



Dialysis: an introduction

Definition: Dialysis is a form of diffusion driven by a concentration gradient that separates substances in solution through a semi-permeable membrane.

Dialysis is the movement of molecules by diffusion from high concentration to low concentration through a semi-permeable membrane. Depending on the pore size of the semi-permeable membrane, only those molecules that are small enough to fit through these pores are able pass the membrane. At equilibrium, there is no net movement of the molecules. The equilibrium state is defined for the entire volume used for dialysis. It describes the state were the molecules move through the pores into and out of the dialysis unit at the same rate. All molecules larger than the membrane pores cannot pass through and will thus remain on the same side of the membrane as they were when dialysis at the point of initiation.

For complete dialysis by means of removal of all unwanted components, the dialysis buffer has to be exchanged for a new concentration gradient to be established. With each change of dialysis buffer, the sample inside the dialysis unit is further purified by a factor equal to the volume of the dialysis buffer.

Example

Dialysis of a 1ml sample against 100ml of dialysis buffer reduces the concentration of the dialyzed substance at equilibrium by a factor of 100. A 1ml sample containing 500mM NaCl contains 5mM of the salt after the first dialysis step. Each new buffer change using 100ml of new dialysis buffer dilutes the sample 100x more.

After three changes of dialysis buffer the diffusible molecules of the sample will be diluted by a factor of 1,000,000 (100x100x100). According to the above mentioned sample that initially contained 500mM NaCl, the salt concentration will decrease to 0.5 μ M, assuming that complete equilibrium was reached at the time of buffer exchange. In practice, dialysis is often used to simply reduce unwanted components to a non-effective minimum. The reduction salt of a 1ml sample that initially contained 500mM NaCl to less than 1mM, a 50x buffer volume with two changes of dialysis buffer can already be sufficient, whereas components disturbing in trace amounts have to be removed by extensive dialysis.

Substances that are significantly smaller than the pores of a dialysis unit will reach equilibrium faster than those that are only slightly smaller. Hence, a buffer change for a sample containing a homogenous pool of purified proteins is faster, than for a sample containing a heterogeneous pool by means of mixtures with proteins of very different molecular masses.

The greater the difference of the molecular mass of the displaceable molecule compared to the molecular weight cut-off (MWCO) of the pore size, the greater the rate of dialysis. The MWCO is the minimum molecular weight of a molecule that will be retained by the membrane pores of a certain dialysis unit. Care must be taken before dialysis is started. An appropriate tubing has to be chosen according to the molecular mass of the components to be kept inside the unit. The MWCO generally applies to globular molecules, such as most proteins. Note that linear proteins may be able to pass through pores although their molecular weight exceeds the stated MWCO. To compensate for this, choose a dialysis device with

a smaller MWCO. As a rule of thumb useful for protein mixtures, a MWCO no greater than one-third the molecular mass should be used in order to prevent sample loss.

Several factors affect the efficiency of dialysis:

Dialysis buffer volume	Time
Buffer & sample composition	Temperature
Number of buffer changes	Particle & pore size

These factors display the difficulties in predicting the dialysis time required for a particular sample. Thorough removal of components requires 100-500x volumes of dialysis buffer including completion of dialysis to equilibrium and three changes of dialysis buffer.

Dialysis is best performed at +4°C to minimize loss of activity.

Special Notes to Dialysis

Especially proteins tend to stick nonspecifically to dialysis membranes made of regenerated cellulose and thus cause sample loss. The amount of protein lost depends to a certain degree on the protein concentration and can be significant with dilute samples (<0.1 mg/ml). It is generally recommendable to yield higher protein concentrations or to concentrate the sample prior to dialysis to at least 0.5mg/ml. In certain cases BSA or other carrier proteins, that should not interact with the protein to be dialyzed, may be added to dilute samples before dialysis is started in order to minimize sample loss. Similarly the membranes of spin concentration units can be coated with a carrier protein. After thorough final washing steps of the membranes after coating, the protein sample is added for concentration. Note, that sample contamination by carrier proteins is not avoidable. Hence, before using carrier proteins for sample treatments, it must be assured that they will not interfere with the experimental processing or analytical evaluation of the sample, as the added proteins may not be completely removable.

During dialysis the molecules pass through a semi-permeable membrane in both directions and each substance reaches its own equilibrium independent of others. As a result, sample dilution occurs for all diffusing components with a rather large difference in concentration between sample and dialysis buffer. If the concentration of a component is higher on the inside of the membrane and if it is small enough to enter the pores, a net movement from the sample into the dialysis buffer occurs. Therefore, essential components like cofactors for example, must be present in both, sample and dialysis buffer to prevent deficiency and protein damage.

Dialysis is driven by a concentration gradient. When dialyzing a high solute concentration against a dilute dialysis buffer, there will be a net movement of water and salts into the dialysis unit. Attention must be paid if high concentrations of glycerol and sugars are present in the sample to be dialyzed and absent in dialysis buffer. These components are very hygroscopic and reach equilibrium very rapidly. More importantly, they strongly affect the osmosis of water into the dialysis unit and increase the volume of the sample. This can be largely avoided by dialyzing the sample first against a more concentrated dialysis buffer and make a stepwise replacement by a less concentrated buffer until the desired final buffer concentration is reached.