Dialysis

Dialysis is a separation technique that facilitates the removal of small, unwanted compounds from macromolecules in solution by selective and passive diffusion through a semipermeable membrane.

Mechanism Dialysis:

Dialysis works by diffusion, a process that results from the thermal, random movement of molecules in solution. 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.

At the end, diffusion of small molecules leads to the net movement from areas of higher to lower concentration, until an equilibrium is reached. The total volume of sample and dialysate determine the final equilibrium concentration of the small molecules on both sides of the membrane. By using the appropriate volume of dialysate and multiple exchanges of the buffer.

The efficiency of dialysis largely depends on the difference between the volumes of the inside (sample, V1) and outside (buffer, V2) liquid spaces. This is why we generally seek to use as large volume (V2) of the dialysate as possible. However, the efficiency of dialysis can be further increased by performing multistep dialysis by exchanging the outer solution after the equilibrium has been reached. In this case, the attainable dilution of the inside solution will be (n: number of steps): $[V1/(V1+V2)]n$.

For example, when dialyzing 1mL of sample against 200mL of dialysate, the concentration of unwanted dialyzable substances will be decreased 200-fold when equilibrium is attained. Following two additional buffer changes of 200mL each, the contaminant level in the sample will be reduced by a factor of 8×10^6 (200 x 200 x 200).

Applications of the dialysis:

Most of these applications occur on the laboratory scale, because on industrial scale the removal of salts can be done more rapidly via diafiltration .However, Dialysis is used for a wide variety of applications:

desalting, buffer exchange, removal of labeling reagents, drug binding studies, cell growth and feeding, virus purification, and blood treatment.

Factors Affecting Dialysis Rate:

Factors that affect the completeness of dialysis include:

1. dialysis buffer volume.
2. Buffer composition.
3. The number of buffer changes.
4. Time.
5. Temperature.
6. Particle size vs. pore size.

Substances that are **very much smaller** than the pore size will reach equilibrium faster than substances that are only **slightly smaller** than the pores.

Advantages of dialysis:

- Simple, easy & quick set-up
- Inexpensive & disposable
- Very gentle separation (no pressure or shear stress)
- Doesn't require monitoring
- Dissolved solutes remain in solution
- Solutes are unaltered or damaged
- Total product recovery

Disadvantages of dialysis:

1. Very slow (1 – 2 days)
2. Molecules should be 100 X size difference

Molecular weight cutoff:

Dialysis is performed with semipermeable membranes. The average or maximum pore sizes of a dialysis membrane determines what size molecules can diffuse across it, which defines its molecular weight cutoff (MWCO). The MWCO describes the molecular weight at which a compound will be 90% retained following overnight (17-hour) dialysis. The MWCO is determined by testing many different proteins of known molecular weight. In general, these MWCO apply to globular molecules, such as most proteins. More linear proteins may be able to pass through the pores, even though their molecular weight exceeds the stated MWCO. To compensate for this, choose a dialysis device with a smaller MWCO. The greater the difference in molecular weight of the unwanted molecule vs. the molecular weight cut-off (MWCO) of the pore size, the greater the rate of dialysis.

For DNA or RNA, a MWCO no greater than one-third the MW should be used in order to prevent excessive sample loss.Thus, a dialysis membrane with a 10000 MWCO will generally retain proteins having a molecular mass of at least 10 kDa.

Dialysis membranes are produced and characterized according to molecular-weight cutoff (MWCO) limits.While membranes with MWCOs ranging from 1-1,000,000 kDa are commercially available, membranes with MWCOs near 10 kDa are most commonly used.

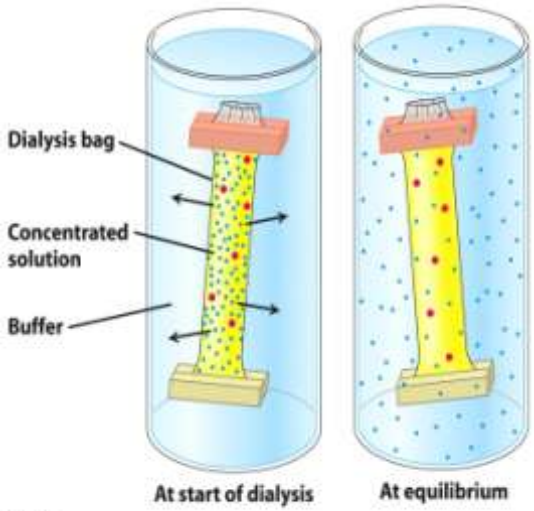
The most common membranes are made of cellulose and are used once because of the high hygiene standards of the usual applications. Membranes have a tendency to contamination and are usually too thin to resist cleaning or high pressure. Because of these characteristics, it is likely not the proper filtration method.

General Protocol of dialysis :

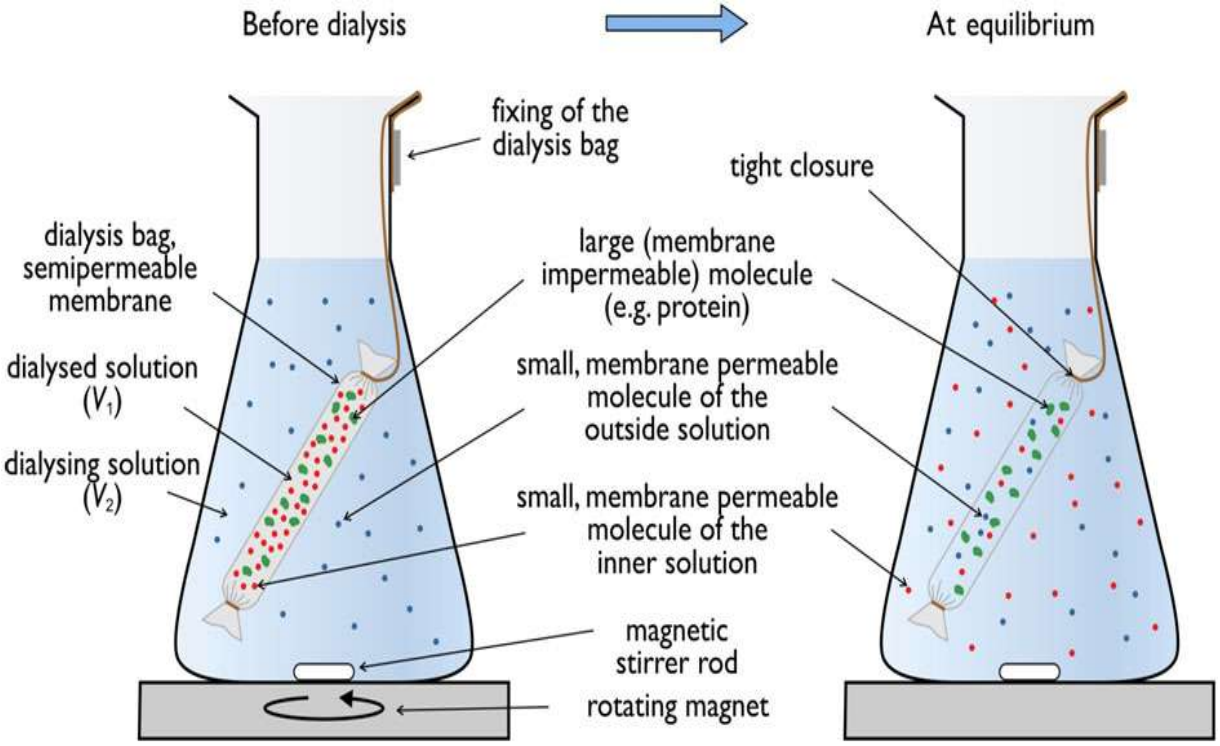
A typical dialysis procedure for protein samples is as follows:

1. Prepare the membrane according to instructions.
2. Load the sample into dialysis tubing, cassette or device.
3. Place sample into an external chamber of dialysis buffer (with gentle stirring of the buffer).
4. Dialyze for 2 hours (at room temperature or 4 °C).
5. Change the dialysis buffer and dialyze for another 2 hours.
6. Change the dialysis buffer and dialyze for 2 hours or overnight.

Dialysis tubing, also known as **Visking tubing**, is a type of semi-permeable membrane tubing used in separation techniques, that facilitates the removal or exchange of small molecules from macromolecules in solution based on differential diffusion. In the context of life science research, dialysis tubing is typically used in the sample clean-up and processing of proteins and DNA samples or complex biological samples such as blood or serums.


Dialysis bag
Concentrated solution
Buffer
At start of dialysis
At equilibrium


Fisherbrand
DIALYSIS
TUBING
Instructions
Fisherbrand


Before dialysis
At equilibrium
fixing of the dialysis bag
tight closure
dialysis bag, semipermeable membrane
large (membrane impermeable) molecule (e.g. protein)
dialysed solution (V_1)
small, membrane permeable molecule of the outside solution
dialysing solution (V_2)
small, membrane permeable molecule of the inner solution
magnetic stirrer rod
rotating magnet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