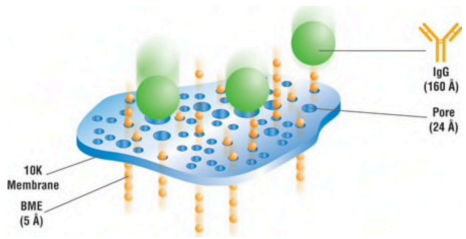
Biochemistry Lab

Protein Dialysis Protocol

Dialysis Methods for Protein Research
(taken in part and edited from ThermoFisher.com) see also https://youtu.be/sOfRMWDF1sg

In working with proteins and nucleic acids, it is often necessary to eliminate small molecular weight substances that might interfere with a subsequent step in the experimental procedure. Similarly, it is often desirable to exchange the protein sample into a different buffer system for downstream application such as electrophoresis, ion exchange or affinity chromatography. Dialysis is one method for accomplishing both contaminant removal and buffer exchange for macromolecular samples such as proteins.

Introduction Dialysis is a separation technique that facilitates the removal of small, unwanted compounds from macromolecules in solution by selective and passive diffusion through a semi-permeable membrane. A sample and a buffer solution (called the dialysate, usually 200 to 500 times the volume of the sample) are placed on opposite sides of the membrane. Sample molecules that are larger than the membrane-pores are retained on the sample side of the membrane, but small molecules and buffer salts pass freely through the membrane, reducing the concentration of those molecules in the sample. Changing the dialysate buffer removes the small molecules that are no longer in the sample and allows more contaminants to diffuse into the dialysate. In this way, the concentration of small contaminants within the sample can be decreased to acceptable or negligible levels.



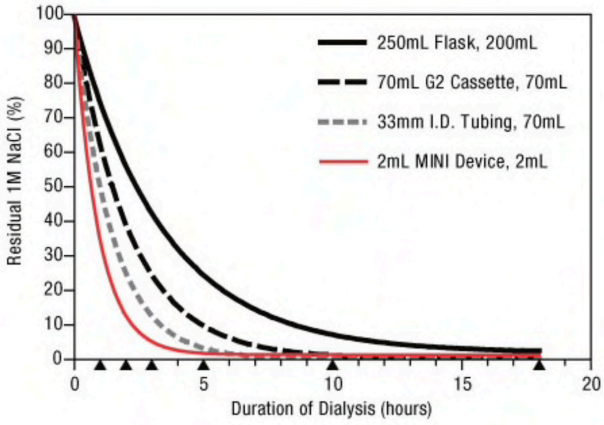
How dialysis membranes work. A dialysis membrane is a semi-permeable film (usually a sheet of regenerated cellulose) containing various sized pores. Molecules larger than the pores cannot pass through the membrane but small molecules can do so freely. In this manner, dialysis may be used to perform purification or buffer exchange for samples containing macromolecules.

Principles of diffusion Dialysis works by diffusion, a process that results from the thermal, random movement of molecules in solution and leads to the net movement from areas of higher to lower concentration (until an equilibrium is reached). In dialysis, unwanted molecules inside a sample-chamber diffuse through a semi-permeable membrane into a second chamber of liquid or dialysate. Because large molecules cannot pass through the pores of the membrane, they will remain in the sample chamber. By contrast, the small molecules will freely diffuse across the membrane and obtain equilibrium across the entire solution volume, effectively reducing the concentration of those small molecules within the sample.

If dialysis is allowed to proceed to equilibrium before each change of dialysate buffer, the substances retained by the membrane are purified by a factor equal to the ratio of buffer volume to sample volume. For example, when dialyzing 1 mL of sample against 200 mL of dialysis buffer, the concentration of unwanted dialyzable substances will be decreased 200-fold when equilibrium is attained. Following two additional buffer changes of 200 mL each, the contaminant level in the sample will be reduced by a factor of 8 x 10^6 (200 x 200 x 200). If the original sample contained 100 mM DTT, this would potentially be decreased in the sample to approximately 12.5 nM following three complete cycles. If a further decrease in concentration is desired, the dialysis process can be continued with additional volumes of dialysate.

Dialysis performance The time required to accomplish dialysis is determined by factors that affect the rate of diffusion of a molecule. Because heat affects the thermodynamics of molecules, increasing temperature speeds diffusion. Therefore, dialysis will proceed faster at room temperature than at 4°C. In selecting the most appropriate temperature, it is important to take into account the thermal stability of the molecule of interest. The rate of diffusion is also directly proportional to the concentration of a molecule, while inversely proportional to its molecular weight. As the concentration of a molecule increases, so does the probability that one of those molecules will contact the dialysis membrane and then diffuse across to the other side. However, as a molecule's molecular weight increases, the rate of movement in solution decreases along with the chance of diffusion through the membrane - even if it the molecule is small enough to pass through the pores.

The rate of dialysis is also directly proportional to the surface area of the membrane and inversely proportional to its thickness. Membranes normally used for laboratory dialysis applications are 0.5 to 1.2 mil (12 to 30µm) thick, providing good diffusion rate as well as structural integrity. While membrane thickness is not a variable that is easily modified, the surface area usually is. The flatter a sample can be spread over a membrane surface, the faster will be its dialysis because all molecules in the sample will be closer to the membrane and a higher proportion of them will be in direct contact with the membrane at any instant. High-performance dialysis products, such as Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, MINI Devices and Flasks, are designed to maximize surface area-to-volume ratios (within practical limits) for different volumes of sample.



Influence of surface area to volume ratio on dialysis rate. Graph displays rates of removal of 1 M NaCl from 2 mL, 70 mL, 70 mL, and 200 mL samples dialyzed in four respective sizes of Thermo Scientific dialysis devices, each equipped with 3.5K MWCO membrane. Dialysis was conducted at room temperature against very large volumes (e.g., 4 L) of water (dialysate). At the indicated times (triangles), the dialysis buffer was changed and the percentage of NaCl removal was determined by measuring the conductivity of the sample. The large device (Flask) has about half the surface-area-to-volume ratio of the other devices, accounting for the slower rate.

Stirring the buffer during the dialysis process also increases the diffusion rate. As low molecular weight compounds exit through the pores on the outer side of the membrane, they form a microenvironment termed a Nernst diffusion layer. In this layer, which is approximately 200-300 molecules thick, the low molecular weight compounds are at a higher concentration in relation to the rest of the dialysate. This high local concentration effectively slows the rate of dialysis because molecules can randomly re-enter the dialysis membrane pores and return to the sample. Stirring efficiently breaks up the macroenvironment outside the Nernst layer, helping to maintain the concentration differential needed to drive the diffusion process.

Membrane molecular weight cutoff Molecular weight cut-off (MWCO) describes membrane pore size measured in angstrom (Å) units. A larger MWCO corresponds to a wider pore size. The MWCO describes the smallest average molecular mass of a molecule that fails to diffuse across the dialysis membrane. For example, a membrane with a 10K MWCO will retain more than 90% of proteins with a molecular mass of 10 kDa or greater. The membranes most commonly used for laboratory dialysis are made of regenerated cellulose, manufactured using either the cuprammonium or viscose process. For both of these methods, dissolved cellulose is extruded as tubing or sheets and then dried. Glycerol is frequently added as a humectant to prevent cracking during drying and to help maintain desired pore structure. Pores range from 15-50 Å for 3.5K, 7K, 10K and 20K MWCO membranes. The membranes have a symmetrical pore structure that allows small molecules to migrate across them in either direction. Regenerated cellulose is hydrophilic and easily saturated in buffer to provide a homogeneous medium for dialysis in aqueous solutes. Membrane diffusion capacity is directly related to hydrophilicity. Thermo Fisher Scientific offers a variety of dialysis devices ranging in size from 2K-20K MWCO.

Dialysis procedure

It is the difference in the composition of sample and dialysis buffer solutions that creates the concentration-differential across the membrane that drives the dialysis process. Using a high buffer-to-sample volume-ratio helps to maintain the concentration gradient. The number of dialysate buffer changes and the dialysis time also affect the outcome achieved in dialysis. Because of the variables associated with each sample, a universal dialysis procedure for all applications cannot be provided, only general guidelines. Similarly, the process completion-point is somewhat subjective. The goal is to reduce the concentration of low molecular weight compounds to a level that will not interfere with subsequent steps in the experiment.

A typical dialysis procedure for protein samples is as follows:

1. Pre-wet or prepare the membrane according to instructions.
2. Load sample into dialysis tubing or device.
3. Dialyze for 1 to 2 h at room temperature.
4. Change the dialysis buffer and dialyze for another 1 to 2 h.
5. Change the dialysis buffer and dialyze overnight at 4°C.

Note: For best results, use a volume of dialysis buffer (dialysate) that is at least 200-fold greater than the sample volume.