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Introduction to Tangential Flow Filtration

Introduction

Tangential flow filtration (TFF), also known as cross flow filtration, is a rapid and efficient method for the separation and purification of biomolecules. The process fluid passes tangentially across the surface of a filter membrane, and as a pressure differential is applied to the system, constituents in the sample that are small enough to travel through the pore structure of the membrane will pass in to the filtrate. Larger constituents will be retained and recirculate around the flow path of the system.

The method can be applied to a wide range of biological fields such as immunology, protein chemistry, molecular biology, biochemistry, and microbiology. TFF can be used to concentrate and desalt sample solutions ranging in volumes from 10 mL to thousands of liters. It can be used to fractionate large from small biomolecules, harvest cell suspensions, and clarify fermentation broths and cell lysates.

What is Tangential Flow Filtration

Membrane filtration is a separation technique widely used in the life science laboratory. Depending on membrane porosity, it can be classified as a microfiltration or ultrafiltration (UF) process. Microfiltration membranes with pore sizes typically between 0.1 µm and 10 µm are generally used for clarification, sterilization, and removal of microparticulates or for cell harvesting. Ultrafiltration membranes, with much smaller pore sizes between 0.001 µm and 0.1 µm, are used for concentrating and desalting dissolved molecules (proteins, peptides, nucleic acids, carbohydrates, and other biomolecules), exchanging buffers, and gross fractionation. Ultrafiltration membranes are typically classified by molecular weight cut-off (MWCO) rather than pore size.

There are two main membrane filtration modes which can use either microfiltration or ultrafiltration membranes:

Direct Flow Filtration (DFF):
Also known as "dead-end" filtration, applies the feed stream perpendicular to the membrane face and attempts to pass 100% of the fluid through the membrane. The resulting filtrate is then collected.

Tangential Flow Filtration (TFF):
Also known as crossflow filtration, where the feed stream passes parallel to the membrane face. Sample constituents that are small enough to travel through the pore structure of the membrane will pass, this is called the permeate or filtrate. Larger constituents will be retained and will recirculate around the system, this is called the retentate. Both the filtrate and retentate may be collected.

An analogy for understanding the theory behind TFF can be seen when trying to separate small particles from large particles using a mesh sieve. The holes in the sieve represent the pores in the membrane.

In DFF, the small and large particulate mixture is forced toward the holes in the mesh sieve. The smaller particles fall through the holes in the sieve, but the larger particles form a layer on the surface of the screen. This prevents small particles at the top of the mixture from moving to and through the holes (Figure 1A).

With DFF, increasing the pressure simply compresses the mixture without increasing the separation. In contrast, operating in a TFF mode prevents the formation of a restrictive layer by recirculating the mixture. The process acts like shaking a sieve. The turbulences remove the large particles that can block the holes in the screen, allowing the small particles at the top of the mixture to fall toward and through the holes (Figure 1B).

Figure 1A
Pressure

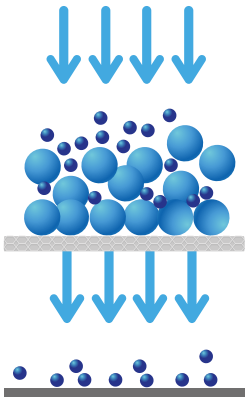
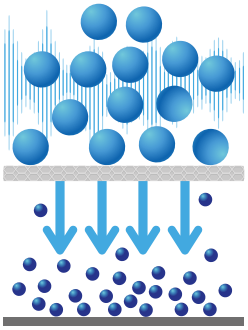


Figure 1B
Turbulence

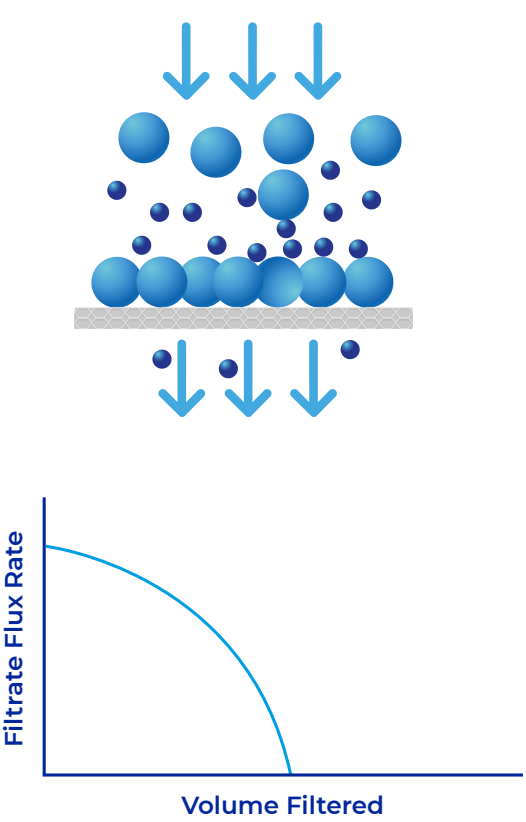


(A) Applying direct pressure to the mixture allows small particles to move through the mesh sieve. A layer of larger particles quickly builds up and prevents further smaller particles from passing through the sieve.

(B) Adding turbulence and shaking the sieve breaks up the aggregated layer of large particles, allowing for the smaller particles to continue to pass through. The crossflow dynamic of the feed stream in tangential flow filtration serves the same purpose as shaking in this example.

When processing a solution the differences between DFF and TFF can be visualized by plotting a graphs of filtrate flux rate against volume filtered. The resulting plots can be seen in figures 2 and 3.

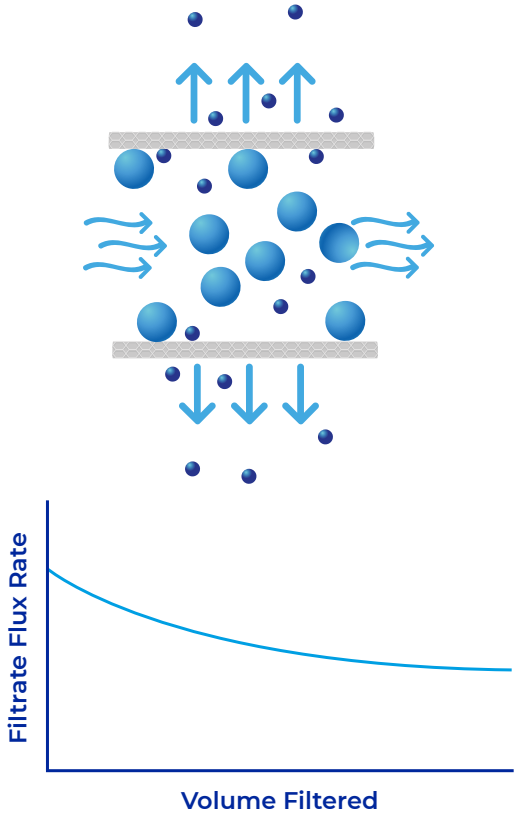
Figure 2
Direct Flow Filtration Process



The feed is directed into the membrane. Molecules larger than the pores accumulate at the membrane surface blocking the flow of liquid and smaller molecules that could pass through. This layer of large molecules is referred to as a “gel layer”.

As the volume filtered increases, fouling increases and the flux rate decreases rapidly, resulting in the membrane blocking and the end of the filtration.

Figure 3
Tangential Flow Filtration Process



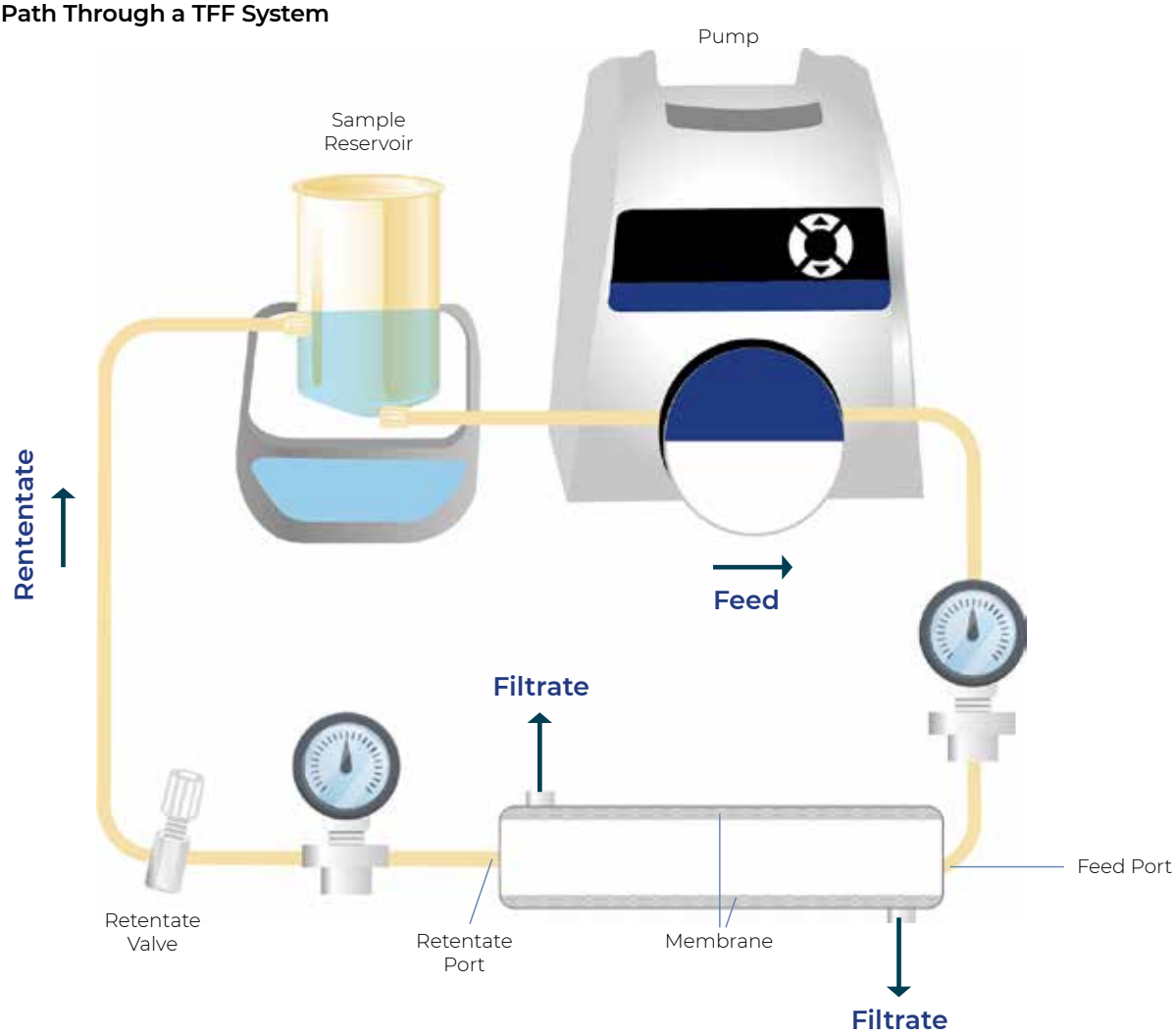
Sample solution flows through the feed channel and tangentially along the surface of the membrane. The crossflow prevents the build-up of molecules at the surface of the membrane. This disruption of the gel layer allows for the flow of molecules that are small enough to pass through the pores of the membrane to continue into the filtrate. The TFF process prevents the rapid decline in flux rate seen in direct flow filtration allowing a greater volume to be processed per unit area of membrane surface.

Tangential Flow Filtration System Configuration

TFF systems typically require a TFF membrane device (capsule or cassette and holder), a pump (peristaltic or equivalent), tubing, valves or clamps, one or more pressure gauges, and a sample reservoir. Pressure gauges are typically installed at the feed, retentate, and filtrate ports in development and process TFF systems.

While it is possible to run a TFF system without pressure gauges, the use of at least one pressure gauge on the feed side (between pump and TFF unit) is strongly recommended. Pressure is an important variable in the TFF process. The ability to monitor and control the pressure leads to more consistent results, and can be very helpful for troubleshooting system problems.

Figure 4
Flow Path Through a TFF System



Two of the important variables involved in all TFF devices are transmembrane pressure (TMP) and crossflow velocity (CF).

1. The transmembrane pressure is the force that drives fluid through the membrane, carrying along the permeable molecules.
2. The crossflow velocity is the rate of the solution flow through the feed channel and across the membrane. It provides the force that sweeps away molecules that can foul the membrane and restrict filtrate flow.

Tangential Flow Filtration System Operation

When operating a TFF system, the fluid is pumped from the sample reservoir into the feed port, across the membrane surface (crossflow), out the retentate port and back into the sample reservoir (Figure 4).

The crossflow sweeps away larger molecules and aggregates that are retained on the surface of the membrane, preventing gel polarization (the formation of a concentrated biomolecule layer on the membrane surface that can foul or plug the membrane).

Liquid flowing through the narrow feed channel creates a pressure difference between the feed and retentate ports. This pressure, which is applied to the membrane, can be further increased by increasing the crossflow rate or by restricting the tubing at the retentate port by tightening the retentate valve. The resulting transmembrane pressure (TMP) is the force that drives liquid through the membrane.

Liquid that flows through the membrane (filtrate or permeate) carries molecules smaller than the membrane pores through the filter. Larger molecules that are unable to pass through the pores of the membrane will remain in the retentate and will continue to circulate around the flow path of the TFF system.

Effectively regulating both the TMP and crossflow rate will prevent membrane fouling, thus allowing a greater volume of product to be processed in the least possible time and improve the operational efficiency of TFF system.

Operation of a TFF system consists of the following steps:

1. Rinse the TFF device before use to remove any storage agent.
2. Establish the normalized water permeability (NWP) of the membrane to establish a baseline for the device performance (note - this step is not necessary but strongly recommended if the device will be cleaned and reused).
3. Condition the system with a sample buffer (conditioning helps remove air from the system, adjusts the systems temperature and prevents possible precipitation or denaturation of biomolecules resulting from contact with flushing solution).
4. Process the sample (concentration and/or diafiltration, or fractionation).
5. Clean the system and TFF device and determine cleaning efficiency.
6. Store TFF Device.

Comprehensive and optimal systems operation including; capsule and cassette conditioning, NWP protocols, recommeded retentate flow rates, cleaning and storage protocols are supplied in Pall's TFF Instructions for Use Guide.

Ultrafiltration Fundamentals

Ultrafiltration (UF) is a membrane separation technique used to separate extremely small particles and dissolved molecules in fluids. The primary basis for separation is molecular size, although other factors such as molecular shape and charge can also play a role. Molecules larger than the membrane pores will be retained at the surface of the membrane and concentrated during the ultrafiltration process.

Compared to non-membrane processes (chromatography, dialysis, solvent extraction, or centrifugation), ultrafiltration:

- Is gentler to the molecules being processed
- Does not require an organic extraction which may denature labile proteins
- Maintains the ionic and pH environment
- Is fast and relatively inexpensive
- May be performed at low temperatures (for example, in the cold room)
- Is very efficient and can simultaneously concentrate and purify molecules

The retention properties of ultrafiltration membranes are expressed as molecular weight cut-off (MWCO). This value refers to the approximate molecular weight (MW) of a dilute globular solute (i.e., a typical protein) which is > 90% retained by the membrane. However, a molecule's shape can have a direct effect on its retention by a membrane. For example, linear molecules like DNA may find their way through pores that will retain a globular species of the same molecular weight.

Pall Omega™ membranes are highly selective. The curves in Figure 5 illustrate the selectivity of these membranes. The narrow pore size distribution results in minimal retention of molecules whose molecular weights fall below the MWCO of the membrane.

There are three generic applications for ultrafiltration:

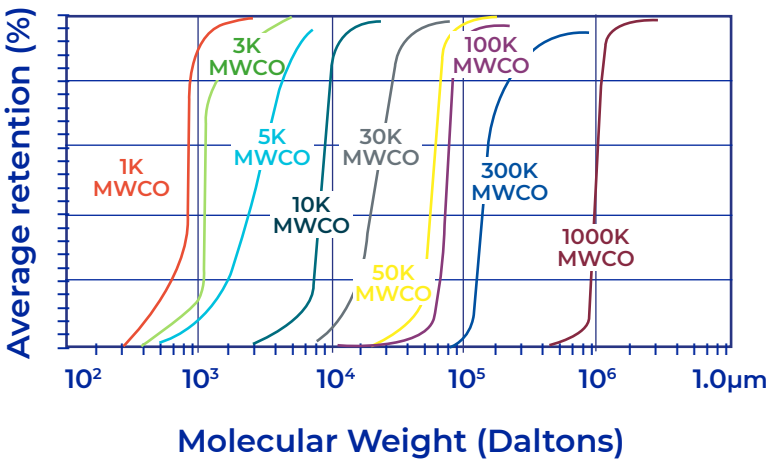
Concentration:
Ultrafiltration is a very convenient method for the concentration of dilute protein or DNA/RNA samples. It is gentle (does not shear DNA as large as 100 Kb or cause loss of enzymatic activity in proteins) and is very efficient.

Desalting and Buffer Exchange (Diafiltration):
Ultrafiltration provides a very convenient and efficient way to remove or exchange salts, remove detergents, separate free from bound molecules, remove low molecular weight materials, or rapidly change the ionic or pH environment.

Fractionation:
Ultrafiltration will not accomplish a sharp separation of two molecules with similar molecular weights. However, ultrafiltration can be used to perform gross fractionation.

The molecules to be separated should differ by at least one order of magnitude (10X) in size for effective separation. Fractionation using ultrafiltration is effective in applications such as the preparation of protein-free filtrates or the separation of unbound and bound constituents.

Figure 5
Selectivity of Ultrafiltration Membranes



Selecting MWCO

Table 1 provides retention characteristics of different MWCO membranes for various solutes.

For proteins, it is recommended that a MWCO be selected that is 3 to 6 times smaller than the molecular weight of the solute being retained. If flow rate is a consideration, choose a membrane with a MWCO at the lower end of this range (3X); if the main concern is retention, choose a tighter membrane (6X).

It is important to recognize that retention of a molecule by a UF membrane is determined by a variety of factors, among which its molecular weight serves only as a general indicator. Therefore, choosing the appropriate MWCO for a specific application requires the consideration of a number of factors including molecular shape, electrical charge, sample concentration, sample composition, and operating conditions.

Common Variables That Increase Molecule Passage:

- Sample concentration less than 1 mg/mL
- Linear versus globular molecules
- High transmembrane pressure
- Buffer composition that favors breakup of molecules
- pH and ionic conditions that change the molecule (for example, cause conformational changes or aggregation)

Common Variables That Decrease Molecule Passage:

- Sample concentration higher than 10 mg/mL
- Buffer conditions that permit molecules to aggregate
- Presence of other molecules that increase sample concentration
- Lower transmembrane pressure
- Adsorption to the membrane or device
- Low temperature (4 °C versus 24 °C)

Because different manufacturers use different molecules to define the MWCO of their membranes, it is important to perform pilot experiments to verify the membrane performance for a particular application. Pall's centrifugal UF devices containing the Omega membrane can aid in offering an indication of membrane performance before scaling up to a larger Pall TFF system. Centrifugal devices allow the testing of small volumes of processing solution in a very easy to use and cost-effective way.

Omega Membrane

Pall's Omega polyethersulfone (PES) membranes offer high flux and selectivities. They have been specifically modified to minimize protein binding to the surface and interstitial structure of the membrane. This polymeric membrane is stable against biological and physical degradation due to the unique chemical properties of PES.

Omega membranes are cast on a highly porous, non-woven polyolefin support. They have an anisotropic structure, a thin-skinned like top layer with a highly porous underlying support.

The structure of the skin determines the porosity and permeability characteristics of the membrane and can typically be cleaned quicker and easier than membranes with a uniform, sub-micron depth structure.

This membrane is compatible with acids, bases and a variety of other cleaning agents. Omega membranes are available in a wide range of nominal molecular weight cut-offs.

Table 1 - Retention Characteristics of Omega Membrane

MWCO Selection for Protein Applications

MWCO	Membrane Nominal Pore Size*	Biomolecule Size	Biomolecule Molecular Weight
1K	-	-	3K - 10K
3K	-	-	10K - 20K
5K	-	-	15K - 30K
10K	-	-	30K - 90K
30K	-	-	90K - 180K
50K	5 nm	15 - 30 nm	150K - 300K
70K	-	-	210K - 420K
100K	10 nm	30 - 90 nm	300K - 900K
300K	35 nm	90 -200 nm	900K - 1800K
500K	-	-	1500K - 3000K
1000K	100 nm	300 - 600 nm	> 3000K

MWCO Selection for Nucleic Acid Applications

MWCO	Base Pairs (DS)	Bases (SS)
1K	5 - 16 Bp	9 - 32 Bs
3K	16 - 32 Bp	32 - 65 Bs
5K	25 - 50 Bp	50 - 95 Bs
10K	50 - 145 Bp	90 - 285 Bs
30K	145 - 285 Bp	285 - 570 Bs
50K	240 - 475 Bp	475 - 950 Bs
100K	475 - 1450 Bp	950 - 2900 Bs
300K	1450 - 2900 Bp	2900 - 5700 Bs
1000K	4800 - 9500 Bp	> 9500 Bs

MWCO Selection for Virus Applications

MWCO	Membrane Nominal Pore Size*	Virus or Particle Diameter
100K	10 nm	30 - 90 nm
300K	35 nm	90 - 200 nm

**Nominal pore size as measured by electron microscopy*

Applications

The primary applications for TFF are concentration, diafiltration (desalting and buffer exchange), and fractionation of large from small biomolecules. In addition, TFF can be used for clarification and removal of cells as well as cellular debris from fermentation or cell culture broths.

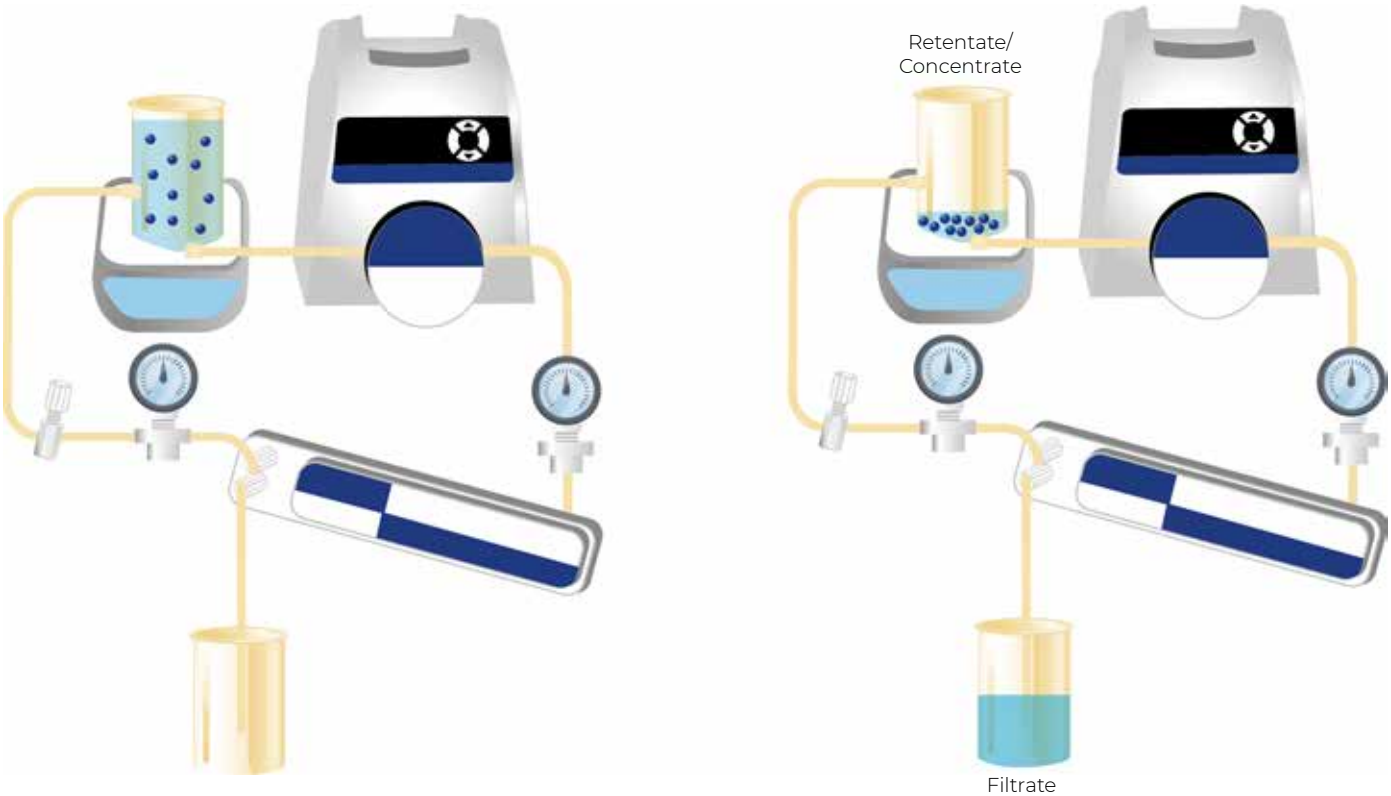
Concentration

Concentration is a simple process that involves removing fluid from a solution while retaining the solute molecules. The concentration of the solute increases in direct proportion to the decrease in solution volume, i.e. halving the volume effectively doubles the concentration.

To concentrate a sample, choose a UF membrane with a MWCO that is substantially lower than the molecular weight (MW) of the molecules to be retained. This is important in order to assure complete retention and high recovery of the target molecule. A good general rule is to select a membrane with a MWCO that is 3 to 6 times lower than the MW of the molecules to be retained. If the sample will only be concentrated, then 3 times lower is sufficient. If significant diafiltration will also be applied to the sample, then an even lower MWCO (i.e. to 6 times lower) may be advisable.

The membrane is installed (or a disposable TFF capsule selected), and the TFF system is initialized. The sample is then added, a crossflow is established, feed and retentate pressures are set, then filtrate is collected. When the desired concentration is reached, the process is stopped, and sample recovered.

Figure 6
TFF Concentration



When recovering the concentrate, it is important to note that a significant portion of the concentrated product could be on the membrane in the form of a gel layer. This will need to be recovered back into the solution before the system is drained.

Pall supply detailed procedures on how to maximize concentrate recovery in the TFF Instructions for Use Guide.

Diafiltration

Diafiltration is a technique that uses ultrafiltration membranes to completely remove, replace, or lower the concentration of salts or solvents from solutions containing proteins, peptides, nucleic acids, and other biomolecules.

Benefits of Diafiltration

Conventional techniques used for salt removal or buffer exchange such as membrane dialysis and column based gel filtration can be effective but have limitations. Dialysis procedures can take up to several days, require large volumes of water for equilibration and risk product loss through manual manipulation of the dialysis bags. Gel filtration results in a dilution of the sample and often requires an additional ultrafiltration step to concentrate it back. Adding steps to a process can risk sample loss or possible contamination.

With diafiltration, salt or solvent removal as well as buffer exchange can be performed quickly and conveniently. Another big advantage of diafiltration is that the sample is concentrated on the same system, minimizing the risk of sample loss or contamination.

There are several ways to perform diafiltration. While the end result may be the same, the time and volume required to complete the process may vary considerably. It is important to understand the differences in the methods used and when to choose one over the other.

Continuous Diafiltration

The technique of continuous diafiltration (also referred to as constant volume diafiltration) involves washing out the original buffer salts (or other low molecular weight species) in the retentate (sample) by adding water or a new buffer to the retentate at the same rate as filtrate is being generated. Figure 7 shows a typical continuous diafiltration system setup using a Minimate™ EVO TFF System. The retentate volume and product concentration does not change during the diafiltration process. If water is used for diafiltering, the salts will be washed out and the conductivity lowered. If a buffer is used for diafiltering, the new buffer salt concentration will increase at a rate inversely proportional to that of the species being removed.

The amount of salt removed is related to the filtrate volume generated, relative to the retentate volume. The filtrate volume generated is usually referred to in terms of “diafiltration volumes”. A single diafiltration volume (DV) is the volume of retentate when diafiltration is started. For continuous diafiltration, liquid is added at the same rate as filtrate is generated. When the volume of filtrate collected equals the starting retentate volume, 1 DV has been processed.

Using continuous diafiltration, greater than 99.5% of a 100% permeable solute can be removed by washing through 6 retentate volumes (6 DV) with the buffer of choice.

Molecules that are larger than salts and solvents, but which are still smaller than the pores in the membrane, can also be washed out. The permeability of these molecules, however, may be less than 100%. In such cases, it will take more liquid, i.e. more DV’s, to completely wash a partially permeable molecule through the membrane, compared to a 100% permeable molecule. Typically, the larger the molecule, the lower the permeability and the greater the wash volume required.

The permeability of a molecule through a specific membrane can be determined by measuring the concentration of the molecule in the filtrate compared to the concentration in the retentate under specified conditions.

$$\% \text{ permeability} = (\text{Conc.}_{\text{FILTRATE}} / \text{Conc.}_{\text{RETENTATE}}) \times 100$$

Permeability is often described in terms of “rejection coefficient” of the membrane, i.e. the membrane’s ability to hold back or reject a given molecule from passing through.

$$\text{Rejection coefficient} = 1 - (\text{Conc.}_{\text{FILTRATE}} / \text{Conc.}_{\text{RETENTATE}})$$

A rejection coefficient of 1 equals 0% permeability

A rejection coefficient of 0 equals 100% permeability

Permeability will be affected by such factors as transmembrane pressure (TMP), crossflow rate, retentate concentration, pH, and ionic strength, and gel layer formation (concentration polarization). Therefore, the permeability may change during the process.

Figure 7
TFF diafiltration example, using the Minimate EVO TFF System

A reservoir lid seals tightly allowing for diafiltration solution to be drawn directly into the reservoir without the need of a transfer pump. Vacuum is created as filtrate is generated through the TFF device allowing continuous diafiltration to be performed.

Table 2 shows the relationship between permeability through a membrane and the number of diafiltration volumes required for removal of permeating species. As noted earlier, a greater volume of buffer is required to remove a molecule that is partially retained. To remove 99.9% of a molecule that is 75% permeable to the membrane requires 9 DV's, while for a 100% permeable species, only 7 DV's are required.

Table 2
Continuous (Constant Volume) Diafiltration

Diafiltration Volumes MWCO	Permeability 100% Rejection Coefficient = 0	Permeability 75% Rejection Coefficient = 0.25
1	63%	53%
2	86%	77%
3	95%	89%
4	98.2%	95%
5	99.3%	97.6%
6	99.7%	98.9%
7	99.9%	99.4%
8		99.7%
9		99.9%

0% - Salts, solvents, buffers, etc.
25% - Molecules lower in MW than MWCO of membrane but bigger than salts

Discontinuous Diafiltration – Sequential Dilution

Discontinuous diafiltration by sequential dilution involves first diluting the sample with water or replacement buffer to a predetermined volume. The diluted sample is then concentrated back to its original volume by ultrafiltration. This process is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent dilution removes more of the small molecules.

As shown in Figure 8, the sample is generally diluted with an equal volume of buffer (1 DV). Alternatively, multiple volumes can be added at once, provided the process tank is large enough to hold the entire volume. Diluting the sample usually lowers the viscosity, which may increase the filtrate flux rate.

Figure 8
Discontinuous Diafiltration – Sequential Dilution

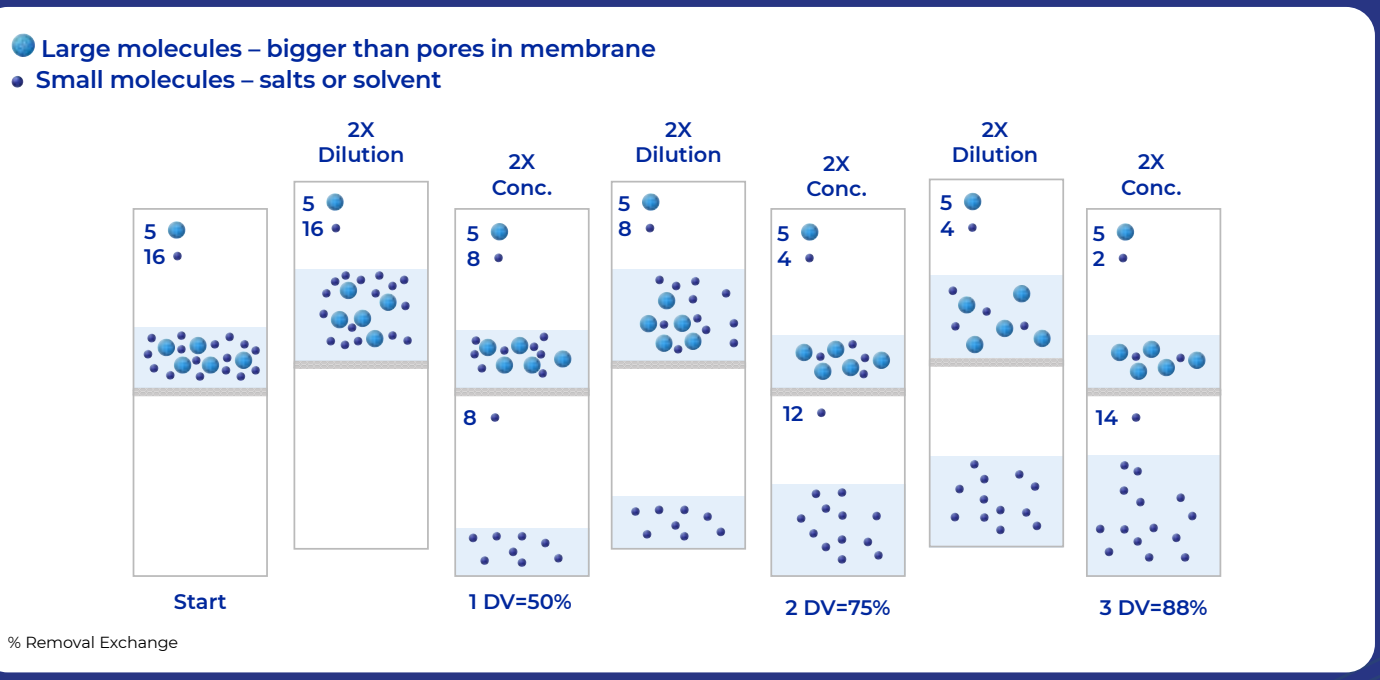
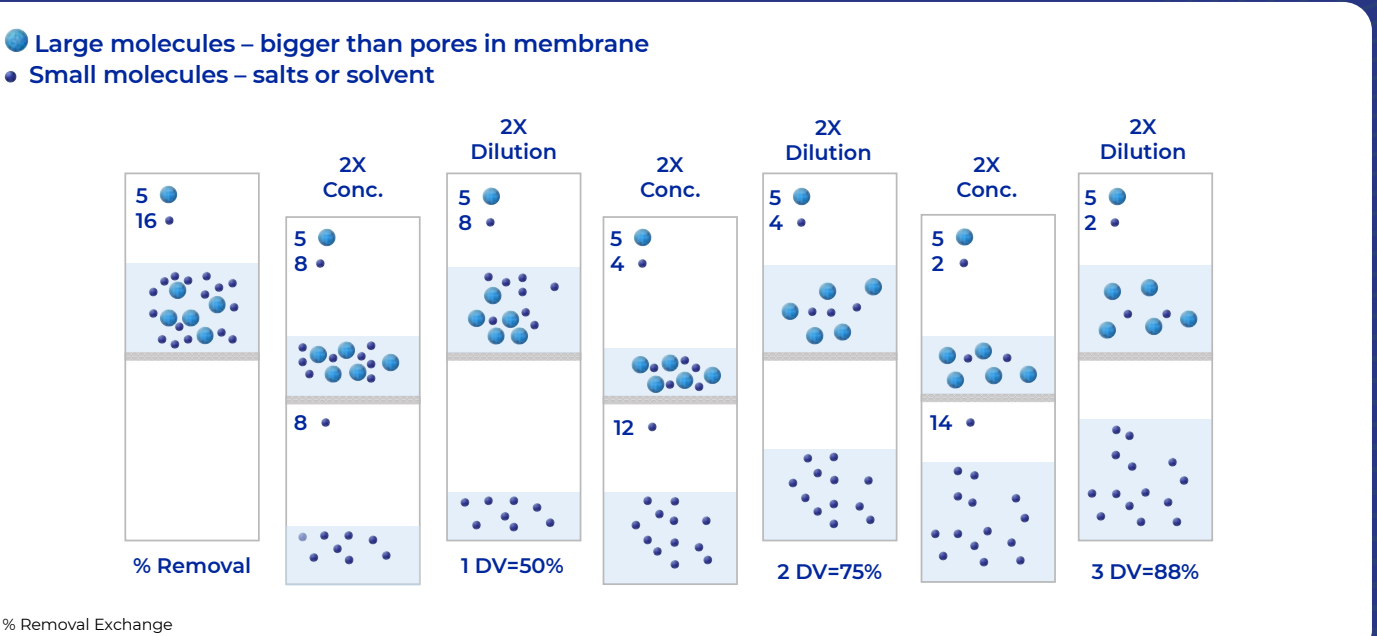


Figure 9
Discontinuous Diafiltration with Volume Reduction



Discontinuous Diafiltration – Volume Reduction

Discontinuous diafiltration by volume reduction reverses this procedure. The sample is first concentrated to a predetermined volume, and then diluted back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent concentration and dilution removes more of the small molecule (Figure 9).

Following diafiltration the sample may be concentrated or the next purification step performed.

The final product, after diafiltration by either method (discontinuous 2X volume reduction or sequential dilution) is at the same volume and concentration as when diafiltration started. The salt concentration has been equally reduced in both examples. However, the volume of diafiltration buffer used by the volume reduction method was half that used in sequential dilution. This is because the initial concentration step reduced the volume in half. A diafiltration volume is equal to the volume where dilution occurs. Therefore, half the volume was required.

This being the case, it would seem that concentrating before diafiltration, by either discontinuous sequential dilution or constant volume diafiltration, should reduce the required diafiltration buffer volume and save time. And in most cases this is true. The factor we have not accounted for is filtrate flux rate, which equates to process time. As the product becomes concentrated, viscosity increases and the filtrate flux rate decreases. The filtrate flux rate varies inversely as the log of the concentration factor.

$$J = k \ln(C_G / C_B)$$

Where:
J = Filtrate Flux Rate
k = constant
C_G = gel layer concentration
C_B = retentate (bulk flow) concentration

This becomes very significant as the product concentration (C_B) increases above a few percent and is dependent on the characteristics of the specific molecules that make up the sample. So, although it might take significantly less volume to diafilter a concentrated sample, it could take considerably more time compared to a less concentrated sample. Simple protocols are available to find optimum conditions to maximize productivity.

Continuous or Discontinuous Diafiltration – Which Technique Should be Used?

When deciding which technique to use and where in the process to perform diafiltration, consider the following factors:

- Initial sample volume, concentration and viscosity
- Required final sample concentration
- Stability of sample at various concentrations
- Volume of buffer required for diafiltration
- Total processing time
- Reservoir size available
- Economics

The choice of which method to use must be based on several criteria. Scale is an important consideration. What we will do at laboratory scale may be very different than at process scale, especially if the process is automated. At lab scale discontinuous diafiltration is often used for simplicity. Continuous diafiltration requires a pump or equipment to add the diafiltration solution at a constant rate. Both techniques can be automated for process applications.

If we eliminate the equipment issue and focus on the process, we can compare the differences.

Table 3
Salt Reduction from Sample using Volume Reduction or Constant Volume Diafiltration

	2X Volume Reduction		Continuous Diafiltration (Constant Volume)	
Diafiltration Volumes	**100% Permeable 0% Retention***	**75% Permeable 25% Retention***	**100% Permeable 0% Retention***	**75% Permeable 25% Retention***
1	50%	41%	63%	53%
2	75%	65%	86%	77%
3	88%	79%	95%	89%
4	94%	88%	98.2%	95%
5	96.9%	93%	99.3%	97.6%
6	98.4%	95.6%	99.7%	98.9%
7	99.2%	97.4%	99.9%	99.4%
8	99.6%	98.4%		99.7%
9	99.8%	99.0%		99.9%
10	99.9%	99.4%		

**Retention of smaller molecules*
0% - Salts, solvents, buffers, etc.
25% - Molecules lower in MW than MWCO of membrane but bigger than salts

The ionic strength, buffer composition and stabilizer concentration can affect stability of the sample. Diafiltration may remove salts or stabilizing molecules, resulting in protein product denaturation and aggregation. The process of concentrating and diluting a protein solution can also affect molecular interactions resulting in denaturation or aggregation as well as subsequent precipitation and product loss. It is necessary to evaluate the effect of concentration on the product to determine where diafiltration is best performed relative to concentration effects.

Continuous diafiltration offers an advantage over discontinuous diafiltration in that the retentate concentration remains constant. It is often seen as a gentler process relative to the stability of the product.

When to Perform Diafiltration – Before or After Concentration?

We have already seen that concentrating a sample first can significantly reduce the volume of diafiltration solution required. We have also seen that continuous diafiltration takes less volume than discontinuous diafiltration with sequential dilution. Therefore, if the sample is first concentrated to the final concentration required and then continuous diafiltration performed, acceptable results should be obtained.

However, above a certain concentration, filtrate flux rates may become prohibitively slow. It may actually take longer to diafilter the concentrated sample than it would if the sample were first diluted to reduce the concentration. In this situation, even though continuous diafiltration of the diluted sample requires a greater diafiltration volume, the total processing time would be less due to the faster filtrate flux rate. (Process Time = Filtrate Flow Rate x Volume)

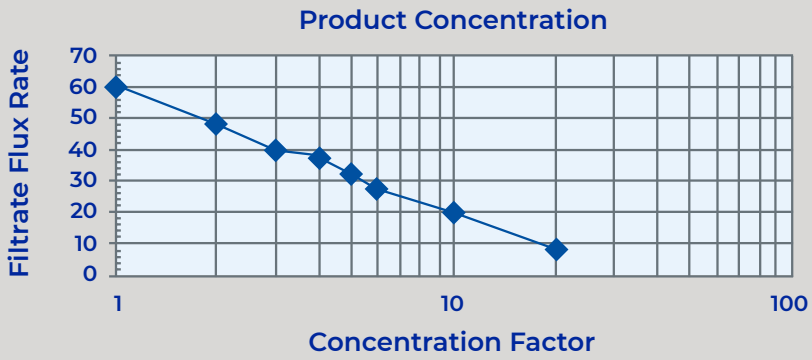
In general, the optimum retentate concentration for performing (continuous) diafiltration is at:

$\ln (C_G/C_R) = 1$ or $C_{R(optimum)} = C_G/e = 0.37C_G$

Where:
C_G = gel layer concentration
C_R = retentate concentration.
$C_{R(optimum)}$ = highest retentate concentration where diafiltration should be performed

The C_G value for a sample can be determined from experimentation by concentrating a sample on a membrane and recording and plotting data for filtrate flux rate vs. log concentration (or concentration factor). The curve can then be extrapolated to filtrate flux rate = "0". The C_G value will be the same for this product regardless of the starting concentration or filtrate flux rate.

Figure 10
Determination of the CG Value for a Product



In this example (Figure 10) the C_G value is a concentration factor of approximately 33X. Therefore the optimal concentration to perform diafiltration would be 0.37 C_G = 12.2X. If the starting product concentration is 5 mg/mL, then diafiltration should be performed when the concentration reaches 61 mg/mL. If the final concentration will be less than 61 mg/mL, then diafiltration should be performed after concentration, unless it is necessary to remove a specific molecule prior to concentration.

The ultrafiltration product selected may dictate choice of continuous or discontinuous diafiltration. Stirred cells and centrifugal devices are best suited for discontinuous diafiltration because of their mode of operation. Tangential flow devices have the advantage of being useful for either diafiltration technique.

Diafiltration Summary

Diafiltration is a fast and effective technique for desalting or buffer exchange of solutions. It can be performed in a continuous or discontinuous mode. Continuous diafiltration usually takes less volume to achieve the same degree of salt reduction as discontinuous diafiltration with sequential dilution and can be easier to perform.

Continuous diafiltration is also perceived as a kinder and gentler process on active biomolecules. On the other hand, discontinuous diafiltration with volume reduction takes less volume than continuous diafiltration.

Concentrating the sample before diafiltration usually reduces the required filtrate volume and saves time. However, if the sample viscosity becomes too great, the filtrate flux rate decreases and the process time can increase substantially. Determining the C_G for the sample can help answer the question - At what concentration should I perform diafiltration?

Fractionation

Ultrafiltration can be used to separate molecules based on their size. The molecules to be separated should differ by at least one order of magnitude (10X) in size for effective separation.

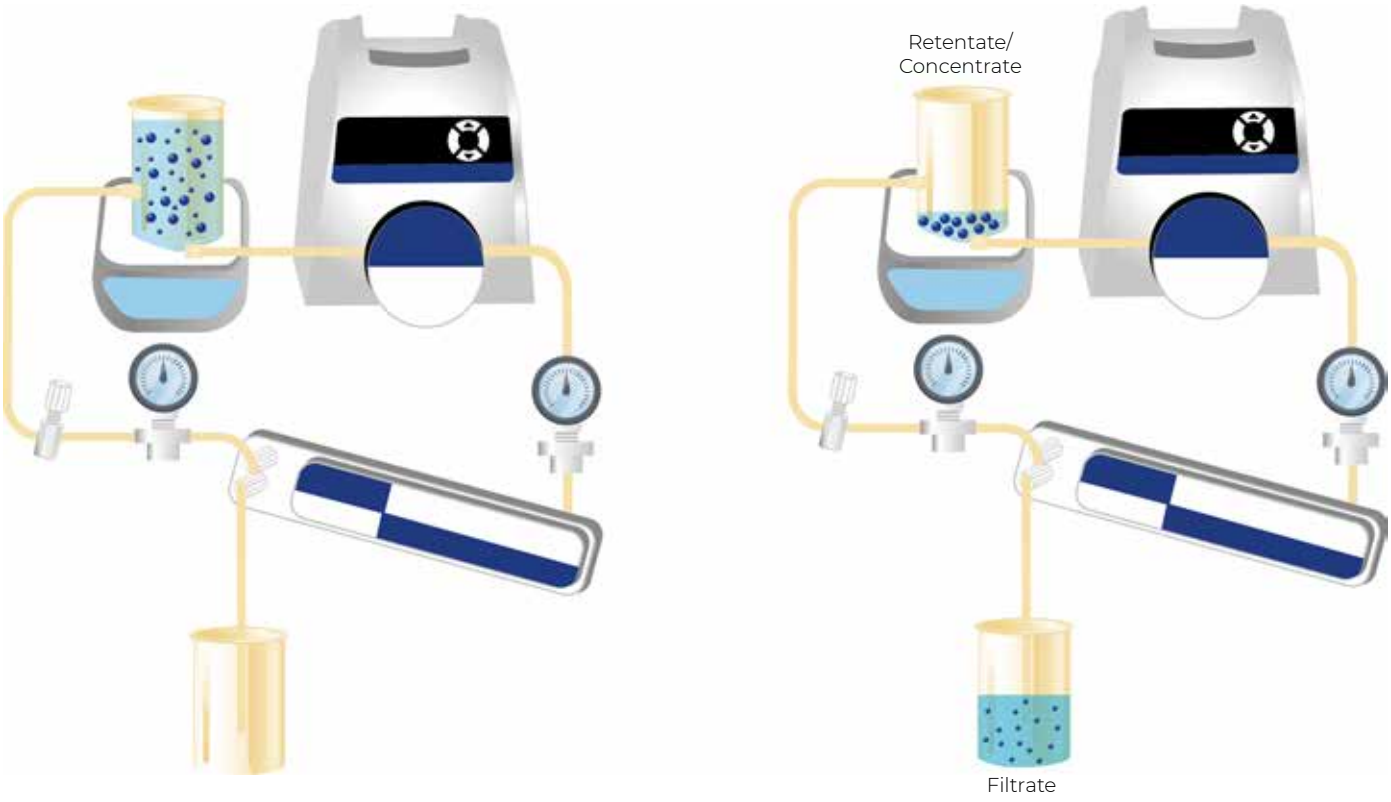
When performing fractionation the larger molecular fraction will remain in the retentate and continue to circulate around the flow path of the system, the smaller molecular fraction with travel through the membrane filter into the filtrate.

The larger molecular fraction will slowly start to concentrate as the buffer solution passes into the filtrate. To avoid concentration of the larger molecular fraction it is possible to add new buffer to the sample feed reservoir at the same rate as filtrate is being generated.

Fractionation using ultrafiltration is effective in applications such as the preparation of protein-free filtrates or the separation of unbound and bound constituents.

It is not possible to not accomplish a sharp separation of two molecules with similar molecular weights using ultrafiltration, more expensive chromatographical methods would have to be used in that instance.

Figure 11
TFF Fractionation



When recovering the filtrate, it is important to note that some solution may remain in the vent ports and tubing of the system.

Pall supply detailed procedures on how to maximize both concentrate and filtrate recovery in the TFF Instructions for Use Guide.

TFF System Selection Considerations

In order to choose the best TFF system for your requirements review and consider the following steps:

Step 1: Define the purpose of the TFF process

The biomolecule of interest in your sample is called a product. Separation can occur by choosing a membrane that retains the product while passing any low molecular weight contaminants. Alternatively, a membrane can be chosen that passes the product while retaining higher molecular weight components in the sample. It is also possible to combine both separations in a two-stage process that will fractionate out the product from both higher and lower molecular weight components. In the first stage, a membrane is chosen that passes the product and retains the higher molecular weight components. The filtrate from the first stage then becomes the sample for the second stage. For the second stage, the membrane is chosen to concentrate the product and remove lower molecular weight substances.

You will need to define your separation goals – **Concentration**, **Diafiltration**, or **Fractionation**. You must also consider the process volumes that you have to work with and any future scale up requirements. It is important to know the concentration factor or the level of salt reduction required in order to choose the most appropriate membrane and system for the process.

Step 2: Choose the membrane molecular weight cut-off

The molecular weight cut-off (MWCO) of a membrane is defined by its ability to retain a given percent of a molecule in solution (typically > 90% retention). As discussed earlier, to retain a product, select a membrane with a MWCO that is 3 to 6 times lower than the MW of the target protein.

For fractionation, select a membrane MWCO that is lower than the MW of the molecule to be retained but higher than the MW of the molecule you are trying to pass.

Please refer to Page 9 to review the retention characteristics of the Omega membrane.

Step 3: Determine the required membrane area for the application

Choosing an appropriate device, capsule or cassette depends on the total sample volume, required process time, and desired final sample volume.

Different TFF devices contain different membrane surface areas.

Use the following equation to calculate the membrane area required for processing a sample in a specified time:

$$A = \frac{V}{J \times T}$$

Where:
A = Membrane area (m^2)
V = Volume of filtrate generated (liters)
J = Filtrate flux rate [liters/m^2/hour (LMH)]
T = Process time (hours)

Examples

Example 1: What TFF system should I use to concentrate 10 liters to 200 mL in 2.5 hours?

Assume the average filtrate flux rate of 50 liters/m^2/hour (L/m^2/h, LMH).

Volumetric throughput (volume of filtrate) = 10 liters – 0.2 liters = 9.8 liters

$$A = \frac{9.8}{50\ L/m^2/h \times 2.5\ h} = \frac{9.8}{125} = 0.08\ m^2$$

Recommended System: Centramate holder with 1 membrane cassette, area of 0.093 m^2 (1 ft^2).

Example 2: You have 50 mL of sample (MW = 54KD) collected from a Mustang™ Q membrane chromatography module that was eluted in a buffer solution (0.05 M Tris, 0.5 M NaCl).

You need to reduce the salt concentration below 0.05 M and then concentrate to 10 mL.

Using a Minimate TFF capsule with a 10KD membrane on a Minimate EVO TFF system, how long will the process time be if the average filtrate flux rate is 40 LMH and 3 diafiltration volumes (constant volume diafiltration) are required to get the salt concentration below 0.05 M?

Minimate Area = 50 cm^2 = 0.005 m^2

Sample Volume = 50 mL

Diafiltration Volume (1DV) = 50 mL

Average Filtrate Flux Rate = 40 LMH

Total filtrate volume (V_T)= V_D+ V_C

Where:
V_D = Filtrate volume from diafiltration step
V_C = Filtrate volume from concentration step
$V_T = V_D + V_C$ = (3 DV x 50 mL) + (50 mL – 10 mL)
$V_T = V_D + V_C$ = 150 mL + 40 mL = 190 mL = 0.19 L
A = V/(JxT)

Rewrite equation to solve for T

T = V/(JxA)

T = 0.19 L / (40 L/m²/h x 0.005 m²)

T = 0.19 / 0.2 = 1 h

When diafiltration is performed, the total volumetric throughput (filtrate volume) equals the initial sample volume multiplied by the number of diafiltration volumes. To save on buffer volume and processing time, very often sample is first concentrated and then subjected to diafiltration.

Example 3: You have a 1 liter sample (0.1 mg/mL) that you need to concentrate 10 times and diafilter to remove at least 99% of the salts. Using a Centramate cassette holder with one cassette 0.093 m² (1 ft²) how much time will it take to process your sample?

The average filtrate flux rate for the process if you concentrate first and then diafilter is 40 LMH. If you do the diafiltration first and then concentrate, the average flux rate is 50 LMH.

Scenario A: The sample is first concentrated 10X (from 1.0 liters to 0.1 liters) followed by continuous diafiltration for 6 DV's to remove salt.

Total filtrate volume (V_T) = V_C + V_D

Where:
V_C = Filtrate volume in concentration step
V_D = Total diafiltration volume (1 DV = 0.1 liters)
V_T = (1.0 - 0.1) + (6 x 0.1) = 0.9 + 0.6 = 1.5 liters

Average filtrate flux rate = 40 LMH

$$\text{Area (A)} = \frac{\text{Filtrate Volume (V)}}{\text{Average Filtrate Flux (J) x Process Time (T)}}$$

Rewrite to solve for T

$$T = \frac{V}{J \times A}$$

$$T = \frac{1.5 \text{ liters}}{40 \text{ L/m}^2\text{/h} \times 0.093 \text{ m}^2} = 0.4 \text{ h}$$

Scenario B: The sample is diafiltered first by continuous diafiltration for 6 DV's to remove salt and then concentrated 10X (from 1.0 liter to 0.1 liter).

Total filtrate volume (V_T) = V_D + V_C

Where:
V_D = Total diafiltration volume (1 DV = 1 liter)
V_C = Filtrate volume in concentration step
V_T = (6 x 1.0) + (1.0 - 0.1) = 6 + 0.9 = 6.9 liters

Average filtrate flux rate = 50 LMH

$$T = \frac{6.9 \text{ liters}}{50 \text{ L/m}^2\text{/h} \times 0.093 \text{ m}^2} = 1.48 \text{ h}$$

In this example, concentrating the sample first followed by diafiltration takes 0.4 hours. Reversing the process and doing diafiltration first takes 1.5 hours. Therefore, concentrating first has saved about 1 hour of process time. If the sample had been fairly concentrated to start, the results may have been very different.

In designing a process it is important to look at the total process and evaluate how filtrate flux rate changes may affect the process requirements.

Table 4 lists TFF products for lab and process development. The table allows you to select a product based on starting sample volume. It gives recommended retentate flow rates and an estimate of filtrate flux rate. Pall supplies additional products (not shown) for larger scale TFF applications.

Table 4
General Product Selection Based on Starting Sample Volume

TFF Capsule or Cassette[1]	Membrane Area	Typical Filtrate Flow Rate[2] at 50 LMH (20 °C)	Recommended Retentate Flow Rate (Screen Channel)	Starting Sample Volume Range	Minimum Concentrated Volume[3]
Lab Scale / Scale-up Devices					
Minimate	50 cm^2	4 mL/min	30 -40 mL/min	25 - 500 mL	< 10 mL
LV Centramate	0.01 m^2 (0.1 ft^2)	8 mL/min	60 - 80 mL/min	40 - 2000 mL	10 mL
LV Centramate	0.02 m^2 (0.2 ft^2)	15 mL/min	120 -160 mL/min	60 - 4000 mL	15 mL
Process Development and Small Scale Production					
Centramate	0.093 m^2 (1.0 ft^2)	4.6 L/hr	600 - 800 mL/min	0.2 - 20 L	100 mL

1) Data is per unit or cassette. Centramate holder can hold 5 cassettes. Other column data can be calculated by multiplying table values by the number of cassettes installed in the holder.

2) Typical filtrate flow rate is based on an average filtrate flow rate of 50 LMH and a process time of about 4 hours. Actual value may be higher or lower depending on the MWCO of membrane, sample composition and viscosity, operating conditions i.e. TMP, cross flow rate, temperature, etc.

3) Minimum concentrate volume depends on system hold-up volume, reservoir design and pump type and speed. Smaller volumes can be achieved by minimizing tubing lengths and use of properly sized components, tubing, fittings, etc.

Capsules, Cassettes and Systems

Choosing the appropriate cassette or device size depends on the total sample volume, the required process time, and the desired final sample volume.

Pall supply an extensive line of TFF holders and cassettes from laboratory friendly plug-and-play devices to fully scalable systems for process development and small scale production.

Minimate Tangential Flow Filtration Capsules

Disposable TFF device for bioprocessing applications accelerates and

- Contains Pall's Omega polyethersulfone (PES) membranes that offer high flux and have been specifically modified to minimize protein binding. This polymeric membrane is stable against biological and physical degradation due to the unique chemical properties of PES. Omega membranes are available in a wide range of molecular weight cut-offs.
- The cost-effective plastic construction of the Minimate TFF capsule and chemical compatibility of the Omega PES ultrafiltration membrane facilitate cleaning and reuse.
- Each Minimate capsule is 100% integrity tested during manufacture to ensure reliable performance. For critical applications, users can re-test the integrity after initial use. A certificate of quality is included with each capsule.
- Features an effective filtration area of 50 cm^2 (0.05 ft^2).

Minimate Tangential Flow Filtration Capsules

Minimate EVO Tangential Flow Filtration System

Plug-and-play benchtop TFF system designed for highly reliable buffer exchange or concentration of samples up to 1 L.

- Designed to work with Minimate TFF capsules.
- A low system working volume achieved through the use of a conical bottom reservoir and compact design enables high concentration factors from up to 1 L or more of sample to be achieved. Concentrate your sample down to as little as 5 mL.
- Roller head peristaltic pumps provide gentle processing and are the choice for critical applications such as fragile biomolecules.
- All wetted components are made from low protein binding, chemically resistant, biologically safe materials.
- The reservoir design allows for either continuous or discontinuous diafiltration to be performed in the same system without sample transfer.
- ·Includes the addition of a downstream pressure gauge to enable accurate transmembrane pressure (TMP) differential calculations, allowing greater user control and easier validation.

Minimate EVO Tangential Flow Filtration System

Centramate T-Series TFF Cassettes with Omega Membrane

Available with 0.02 m^2 (0.2 ft^2) or 0.1 m^2 (1.1 ft^2) effective filtration areas and feature Omega polyethersulfone (PES) membranes.

- Feature a durable and stable construction that exhibits very low extractables and offer broad chemical compatibility. The cassettes are designed to deliver optimal mass transfer to improve your process economics.
- Omega PES membrane provides superior performance and is stable against biological and physical degradation, offering high flux, high selectivity, and low protein binding. Omega membranes are available in a wide range of molecular weight cut-offs.
- Centramate cassettes offer easy scale-up for robust purification processes, they feature the same materials of construction from development to production-scale processes.
- All materials of construction in the T-Series cassettes have been tested and meet requirements for United States Pharmacopeia (USP) Biological Reactivity Test, In Vivo at 70 °C (158 °F).

Centramate T-Series TFF Cassette

LV Centramate Lab Tangential Flow Filtration Holder

Designed for maximum product recovery for lab-scale or scale-up process volumes up to 4 L.

- Stainless steel holder designed for use with 0.02 m^2 Centramate cassettes.
- Designed for a low hold-up volume allowing for high concentration factors to be achieved from small starting volumes.
- Features easy connections through luer lock fitted ports with polished 316L stainless steel to ensure the same compatibility characteristics as production-scale holders.
- Can be used with the Minimate EVO Tangential Flow Filtration System.

LV Centramate Lab Tangential Flow Filtration Holder

Centramate and Centramate PE Lab Tangential Flow Filtration Holders and Systems

Suitable for process development and small-scale production of 1 to 125 L.

- Holders designed for use with Centramate cassettes.
- Holders are available with Type 316L stainless steel (Centramate holder) or economically priced, extremely durable, ultra-high molecular weight polyethylene (Centramate PE holder).
- Filtration area is easily expanded by adding additional membrane cassettes.
- Identical fluid path lengths provide precise linear scale-up to larger process systems.
- Fittings kits containing clamps, tubing and gauges can be purchased separately or as part of complete Centramate or Centramate PE systems.

Centramate SS System

Centramate PE System

Ultralab™ Systems and Ultrareservoir™ Containers

Simplify processing of volumes up to 5 L.

- Ultrareservoir Containers are available in 2 L or 5 L volume capacities. The self-contained siphoning units allow for continuous diafiltration.
- Constructed of clear acrylic with easy-to-read volume graduations. Connections located in base plate for easy access and minimal sample hold-up.
- Ultralab systems feature a peristaltic pump that minimizes shear of sensitive molecules, eliminates air in the system, and reduces foaming. A variable speed controller allows precise adjustment of crossflow during sample processing.

Ultrareservoir Containers and Ultralab Systems

Frequenty Asked Questions

In a recent interview with Dan Rayner, Global Product Manager - Cellular, he answered a number of frequently asked questions about TFF and the use of TFF systems.

Centrifugal devices are often used in research to perform ultrafiltration applications, but when and why would researchers choose to move to using TFF?
UF centrifugal devices are ideal for the processing of small volumes of samples, however, centrifugal devices are dead-end filters, meaning that the membrane will suffer from fouling and will eventually block.

As users scale up to processing larger volumes of solution then TFF systems can offer greater efficiencies, the tangential flow of fluid across the surface of the membrane in TFF causes a sweeping action which reduces the potential for membrane fouling and therefore gives far more efficient processing. TFF can also be scaled up from research to pilot and then to process scale, operating with volumes in excess of 10,000 liters.

TFF systems can offer more versatility than centrifugal devices. For example, proteins can be extremely sensitive to solution conditions, they tend to aggregate at high concentrations. TFF offers more control over processing parameters, so it's possible to accurately achieve desired concentration factors without proteins crashing out of solution.

The versatility of TFF also allows for multiple processing steps to be performed in one system, this prevents excess sample handling and potential sample loss. For example, it's very simple to perform continuous diafiltration followed by a concentration step all within the same TFF system.

What advantages does TFF have over older ultrafiltration methods using stirred cells?
Stirred cells are simply devices for performing ultrafiltration applications, however, as with centrifugal devices they operate under direct flow filtration, also known as "dead-end" filtration, which can fall prey to problems with membrane fouling.

To try and reduce the formation of a gel layer, stirred cell devices utilize a floating stir bar on the upside of the membrane that generates turbulence, however, they are limited in performance since the velocity and subsequent level of agitation is dependant on the sweep of the bar, and that varies along the radius of the sweep. In contrast TFF operation allows for a uniform and gentle recirculation of sample over the entire membrane surface resulting in improved flux rates, significantly reducing processing times and increasing productivity.

There are a number of major differences between the operation of stirred cell devices and TFF systems. Firstly, many of the commercially available stirred cell devices require researchers to assemble the units themselves, manoeuvring UF membrane disks into the housing and screwing in place. This manual handing may create leaks or fluid bypass and could even damage the structure of the membrane. Major leaks can be easily identified when visualizing abnormally high filtrate flow rates, more critical however, is that a partial bypass can look like a normal run, but the loss of molecule would not be detected until it was too late or even at the end of a run.

Pall have undertaken studies and generated data comparing the processing times and device integrity of laboratory scale stirred cell devices and the Minimate TFF capsule (See Figure 12A and 12B).

Finally, one of the main differences between the operation of stirred cell devices and TFF systems is the need for a pressure vessel or a nitrogen tank which creates the desired pressure to run a stirred cell device. This can require more specialist handling for stirred cell systems and raise laboratory safety concerns.

Centrifugal devices can actually be very useful when wanting to work with TFF systems from Pall.

Pall offer a range of centrifugal devices, these devices contain the Omega (modified polyethersulfone) membrane that is found in the larger TFF devices. So when trying to select the best MWCO membrane our centrifugal devices can offer a cost-effective way to predict membrane performance, and help you maximize protein concentration and recovery. Obviously, it should be noted that this type of test will only offer an indication of performance as proteins can behave differently based on the volume of solution they are in, pH conditions and concentration factors.

Figure 12A

Use of the Minimate Capsule Significantly Reduces Processing Times

A 2 mg/mL BSA solution was concentrated ten-fold (1000 to 100 mL) in either a 350 mL stirred cell device or Minimate capsule. The Minimate contains a pre-assembled Omega 10K membrane. The crossflow, set at 50 mL/min with retentate loop backpressure applied to create an initial filtrate flow of about 15 mL/min. The stirred cell devices used polyethersulfone (PES) or regenerated cellulose (RC) disks and were pressurized with filtered air at 55 psi giving a starting filtrate flow of about 6 mL/min. Error bars indicate standard error for five independent runs.

Measuring the absorbance at 260 nm for both the filtrate and retentate fractions provided verification of protein concentration process. Using stirred cell devices it was observed that BSA leaked through the 10K PES membrane during processing indicating a failure in integrity. Subsequently, in order to ensure a statistically significant comparison between configurations additional experiments were performed until a total of five successful stirred-cell runs were completed.

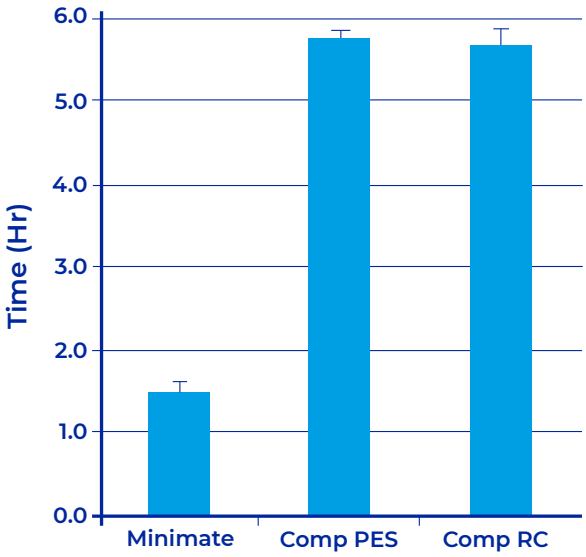
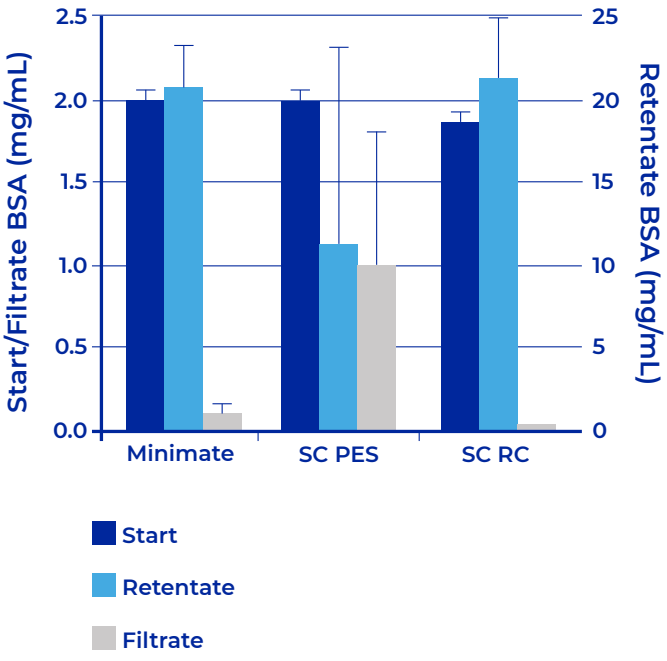


Figure 12B

The Pre-assembled Minimate Eliminates Integrity Failures

Sample concentration was performed as described in Figure 12a. Aliquots of the starting material, final retentate, and final filtrate pools were analyzed for protein concentration at 280 nm. Average concentrations are plotted with error bars indicating standard error for (5 - Minimate), (10 - SC PES), and (5 - SC RC) runs respectively. For simplicity in the graphical representation, the retentate values are plotted on the Y2 axis to accommodate the ten-fold concentration that occurred in the processing.

By monitoring the filtrate for protein bypass, it was observed that the competitive PES membrane in stirred cell operation suffered significant integrity failures. The Minimate TFF and regenerated cellulose stirred cell devices were all integral, however, the competitive PES membranes failed in 5 out of 10 runs each at different stages in the processing.



What are the advantages of using a bespoke system, such as the Minimate EVO TFF System over putting a basic system together?

While it is possible to put together a simple TFF system using a pump, tubing and gauges, a bespoke system such as the Minimate EVO TFF System offers numerous advantages. The Minimate EVO TFF System is delivered with all the components needed to set up the system, Pall supply a quick start guide that takes a researcher through the simple steps to assemble the system. The system is designed to be plug-and-play using luer connections, making installation rapid and easy.

The Minimate EVO TFF System features an optimized flow path design, we guide users as to the tubing lengths to use on it, this helps provide higher product recoveries and the ability to operate at low working volumes, achieve low volumes of concentrate and ensure low hold-up volumes within the system.

Included with the system is a variable-speed, roller-head peristaltic pump for gentle processing, two pressure gauges, valves, tubing, and 500 mL reservoir with a magnetic stir bar and stir plate, all assembled on a compact drip tray. All wetted components are made from chemically resistant materials.

How many times can a typical TFF cassette be used?

There are no specific guidelines for the total number of times a TFF capsule or cassette can be reused. The lifespan of a device is very much dependent on usage frequency, particle load of process fluids, storage conditions and execution of clean-in-place (CIP) procedures.

We recommend that a normalized water permeability (NWP) value is taken on new Minimate capsules and Centramate cassettes prior to use. Then after each use and following a CIP procedure the NWP can be re-evaluated.

Typically, after a CIP procedure the NWP of capsule or cassette should be greater than 75% of the original NWP value, if not then we would recommend performing the CIP procedure again. Once the NWP value on a used device drops below 50% of the original NWP value then we would suggest using a new capsule or cassette.

Our comprehensive instructions for use documents contain information on how to correctly handle our TFF devices, how to perform both CIP and NWP procedures, and how to store devices when not in use.

What is scale up and how can it help researchers?

Scalability is the ability to scale up or even scale down products and systems based on the volume and processing requirements of a solution. Scalability can be viewed in different ways, firstly from a proof-of-concept perspective, or secondly where devices and systems can be scaled in a truly linear way. Scale up can help remove bottlenecks, reducing evaluation times, freeing up resources to focus on process optimization.

Proof-of-concept testing can be very valuable when performing ultrafiltration applications. A good example is ultilizing a Pall UF centrifugal device to gain an indication of MWCO performance before moving to a TFF capsule or cassette.

Minimate capsules have the same path length and materials of construction as the larger Centramate cassette product range that can be used in pilot and production plants. While the Minimate may not be linear scalable to the Centramate cassette product range it offers the ability for super fast set up and easy plug-and-play lab scale processing or proof-of-concept TFF testing, offering predictable performance, which saves time when scaling up a process.

It is also possible to scale up the Minimate system itself. By connecting several Minimate capsules in parallel you will achieve a greater membrane surface area, allowing for an increase in initial process volume or an increase in the speed of processing time.

The LV Centramate and Centramate cassette range feature identical fluid path lengths providing precise linear scalability.

Can I attach an LV Centramate holder and cassette to the Minimate EVO TFF System, and what would I need to do so?

Yes, you can use a LV Centramate with the Minimate EVO TFF System. We recommend ordering the Minimate EVO TFF Fittings Kit, part number 97014, this will provide the additional 3-way stop cocks, hose barb adapters and tubing needed to properly connect the LV Centramate holder (See Figure 13).

How would you summarize the benefits of Tangential Flow Filtration?

It's easy to set up and use, especially if you are using a purpose-built system such as the Minimate EVO TFF System.

TFF is fast and efficient, the tangential flow of solution across the surface of membrane reduces membrane fouling allowing for efficient and gentle sample processing.

Multiple processing steps can be performed in one system without the need to transfer samples. You can perform diafiltraion and concentration of a sample in the same system, saving time and avoiding product loss.

TFF can be scaled up or scaled down, either for proof-of-concept or in the case of the LV Centramate and Centramate cassette range by precise linear scalability. This allows TFF to be performed at lab, pilot or process scale.

Finally, TFF is very economical, devices and cassettes can be cleaned and reused.

Figure 13
Minimate EVO TFF System with LV Centramate Holder and Cassette

Glossary

Concentration Polarization: The accumulation of the retained molecules (gel layer) on the surface of the membrane caused by a combination of the following factors: transmembrane pressure, crossflow velocity, sample viscosity, and solute concentration.

Continuous Diafiltration: The technique of continuous diafiltration (also referred to as constant volume diafiltration) involves washing out the original buffer salts (or other low molecular weight species) in the retentate (sample) by adding water or a new buffer to the retentate at the same rate as filtrate is being generated.

Crossflow Rate (CF): The retentate flow rate; Units in L/min. Provides the "sweeping" effect to reduce concentration polarization. The pressure drop, P (P_{FEED} – $R_{RETENTATE}$), is directly related to the CF.

Crossflow Flux Rate (CFR): Crossflow rate per unit area of membrane, units L/min/ft^2 or L/min/m^2.

Diafiltration: The fractionation process that washes smaller molecules through a membrane and leaves larger molecules in the retentate (concentrate). It can be used to remove salts or exchange buffers, remove ethanol or other small molecules such as detergents, small peptides or nucleic acids.

Discontinuous Diafiltration-Sequential Dilution: Discontinuous diafiltration by sequential dilution involves first diluting the sample to a predetermined volume, then concentrating the sample back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent dilution removes more of the small molecules.

Discontinuous Diafiltration-Volume Reduction: Discontinuous diafiltration by volume reduction involves first concentrating the sample to a predetermined volume, then diluting the sample back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent concentration and dilution removes more of the small molecule.

Concentration Polarization: The accumulation of the retained molecules (gel layer) on the surface of the membrane caused by a combination of the following factors: trans-membrane pressure, crossflow velocity, sample viscosity, and solute concentration.

Filtrate: The solution that passes through the membrane.

Filtrate Flux Rate: Filtrate flow rate per unit area, unit L/m^2/h (LMH). Filtrate flux rate is affected by crossflow rate, TMP and viscosity.

Gel Layer: The microscopically thin layer of molecules that forms on the top of the membrane. It causes a reduction in the filtrate flow rate and may increase the retention of molecules that would normally cross into the filtrate.

Hold-up Volume: The volume of retentate fluid remaining in the filter and system tubing after sample recovery.

Microfiltration (MF): Microfiltration refers to filtration using filter media with pore sizes typically between 0.1 µm and 10 µm. Generally used for clarification, sterilization, and removal of microparticulates or for cell harvesting in life science applications.

Minimal Operating Volume: The minimal volume of process fluid that can be handled effectively by the TFF system.

Molecular Weight Cut-Off (MWCO): The molecular weight cut-off of a membrane sometimes called Nominal Molecular Weight Limit (NMWL) is defined by its ability to retain a given percent of a molecule in solution (typically 90% retention).

Normalized Water Permeability (NWP): Membrane water permeability corrected to a temperature of 20°C.

Membrane Water Permeability: Filtrate flux rate for water per unit of applied TMP. The water permeability is related to the membrane hydraulic resistance. It is significantly affected by temperature.

Membrane Recovery: Measure of the water permeability of the membrane after processing and cleaning compared to the water permeability of the "original" membrane.

% Membrane Recovery = (NWP_{CLEAN} / $NWP_{ORIGINAL}$) x 100%

Product Recovery: The amount of product (mass or activity) recovered after processing compared to the amount in the starting sample. Usually expressed as a percentage of starting material.

Retentate: The sample that passes through the feed channel (does not pass through the membrane). Also known as the concentrate.

Tangential Flow Filtration (TFF) or Crossflow Filtration: A process where the feed stream flows parallel to the membrane face. Applied pressure causes one portion of the flow stream to pass through the membrane (filtrate) while the remainder (retentate) is recirculated back to the feed reservoir.

Transmembrane Pressure (TMP): It is the driving force for liquid transport through the ultrafiltration membrane. Calculated as the average pressure applied to the membrane minus any filtrate pressure.

$TMP = (P_{FEED} - P_{RETENTATE})/2 - P_{PERMEATE}$

In most cases, pressure at filtrate port equals zero.

Ultrafiltration (UF): A process that separates solutes based on their molecular weight or size. Ultrafiltration membranes have pore sizes between 0.001 and 0.1 µm, and are typically used for concentrating and desalting dissolved molecules (protein, peptides, nucleic acids, carbohydrates, and other biomolecules), exchanging buffers, and gross fractionation. Ultrafiltration membranes are typically classified by molecular weight cut-off (MWCO) rather than pore size.

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