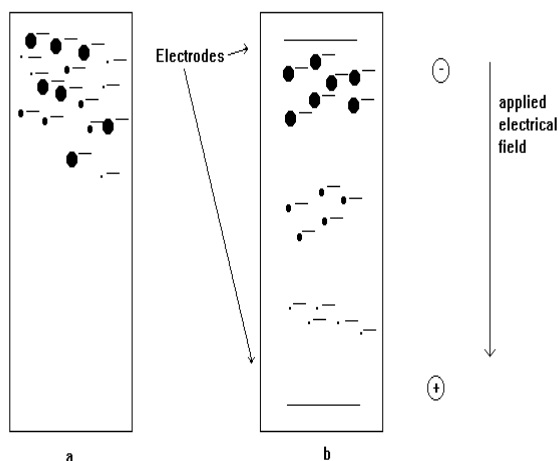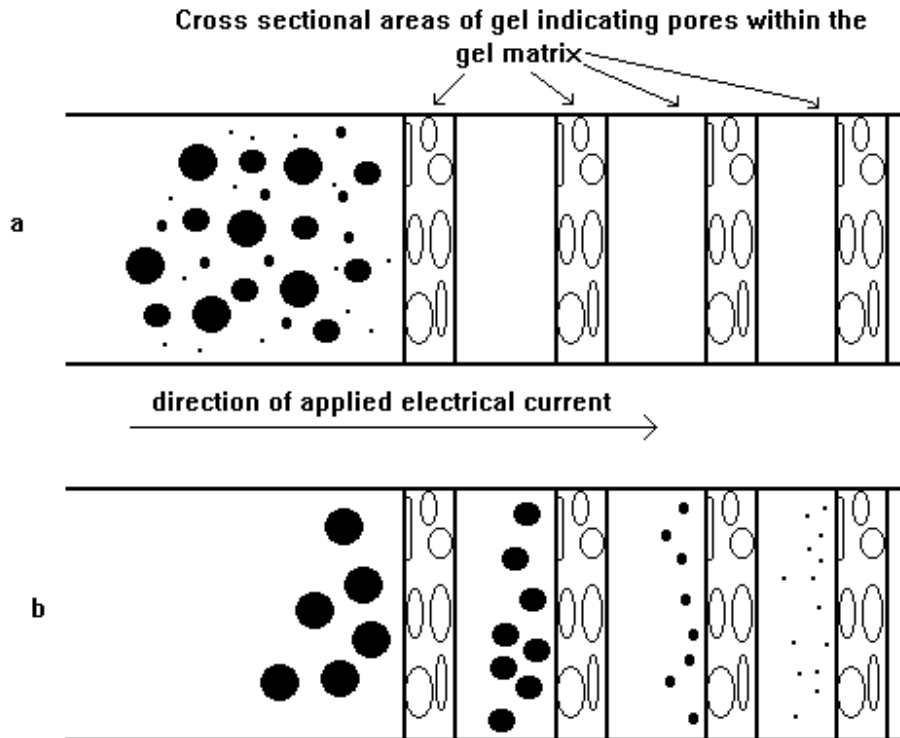

Figure 1.a) Solution of charged ions before electrical field applied
        b) Solution after application of electrical field

While this type of separation can take place in solution, it would be hard to isolate and characterize the compounds once the electric field was removed. At this point the molecules of interest would be free to move, diffuse, and interact with each other.

For this reason, electrophoresis techniques are generally performed within some type of supporting matrix or medium. In general, the separations are usually run in gels which are cast (or poured) from either agarose or acrylamide. For high school chemistry purposes, either agarose gels or prepared plates coated with either cellulose or silica will be used.

In both cases, the separation of the components is aided by the interaction of the compounds with the medium. The medium interacts with the separating molecules and can be thought of as a porous material in which the pore size is similar to that of the molecules. Thus, in conjunction with the charge interaction, separation occurs due to a molecular sieving or separation by size.

Cross sectional areas of gel indicating pores within the
gel matrix

direction of applied electrical current

Figure 2. a) Gel before electrical current is applied. b) Gel after electrical
current is applied indicating the migration of the components of the mixture.
Those molecules which are smaller and do not interact with the matrix as much
move further along the gel more quickly than those who intract to a greater extent.

While the molecular sieving occurs, the charge of the molecule continues to play a role in the separation. Electrical current from one electrode is repelling the molecules while the other electrode is attracting it. The separation of the components is due to both size and charge. The molecules are forced through the pores of the medium by the applied electrical current. This interaction causes the different components of the mixture to become distinct bands on the medium. Thus, two components of identical charge but different sizes will separate due to molecular sieving based on molecular weight. Those of identical weight but different charge density will separate based on charge under certain running conditions.

The gels themselves usually consist of agarose or polyacrylamide. Agarose gels are made by heating solid, powdered agarose in the presence of an electrolyte buffer, pouring the resulting mixture into the desired gel container, and allowing it to harden (this is analogous to making jello). To some extent, resolving power will depend on concentration. The higher the concentration, the lower the molecular weights which can be resolved -- while with lower concentrations, higher molecular weights can be separated. In general, for agarose gels the pore size is very large, and overall, molecular sieving is a minimum. Separation is based mainly on charge density; mixtures of higher molecular weights up to 200,000 g/mol can separate. Due to this separating ability, agarose gels have been very useful for separating nucleic acids or DNA.

In acrylamide gels the effective pore size is greatly influenced by total acrylamide concentration, and separation of molecules is greatly influenced by pore size (see below). At 2.5% acrylamide concentration, molecules of molecular weights around 10^6 g/mol can be separated successfully, while a concentration of 30% allows molecular weights as low as 2,000 g/mol to be separated successfully. This range allows for polypeptides and proteins to be separated successfully and allows for enzyme analysis and gene sequencing.
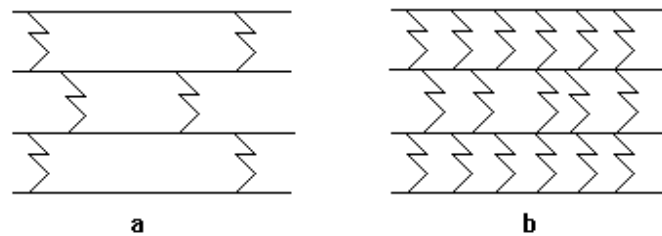
Figure 3. a) Diagram showing gel prepared by low concentration of monomer and crosslinker having large pore sizes for separating high molecular weights. b) Diagram showing gel prepared by high concentration of monomer and crosslinker having small pore sizes for separating low molecular weights.

For any given concentration of acrylamide, the effective pore size, stiffness, brittleness, and swelling properties vary with the proportion of crosslinker used. Without bisacrylamide crosslinker in polymerization, the resultant material would be a viscous solution. With bisacrylamide, random gelation of acrylamide occurs giving a meshwork.

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