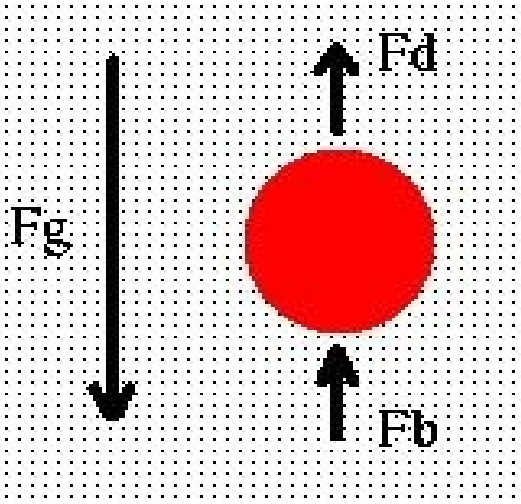
SEDIMENTATION AND ELECTROPHORETIC METHODS

An important tool in biochemical research is the centrifuge, which through rapid spinning imposes high centrifugal forces on suspended particles, or molecules in solution, and separates them based on differences in weight (e.g. red blood cells may be separated from plasma, nuclei from mitochondria in cell homogenates, and one protein from another in complex mixtures).

Biological molecules can be separated based on their electrical charge as well. Amino acids and proteins possess net positive or negative charge according to the acidity of the solution in which they are dissolved. In an electric field, such molecules adopt different rates of migration toward positively or negatively charged poles and permit separation.

Sedimentation Velocity Method

Sedimentation velocity is an analytical ultracentrifugation method that measures the rate at which molecules move in response to centrifugal force generated in a centrifuge. The ultracentrifuge is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 1,000,000 g (g = 9.800 m/s²). Theodor Svedberg invented the analytical ultracentrifuge in 1923, and won the Nobel Prize in Chemistry in 1926 for his research on colloids and proteins using the ultracentrifuge.



Consider an object sinking in a liquid with a constant speed. The forces that act on it are: gravity (F_G = m·g), drag force (F_D = f·v), and buoyant force ($F_B = \frac{m}{\rho_{object}} \cdot \rho_{liquid} \cdot g$). Here m is the mass of the object, v is the velocity of the object, f the shape factor, ρ is the density of the medium, and g is the gravitational acceleration.

If the object is moving at a constant speed:

$F_G = F_D + F_B$;

$f \cdot v = m \cdot g \cdot (1 - \frac{\rho_{liquid}}{\rho_{object}})$.

In a centrifuge we can get much larger accelerations than the g = 9.800 m/s² gravitational acceleration on the surface of the Earth. In a centrifuge the acceleration can be calculated as:

$a = r \cdot \omega^2$.

For a particle in a centrifuge:

$f \cdot v = m \cdot a \cdot (1 - \frac{\rho_{liquid}}{\rho_{object}})$.

The velocity of movement of the particle is proportional to the acceleration. In other words, the ratio $\frac{v}{a}$ is constant.

$\frac{v}{a} = \frac{m}{f} \cdot (1 - \frac{\rho_{liquid}}{\rho_{object}}) = S$.

S is called the sedimentation constant. The non-SI unit of the sedimentation constant is [S] = Sv (Svedberg) = 10^{-13} s.

There is a straightforward relationship between the shape factor (f) and the diffusion constant (D):

$f = \frac{k \cdot T}{D}$;

where k denotes the Boltzmann constant, T the absolute temperature.

If the sedimentation experiment is combined with diffusion measurements, and both S and D are determined experimentally, the mass of the object can be calculated.

Sedimentation Equilibrium Method

Sedimentation equilibrium is an analytical ultracentrifugation method for measuring molecular masses in solution. With this method we need to wait until the equilibrium between the sedimentation and random thermal movement is reached. In thermal equilibrium Boltzmann's law will describe the distribution of the particles in the centrifuge.

$\frac{n_1}{n_2} = e^{-\frac{E_1 - E_2}{k \cdot T}}$;

where n_1 and n_2 are the number of particles at distance r_1 and r_2, respectively, while E_1-E_2 is the work done by the centrifugal force, when the particle is transferred from r_1 to r_2. Knowing that:

$E_1 - E_2 = \frac{m}{2} \cdot (r_1^2 - r_2^2) \cdot \omega^2 . (1 - \frac{\rho_{liquid}}{\rho_{object}})$;

we get:

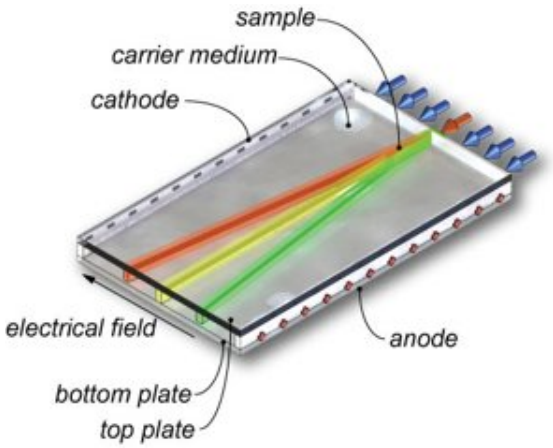
$\ln(\frac{n_1}{n_2}) = \frac{m}{2 \cdot k \cdot T} \cdot (r_1^2 - r_2^2) \cdot \omega^2 . (1 - \frac{\rho_{liquid}}{\rho_{object}})$.

The above formula allows to determine the mass of the particle without the measurement of the diffusion constant.

Free Flow Electrophoresis

Electrophoresis means techniques that use the electric field driven motion of charged particles to separate them. Free flow electrophoresis is an electrophoresis procedure working continuously in the absence of a stationary phase (or solid support material such as a gel). If the charged particle is moving at a constant speed in the electric field, the speed of this motion (v) is proportional to the electric field strength (E) if the field is not too strong. Using this assumption makes possible the introduction of electrophoretic mobility μ_e as coefficient of proportionality between particle speed and electric field strength:

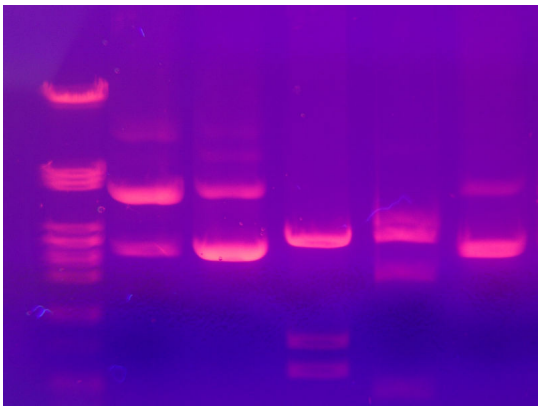
$\mu_e = \frac{v}{E}$.



Gel Electrophoresis

Gel electrophoresis is a technique used for the separation of DNA, RNA, or protein molecules using an electric voltage applied to a gel matrix. It is usually performed for analytical purposes, but it can be used as a preparative technique as well.

The gel is a solid, yet porous matrix. By placing the molecules in wells in the gel and applying an electric force, the molecules will move through the matrix at different rates, determined by their mass and charge. After the electrophoresis is complete, the molecules in the gel can be stained to make them visible.

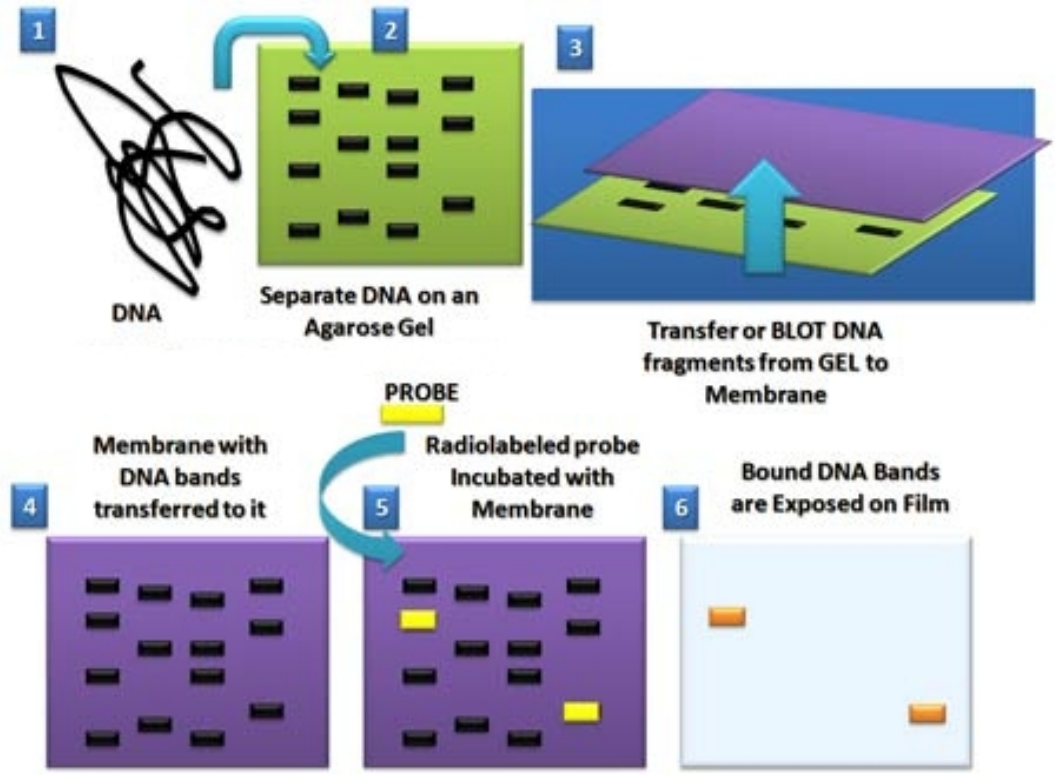


Isoelectric Focusing

Isoelectric focusing is a technique for separating different molecules by their isoelectric point. The technique uses a gel in which the pH depends on the position inside the gel, and takes advantage of the fact that a molecule's charge changes with the pH of its surroundings. Negatively charged molecules migrate through the pH gradient in the medium toward the positive end, while positively charged molecules move toward the negative end. As a particle moves towards the pole opposite of its charge it moves through the changing pH gradient until it reaches the pH at which the molecule is net neutral (isoelectric point). At this point the

molecule has no net charge and so migration ceases. As a result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its isoelectric point. The technique is capable of extremely high resolution with proteins differing by a single charge being resolved into separate bands.

Southern Blot



A Southern blot is a method routinely used in molecular biology to check for the presence of a DNA sequence in a DNA sample. Southern blotting combines gel electrophoresis for size separation of DNA with methods to transfer the size-separated DNA to a filter membrane for probe hybridization. The method is named after its inventor, the British biologist Edwin Southern. Other blotting methods (i.e., western blot, northern blot) that employ similar principles, but using RNA or protein, have later been named in reference to Southern's name. Other blotting methods (i.e., western blot, northern blot) that employ similar principles, but using RNA or protein, have later been named in reference to Southern's name.