

Diafiltration (Buffer Exchange) Using Hollow Fiber Membranes instead of Dialysis Tubing - Automated Diafiltration

Diafiltration is term used reflecting tangential flow filtration (TFF) method of “washing” or removing a permeable molecule (impurities, salts, solvents, small proteins, etc) from a solution in an accelerated method when compared to dialysis tubing. By selecting the appropriate membrane(s), it can also be used to exchange buffers, narrow particle size distributions, modify salt concentrations or maximize protein recovery in a clarification process. Buffer exchange, the process of using an ultrafiltration membrane to rapidly and gently replace one buffer of a protein solution with another, is one of the most common applications of diafiltration with Spectrum Laboratories, Inc. hollow fiber modules.

The success of a diafiltration for performing a fractionation process is largely determined by the selection of an appropriate membrane. The membrane pores must be large enough to allow the permeable species to pass through (when fractionating) and small enough to retain the larger species. A large variety of membranes are available in the ultrafiltration and microfiltration range for this purpose.

Tangential Flow Filtration using a hollow fiber membrane is accomplished by pumping the process solution into the inner diameter of a tubular fiber. The pores of the membrane in the walls of the fiber allow the permeable species to pass through, while the larger species is retained in the bulk flow and continues through to the ‘retentate’ end of the fiber (figure 1). Diafiltration occurs by adding the replacement buffer, or washing solution, to the bulk flow either at the same rate of permeable species (continuous diafiltration) or after a certain level of concentration then re-suspension (discontinuous diafiltration). The result is a decrease in concentration of the permeable species while the retained species remains in solution which is gently circulating through the tangential flow system.

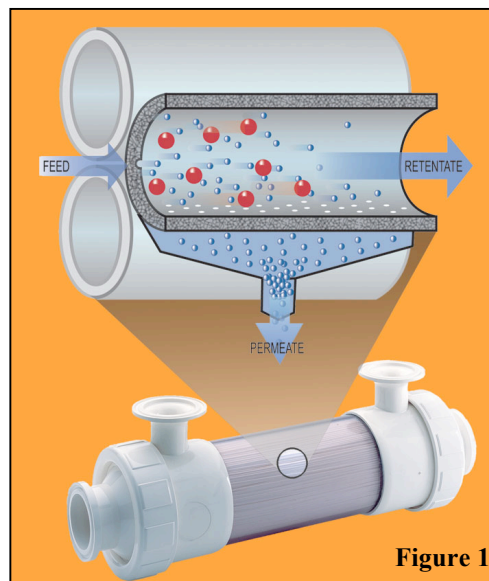


Figure 1

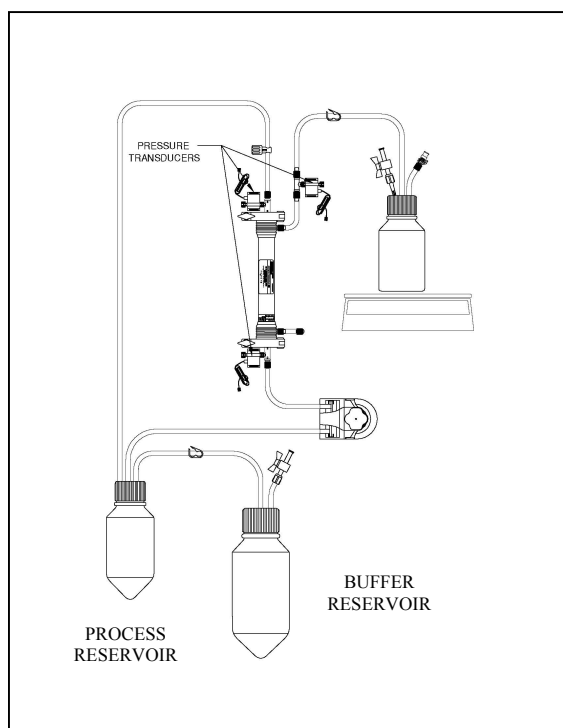


Figure 2

The typical TFF system includes a pump, pressure measurement device, flow measurement device, process reservoir, buffer reservoir and hollow fiber filter module. The pump circulates the process solution from the process reservoir, through the filter and back to the process vessel at a controlled flow / shear rate. Pressure measurements are acquired in this re-circulation loop to control the driving force through the membrane. Careful measurement of the permeate flow rate enables accurate process scale up and process optimization. Diafiltration occurs simply by adding the diafiltration buffer to the circulation directly into the process vessel or within the recirculation loop. Figure 2 shows a typical diafiltration system where the buffer is continuously added back into the process reservoir by means of vacuum flow rate based upon permeate flow (continuous diafiltration).

There are multiple ways of performing a diafiltration. Working with hollow fiber modules, tubing and air-tight sealable bottles is an excellent (easy) means of performing the more efficient “continuous” method of diafiltration. In continuous diafiltration, the diafiltration buffer is added to the process vessel (or process loop) at the same rate permeate is being extracted.

Concentration Before Diafiltration Considerations

There is a relationship / correlation between the process solution volume and the amount of buffer required to complete a diafiltration. This will be discussed in the following section.

To minimize the volume (time, cost) of buffer used, it is sometimes better to *concentrate* the product prior to diafiltration. Concentration is the volume reduction of the starting process

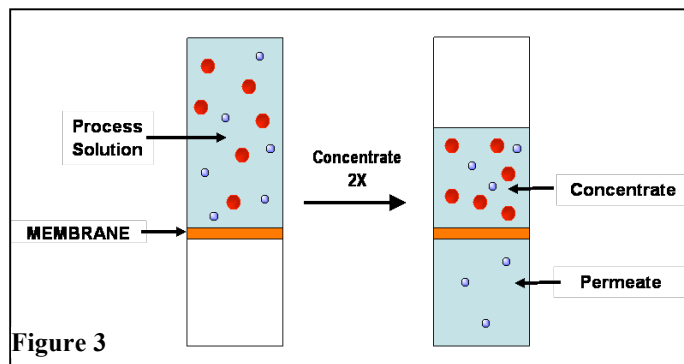


Figure 3

solution. It is accomplished by collecting permeate without adding any diafiltration buffer to the process vessel. Because the larger molecule is retained by the membrane, the concentration of that molecule will increase. However, the concentration of a freely permeable molecule (salts or solvents) would remain the same. Figure 3 shows a 2X concentration, in which the process volume is reduced to half of its initial

volume and the concentration of the retained molecule is doubled.

Concentration can affect the performance of the diafiltration in two ways: process flux (permeate flow rate per unit area) and transmission of the permeable molecules when utilizing diafiltration to fractionate the process solution. As the concentration of the retained molecule increases at the membrane surface, there is a higher resistance to flow through the membrane. This increase in resistance results in a decrease in the permeate flow rate, also referred to as the *process flux*, J (equation 1).

$$J, (L/M^2/hr) = \frac{\text{Permeate Flow rate}}{\text{Membrane Area}}$$

Equation 1

In some applications, the higher concentration of retained molecules at the membrane surface may also result in difficulty for the smaller permeable species to pass through the membrane (fractionation). This critical relationship called Transmission, %T, can be characterized by sampling from the feed and permeate during a concentration process.

$$T = \frac{C_{\text{PERMEATE}}}{C_{\text{FEED}}} \quad (2a) \quad T (\%) = \frac{C_{\text{PERMEATE}}}{C_{\text{FEED}}} \times 100 \quad (2b)$$

Equation 2: Transmission coefficient (2a) and %Transmission (2b)

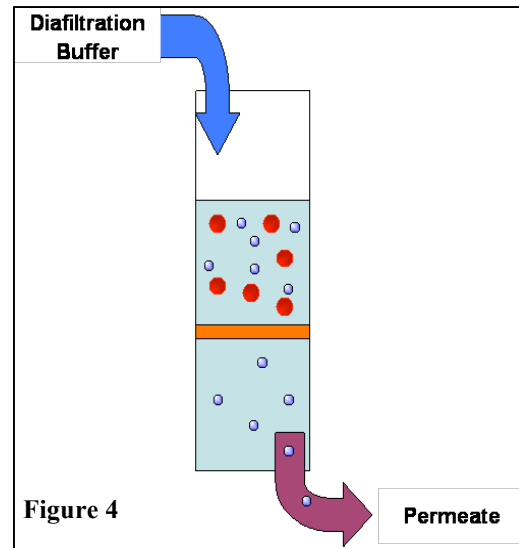
Understanding the effect of concentration on these two parameters, process flux and transmission, makes it possible to optimize a diafiltration and fractionation process.

Diafiltration (Buffer Exchange)

To summarize, diafiltration is a process during which clean buffer is added to the process reservoir with the goal of “washing out” a permeable species. Continuous diafiltration is one technique or type of diafiltration. This process of continuous diafiltration is when buffer is added to the process material at the same rate permeate is exiting the hollow fiber module through the permeate. Figure 4 is a simple representation of this.

Continuous diafiltration in an airtight system is a self-regulating and constant flow process.

[Note: a similar set up can be done using a pump assisted process where a double headed secondary pump is used to both regulate permeate flow while adding buffer back into the process reservoir at the same rate (this is commonly done in MF-TFF where fractionation / clarification of components are performed)].



When the tubing to the buffer reservoir (figure 2) is submerged in the clean diafiltration buffer, a vacuum forms in the process reservoir. This vacuum pulls buffer into the process vessel at a flow rate equal to the process flux (permeate flow rate per unit area). When the target volume of diafiltration buffer has been collected in the permeate vessel, the process is stopped simply by removing the tubing from secondary / buffer reservoir or breaking the seal on the feed reservoir (open vent valve). This method maintains gentle conditions and will continue if required the constant concentration of the retained molecule.

This can be particularly important for some sensitive products.

It is important to understand is the term *Diafiltration Volume*, V_D . Each diafiltration volume is a volume of buffer equal to the volume of process solution in the reservoir and hold up volume of the process recirculation loop at the start of the diafiltration.

Therefore, the total volume of buffer required for a diafiltration process can be calculated as follows:

$$\text{Buffer Volume} = V_D \times V_{\text{INITIAL}}$$

Equation 3

To predict the number of diafiltration volumes required to “wash out” a permeable molecule when fractionating, the chart in Figure 5 may be used. The equation following the chart, Equation 4, is the well-accepted formula used to derive it.

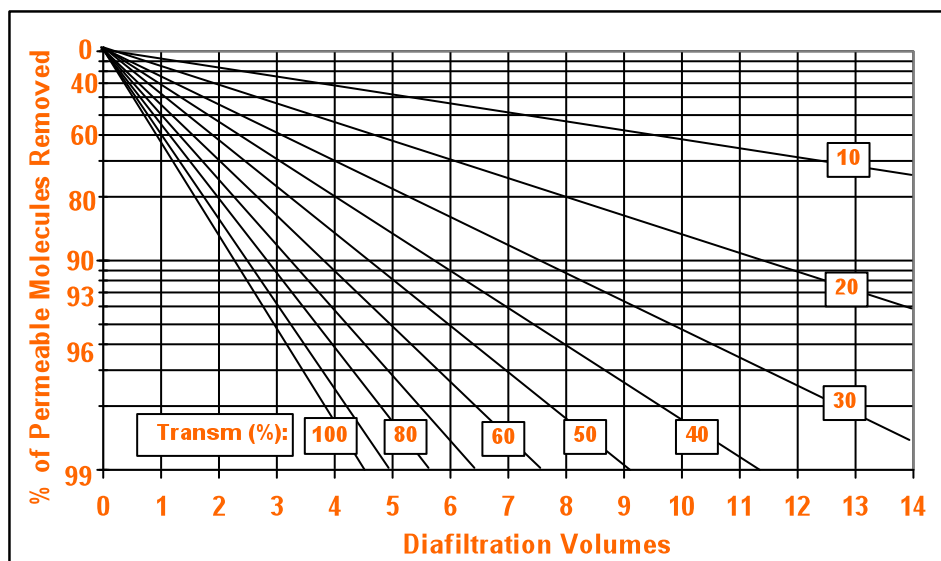


Figure 5

With the transmission coefficient, T, and the number of diafiltration volumes utilized, it is possible to calculate the percentage of the permeable molecules that have been removed. The actual can be verified through analytical methods for verification.

$$\% \text{ Permeable Molecules Removed} = 1 - \left[e^{-V_D \times T} \right]^{-1}$$

Equation 4

Summary

Diafiltration, or Concentration/Diafiltration, is a rapid as well as gentle and scalable method of “washing” out a molecule, replace salts etc that is smaller than those being retained by the tangential flow filtration membrane (UF or MF). The small species may be low molecular weight impurities (like serum base proteins, etc), buffer salts, non-reacted components, or even the product of interest (MF-TFF, microporous tangential flow filtration). Optimizing the diafiltration process by selecting the correct membrane pore size and characterizing the relationship between concentration and transmission will provide a stable and reproducible purification process and, as a result, a reproducible product with each filtration operation.

Technical Bulletin

Accelerated Method for Diafiltration when a Buffer Cannot be utilized: “Co-Flush” Tangential Flow Filtration

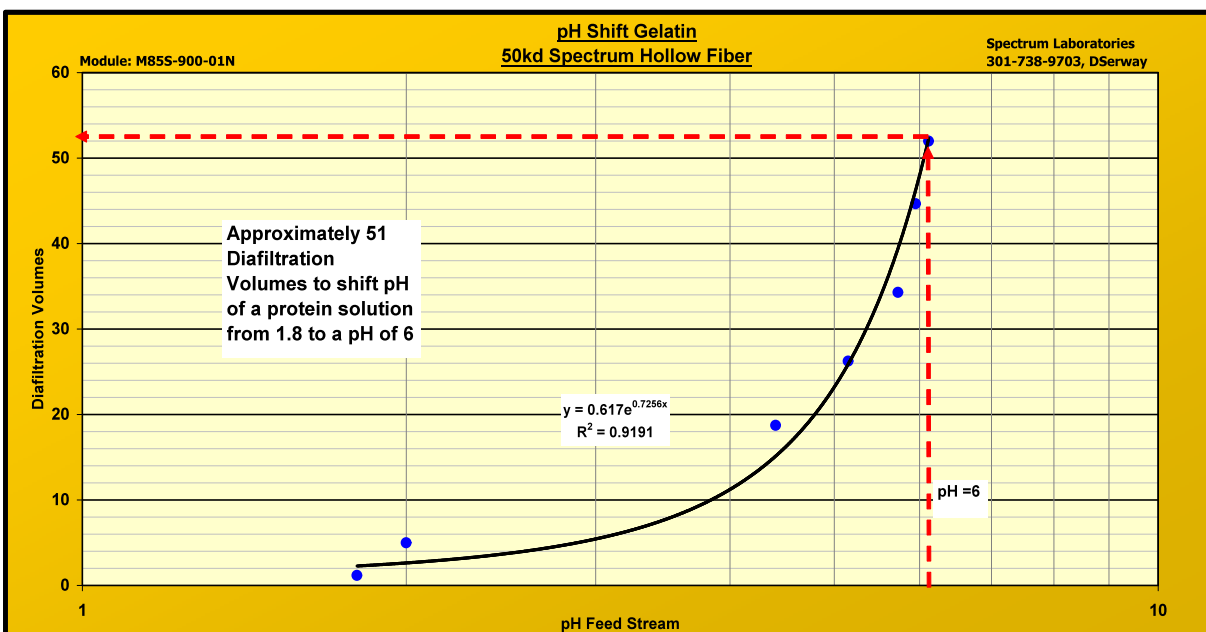
Several applications currently within the market require removal of reactives (acid’s, base solutions) without the addition of a salt solution. Acceptable practice is to utilize water for injection as the “buffer” or solution to exchange out the acid or base with water to bring the solution back to a neutral or close to neutral pH.

Common practice at a laboratory scale is utilization of Spectrum Laboratories, Inc. dialysis tubing with many bath changes over a period of days. However, this is not a scaleable practice for the biopharmaceutical industry.

Since water is not a buffer simple diafiltration via continuous or discontinuous method on a hollow fiber is not readily achieved since the freely passing ions can transfer both through the pore of the membrane readily in either direction. Understanding this, when performing a discontinuous or continuous diafiltration with WFI / water additional considerations must be performed to actively remove the ions from the hollow fiber module.

Utilization of a double headed secondary pump comes into practice when performing this process of “diafiltering” an acidic or basic solution with WFI or like water. With Spectrum Laboratories, Inc. hollow fiber modules water is brought into the lower permeate port as well as into the process reservoir or feed stream at the same rate while maintaining the targeted process material concentration. This method flushes the permeable species outside the lumen of fibers while the lingering ions are then continual flushed from the ECS (extracapulary space / housing) to drain. It is important to note that the amount of WFI or water required for this process depends upon the starting pH of the feed stream.

Below shows this relationship when removing acid (pH 1.8) within a protein stream with WFI water:



Summary:

This method of “co-flush” diafiltration is an ideal method for performing a buffer exchange with a non-ionic solution like WFI or DI water. Without this practice, the shift of the pH in this process solution can not be effectively achieved for a scaleable process.

This improvement using Spectrum's co-flush tangential flow diafiltration method will reduce buffer exchange time from day's to hours.