

A Hands-On Guide to Ultrafiltration/ Diafiltration Optimization using Pellicon® Cassettes

In ultrafiltration (UF) tangential flow filtration (TFF) systems, operating parameter selection will have a far reaching impact as the process is scaled to full-scale manufacturing levels. While there are many factors that contribute to final system design, several key parameters should be optimized early in the process development phase. The goal is to develop a robust process with the following success criteria: superior product quality, consistent and high product yield, reproducible process flux and time, and a cleaning regime that allows extended membrane reuse.

The following basic experiments should be considered during development of processing methodology:

- Optimization
 - Impact of transmembrane pressure (TMP) and feed flow on process flux and retention
 - Impact of product concentration and buffer conditions on process flux and retention
 - Impact of diavolumes on buffer exchange and contaminant removal
- Paper design and full process simulation with chosen processing parameters

Typically, the first three experiments are performed sequentially to bracket process performance and obtain data for analysis. This information is then combined with actual manufacturing considerations (batch volume, process time, etc.) to design a process simulation.

The purpose of a process simulation is to duplicate the entire manufacturing process in a scale-down format, to confirm sizing, and to assess preliminary product quality and yield. The intent is to develop an optimized process (Figure 1), on the bench, that will efficiently scale-up to meet full-scale manufacturing expectations.

Sequence	Purpose
1. TMP Excursion at Initial Concentration ($C_{b \text{ initial}}$) ↓	• Determine TMP for UF/DF • Determine Feed Flow (Q_F) for UF/DF • Demonstrate Flux Stability • Confirm Retention of Product
2. Concentration/Volume Reduction ($C_{b \text{ initial}} \rightarrow C_{b \text{ final}}$) ↓	• Determine Flux as Function of Concentration • Determine Placement of Diafiltration Step • Determine Flux as Function of Buffer Conditions
3. TMP Excursion at Final Concentration ($C_{b \text{ final}}$) ↓	• Determine TMP for UF/DF • Determine Feed Flow (Q_F) for UF/DF • Confirm Retention of Product
4. Diafiltration/Buffer Exchange ↓	• Determine Diavolume Requirement • Confirm Retention of Product during DF
5. Product Recovery	• Crude Assessment of Step Yield • Product Quality Evaluation

Figure 1. Basic Optimization Experiments

Use this step-by-step guide to develop a robust UF/DF process with Pellicon® cassettes (cutoffs of 100 kD and lower) that will deliver superior product quality, reproducible results, and high yields.



The following are step-by-step protocols for basic optimization experiments.

Set-up and Installation Procedure

Refer to the Pellicon® 2 Cassettes Installation, User, and Maintenance Guide (UG2996EN) or the Pellicon® 3 Cassettes Installation and User Guide (AN1065EN00) when performing actual set-up and installation of Pellicon® cassettes.

1. Assemble the TFF system as shown in Figure 2.
2. Install the Pellicon® cassette(s) (Pellicon® 2 Mini with 0.1 m² membrane area, Pellicon® 3 with 0.11 m² membrane area) in the appropriate Pellicon® holder.
3. Flush the system with water, clean with the appropriate cleaning agent (per appropriate maintenance guide), and flush again.

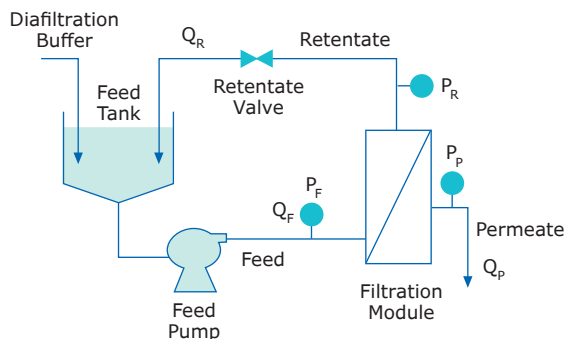


Figure 2. Schematic of a TFF System

Equilibration Procedure

1. Add 3 L/m² of the appropriate buffer to the feed tank.
Example: 0.1 m² membrane area x 3 L/m² = 0.3 L buffer
2. Direct the retentate and permeate to a waste container.
3. Start the feed pump and achieve the following conditions by partially closing the retentate valve and adjusting the pump speed:
 - Feed flow of 5 L/min/m²
 - Retentate pressure of 2–15 psi (0.14–1.03 bar) to achieve approximately 30% conversion
4. When half the buffer has been flushed, put the system in total recycle mode 1 and recirculate for 10 minutes; verify that the pH and conductivity in the system have been equilibrated to the level of the starting buffer.
5. Direct the retentate and permeate to a waste container.
6. When the feed tank level reaches the minimum level, open the retentate valve fully and stop the feed pump to prevent the introduction of air into the system.

Part 1. TMP Excursion at Initial Concentration

1. Add sufficient volume of product to the feed reservoir such that final volume or concentration target can be reached or slightly exceeded (approximately 1–1.5 L of final product at final concentration per m²). Example: if $C_{\text{initial}} = 10 \text{ g/L}$ and $C_{\text{retentate}} = 80 \text{ g/L}$, then the concentration factor is 8X. If the minimum achievable final volume for 0.1 m² is 0.1 L, calculate the required initial volume:
$$V_{\text{initial}} = V_{\text{minimum}} \times \text{VCF} = 0.1 \text{ L} \times 8X = 0.8 \text{ L}$$
2. Open the retentate valve fully and configure system in total recycle mode.
3. Start the feed pump and achieve the following conditions by partially closing the retentate valve and adjusting the pump speed:
 - Recommended feed flow (Q_F) rate for the membrane device, typically 5 L/min/m² for Pellicon® 2 and 3 cassettes
 - Minimal TMP, typically 2–5 psi (0.14–0.34 bar) for more open membranes and 10 psi (0.69 bar) for tighter membranes.
4. Recirculate the product for 10–15 minutes and ensure that stable process flux is achieved².
5. Record temperatures, pressures, and flows; sample feed and permeate for product retention³.
6. Increase TMP by 5–10 psid (0.34–0.69 bar) by manipulating the retentate valve while keeping the feed flow constant. For more open membranes increase by 2–5 psid (0.14–0.34 bar). Repeat steps 4 and 5.
7. Repeat step 6 until flux begins to level off⁴; typically 4–6 TMP values are evaluated in total.
8. Open the retentate valve fully and allow system to continue in a total recycle.
9. Increase or decrease the feed flow by 2–3 L/min/m² and repeat steps 4 through 8. If desired, a third feed flow rate can be investigated.
10. Plot the data as shown in Figure 3.

Tight membranes (1 kD, 5 kD, etc.)

Can use large TMP increases since optimum is typically >30 psi

Open membranes (50 kD, 100 kD, etc.)

Can use small TMP increases since optimum is typically <10 psi

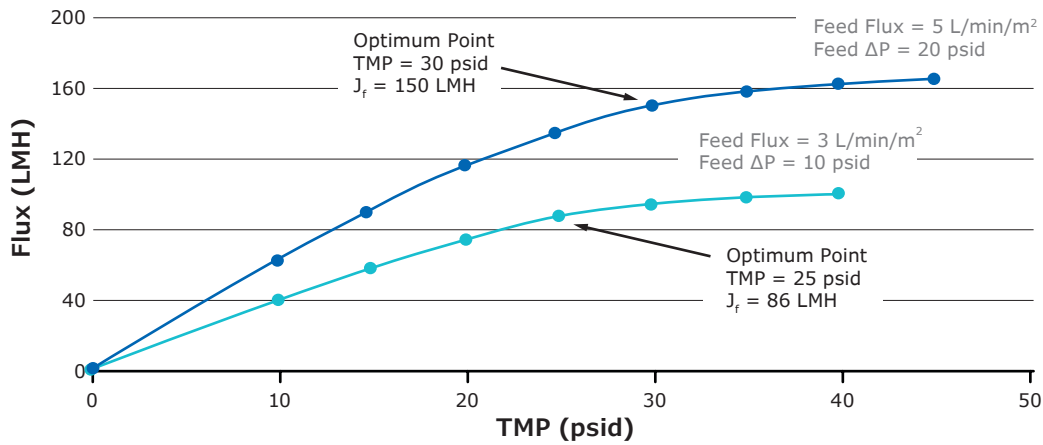


Figure 3. TMP Excursion at Two Feed Flows

	$Q_1 = 5 \text{ L/min/m}^2$	$Q_2 = 3 \text{ L/min/m}^2$	Q_1/Q_2
A [m ²]	Volume/Time/150 LMH	Volume/Time/86 LMH	0.57
Q_F [L/min]	(5 L/min/m ²) x Volume/Time/150 LMH	(3 L/min/m ²) x Volume/Time/86 LMH	0.95

Table 1. Membrane Area vs. Pump Feed Rate (Figure 3)

Calculations

The appropriate combination of feed flow rate and TMP will maximize flux while minimizing the impact of pumping and shear on the product. The appropriate combination of these two parameters will also minimize processing time and/or membrane area. To calculate the optimum feed flow, compare the required membrane area with the required pump rate at each of the two feed flow conditions, as shown in Table 1.

$$\text{Membrane Area [m}^2\text{]} = \frac{\text{Process Volume [L]}}{(\text{Flux [LMH]} \times \text{Process Time [h]})}$$

In Figure 3:

$$\text{Area}_{Q_1} = 0.57 \times \text{Area}_{Q_2}$$

$$\text{Pump feed rate [L/min]} =$$

$$\text{Feed flux [L/min/m}^2\text{]} \times \text{Area [m}^2\text{]}$$

In Figure 3:

$$\text{Pump feed rate}_{Q_1} = 0.95 \times \text{Pump feed rate}_{Q_2}$$

In this example, it is advantageous to run at the higher feed flow, Q_1 , since it only requires 57% of the membrane area used at the lower feed flow rate at almost the identical pump feed rate.

Note:

- Anticipated final volume of over-concentrated product must exceed minimum working volume of membrane system at selected feed flow rate (Q_F); avoid introduction of air and maintain uniform mixing at end of volume reduction.
- Move from least to greatest fouling conditions:
 - Do not test into pressure-independent regime (past the knee of the flux vs. TMP curve)⁴
 - Avoid exceeding 30–40% conversion ratios
- Check hysteresis if possible by returning the system to the initial conditions and taking a final flux measurement; compare initial flux performance to final flux performance at initial conditions.
- Ensure that choice of TMP and feed flow have corresponding retention values that are acceptable (> 0.998) at both initial and final product concentration and in each buffer⁵.
- There is often very little performance difference versus feed flow rate at low product concentration. However, at the higher concentrations that will be investigated in Parts 2 and 3, the benefits of different feed flow rates should become more pronounced.

Part 2. Concentration

1. Use the product from Part 1 in the starting buffer. Based on desired final product concentration factor, add additional feed volume as needed to ensure sufficient volume at end of concentration⁶.
 2. Sample feed to confirm product concentration.
 3. Put the system in total recycle.
 4. Start the feed pump and achieve the optimum TMP and feed flow as determined in Part 1 by partially closing the retentate valve and adjusting the pump speed.
 5. Direct the permeate to a separate container to concentrate product and reduce volume.
 6. Record temperatures, pressures, and flows throughout the concentration; sample feed and permeate for product retention⁷.
 7. Concentrate slightly beyond desired final product concentration.
 8. Repeat the TMP excursion outlined in Part 1 to determine optimum TMP at the final concentration in the starting buffer.
 9. Diafilter with one diavolume to get product into final buffer and dilute with final buffer back to initial concentration.
 10. Repeat the TMP excursion to determine the optimum TMP at the initial concentration in the final buffer.
 11. Repeat Part 2 steps 2–7 once in final buffer using the optimum TMP as determined above.
 12. Plot the data as shown in Figure 4, remembering to apply a temperature correction in the flux calculations⁸.
- Note, concentration should be plotted in a logarithmic manner.

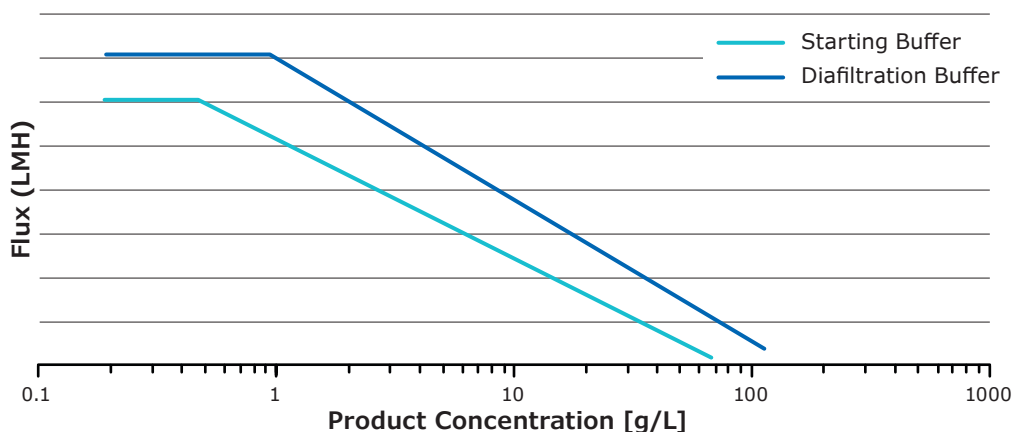


Figure 4. Flux vs. Concentration

Calculations

The tradeoff between flux and diafiltration buffer volume create an optimum bulk concentration at which to perform diafiltration; this can be calculated using the DF optimization parameter at each data point:

$$\text{DF Optimization Parameter} = \text{Concentration [g/L]} \times \text{Flux [LMH]}$$

Plotting the DF optimization parameter as a function of product concentration yields the optimum concentrations for diafiltration in both the starting and final buffers, as shown in Figure 5.

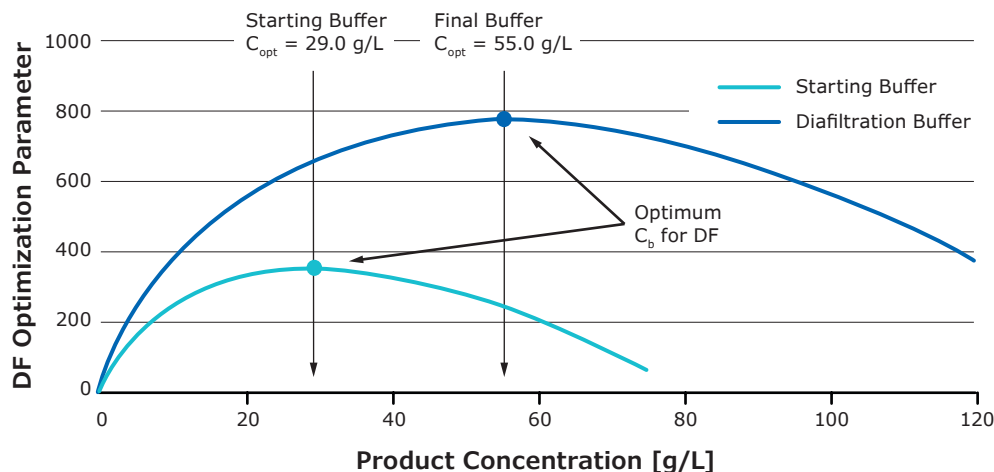


Figure 5. DF Optimization

There is an alternative approach that may be used to calculate the optimum concentration at which to perform diafiltration (C_{opt}). It assumes that the product is completely retained and that the passage of the permeating species is constant.

If the flux versus concentration data is plotted as shown in Figure 4, then the gel concentration, C_g , is the concentration at which the permeate flux reaches zero (example: ~80 g/L in the starting buffer, ~110 g/L in the final buffer). The optimum concentration at which to perform diafiltration is then calculated as⁹:

$$C_{opt} \text{ [g/L]} = C_g \text{ [g/L]} / e$$

In Figure 4:

$$\text{Starting buffer } C_{opt} = 80 / 2.71828 = 29.4 \text{ g/L}$$

$$\text{Final buffer } C_{opt} = 110 / 2.71828 = 40.5 \text{ g/L}$$

The C_g/e method can only be used when the flux vs. concentration data allows for accurate extrapolation to zero flux.

Note:

- Ensure enough feed material and appropriate system working volume in order to achieve the final concentration.
- Based on the results of the additional TMP excursions performed in Part 2, the TMPs used for concentration in both the starting and final buffers should be changed and the concentration should be repeated to obtain more accurate data.
 - If the optimum TMP for the dilute solution occurs in the pressure-independent region (past the knee of the curve) for the concentrated solution, then the TMP should be decreased to the lowest optimum value.
 - If the optimum TMP for the dilute solution occurs within the pressure-dependent region (before the knee of the curve) for the concentrated solution, then the TMP may be increased to the highest optimum value to further optimize the flux and reduce the processing time.
- Optimum concentration for diafiltration will be different for each buffer; choose an average or the most conservative.
 - Restrictions on buffer usage or minimum recirculation volume often dictate the concentration at which diafiltration occurs.
 - If the required final concentration is significantly less than the optimum concentration for diafiltration, over-concentration followed by dilution is a possible option, although rarely chosen. It should only be considered in cases where diafiltration buffer is limited and the product is stable at the higher concentrations.

Part 3. TMP Excursion at Final Concentration

1. Use the product from Part 2 at the final concentration in the final buffer.
2. Repeat steps 2–10 of Part 1.

Calculations

Reference Part 1.

Note: Reference Part 1 and Part 2 notes.

Part 4. Diafiltration

1. Use the product from Part 3 at the optimum concentration for diafiltration; dilute as needed using the final buffer.
 2. Configure the system for constant volume diafiltration.
 3. Start the feed pump and achieve the optimum TMP and feed flow as determined in Part 1 and Part 3.
 4. Diafilter the product with the chosen number of diavolumes:
 - Choose the number of diavolumes based on the product purity specifications (if known, see calculation below) and add a safety factor of 2 diavolumes, or
 5. Record temperatures, pressures, and flows at every diavolume; sample feed and permeate for both product retention, and retention and concentration of the contaminant of interest.
 6. Plot the data as shown in Figure 6.
- Use 3–5 diavolumes as an initial estimate for upstream UF/DF steps, or
 - Use 7–12 diavolumes as an initial estimate for final formulation UF/DF steps

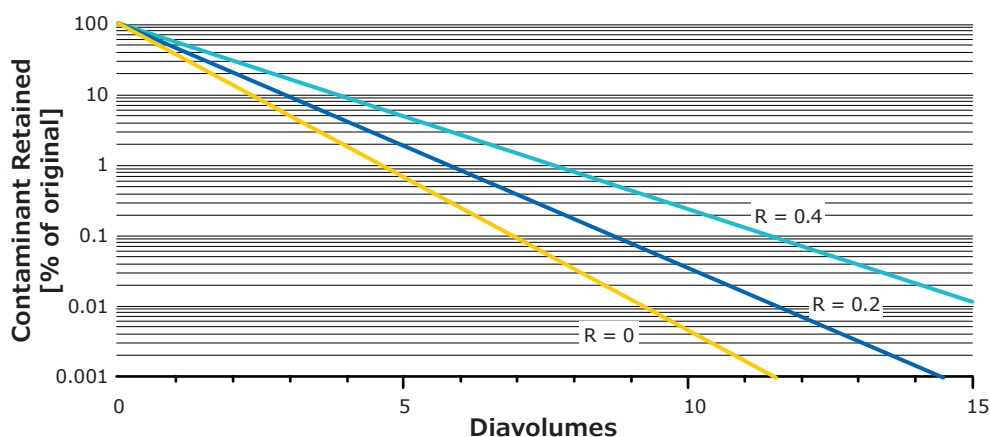


Figure 6. Contaminant removal vs. Diavolumes

Calculations

The percentage of the original contaminant in the retentate at each diavolume can be calculated from the retention values using the following:

$$\text{Remaining Contaminant [\%]} = 100 \times e^{(\text{Retention} - 1) \times N}$$

where N is the number of diavolumes.

However, since contaminant concentration is being directly measured in each feed sample throughout diafiltration, plot these concentrations as a percentage of the original and use the above equation to plot several lines of theoretical retention, as shown in Figure 6. This plot will help demonstrate the contaminant removal at various retentions.

Select the whole number of diavolumes based on the acceptable contaminant levels for the product; always add 2–3 diavolumes as a 10-fold safety factor for critical diafiltration steps, such as final formulation.

For upstream steps, add 1–2 diavolumes. If the goal of diafiltration is not to wash out a contaminant but rather to reach a target pH or conductivity, then the measurement of that quality can be plotted against the number of diavolumes instead.

Note:

- If it appears necessary to diafilter past ~14 diavolumes, any dead-legs or poor mixing areas in the system will increase the apparent retention of the contaminant and make further removal difficult.
- Ensure that choice of TMP and feed flow have corresponding product retention values that are acceptable (>0.998) throughout diafiltration.

Part 5. Product Recovery

There are various methods for product recovery at large-scale¹⁰. However, at small-scale, sufficient product recovery can be achieved by manually tilting the system and breaking the piping at low-points to drain the product. Samples of the final retentate should then be analyzed for product concentration and quality.

1. After the product has been drained from the system, add one system volume of diafiltration buffer to the feed tank.
2. Recirculate at the selected feed flow rate with the retentate valve fully open for 10 minutes.
3. Recover the buffer in a separate container using the same methods that were used to recover the product. Samples of this buffer rinse should be analyzed for product concentration.
4. After the product is recovered, the system should be cleaned with the appropriate solutions⁸.

Calculations

Ideally, the total product mass recovered in the retentate, permeate, and buffer flush as well as unrecoverable holdup volume should equal the total mass of product in the feed. If the total product mass recovered is less than the initial product mass, it is typically due to adsorption and/or solubility losses during processing¹¹. However, it is important to perform a mass balance and calculate total yield to ensure optimum process parameters.

Actual Yield [%] =

$$100 \times (V_{\text{retentate}} [\text{L}] \times C_{\text{retentate}} [\text{g/L}]) / (V_{\text{initial}} [\text{L}] \times C_{\text{initial}} [\text{g/L}])$$

Mass Balance [%] =

$$100 \times \{(V_{\text{retentate}} [\text{L}] \times C_{\text{retentate}} [\text{g/L}]) + (V_{\text{permeate}} [\text{L}] \times C_{\text{permeate}} [\text{g/L}]) + (V_{\text{rinse}} [\text{L}] \times C_{\text{rinse}} [\text{g/L}])\} / (V_{\text{initial}} [\text{L}] \times C_{\text{initial}} [\text{g/L}])$$

The theoretical yield can also be calculated based on the membrane retention and compared to the actual yield.

$$\text{Theoretical Yield [\%]} = 100 \times e^{(\text{Retention} - 1)(N + \ln X)}$$

where N = number of diavolumes and X = concentration factor.

Note:

- All calculations are estimates; during these optimization steps, the product has undergone more processing than normal. Product degradation and yield may be slightly affected. For a true indication of processing on product quality, perform the entire optimized process using fresh feed and new membranes.
- Product can be very viscous when recovered and may affect assays; perform serial dilutions for more accurate assay results.
- Actual yield and mass balance percentages should be close to 100% and/or theoretical yield. If significant losses occur, process parameters (including membrane type) may have to be changed and then re-optimized.
- In a robust process, adsorption and solubility losses should be very low.

Paper Design and Process Simulation

The optimization parameters obtained from the previous experiments can be combined to design a full process simulation: concentration, diafiltration, (concentration,) and recovery. If time permits, a process simulation should be run immediately following the optimization work, and should employ the following:

- New set of cassettes; same membrane type, same cassette path length
- Fresh feedstock
- Fresh buffer(s)
- Optimized process parameters
- See detailed process simulation calculations below.

Calculations

The membrane area can be optimized to allow the entire process (both concentration and diafiltration) to be completed in the specified timeframe (3–4 hours is recommended). The average flux for each concentration and diafiltration step can be estimated from the optimization data and combined with the desired volumes to be processed. The approximate required membrane area can then be calculated for both manufacturing scale and scale-down runs.

Assume an example process scenario (this would have been determined by optimization data, DF parameter, etc.):

- 2.9X Concentration:
10 g/L to 29 g/L; flux decreases from 150 LMH to 80 LMH
- 7X Diafiltration:
29 g/L; flux increases from 80 LMH to 85 LMH
- 3.4X Concentration:
29 g/L to 100 g/L; flux decreases from 85 LMH to 20 LMH
- Desired process time is 4 hours

After performing the process simulation, the system should be cleaned with the appropriate solution⁸. If possible, the process should be rerun using the cleaned membranes to determine the effectiveness of the cleaning cycle and the consistency of membrane performance from run-to-run. If the cleaning cycle does not prove effective, the cleaning parameters or cleaning solutions will need to be changed and the cleaning cycle will have to be tested again.

Manufacturing scale volumes as determined by the user:

- Feed volume = 5000 L
- Retentate volume at end of 2.9X concentration = $5000 \text{ L} / 2.9 = 1724 \text{ L}$
- Permeate volume removed during 2.9X concentration = $5000 \text{ L} - 1724 \text{ L} = 3276 \text{ L}$
- 7X Diafiltration buffer volume = $7 \times 1724 \text{ L} = 12,068 \text{ L}$
- Retentate volume at end of 3.4X Concentration = $1724 \text{ L} / 3.4 = 507 \text{ L}$
- Permeate volume removed during 3.4X concentration = $1724 \text{ L} - 507 \text{ L} = 1217 \text{ L}$

Average process flux for concentration step¹²:

$$J_{avg} = J_{final} + 0.33 (J_{initial} - J_{final}) = J_{initial} \times 0.33 + J_{final} \times 0.67$$

For 2.9X concentration:

$$J_{avg} = 150 \text{ LMH} \times 0.33 + 80 \text{ LMH} \times 0.67 = 103 \text{ LMH}$$

For 3.4X concentration:

$$J_{avg} = 85 \text{ LMH} \times 0.33 + 20 \text{ LMH} \times 0.67 = 41 \text{ LMH}$$

Average process flux for diafiltration step:

For diafiltration the average flux can be estimated as the initial and final process flux during the diafiltration step.

Required area:

$$\text{Area} = [(\text{Permeate volume}/\text{Average flux})_{\text{Concentration}} + (\text{Permeate volume}/\text{Average flux})_{\text{Diafiltration}} + \dots] / \text{Time}$$

In this example:

$$\text{Area} = [(3,276 \text{ L}/103 \text{ LMH}) + (12,068 \text{ L}/83 \text{ LMH}) + (1,217 \text{ L}/41 \text{ LMH})] / 4 \text{ hours} = 51.6 \text{ m}^2$$

Add 20% safety factor:

$$\text{Area} = 62 \text{ m}^2$$

To perform a scale-down process simulation, the same volume to area ratio is used and scaled based on either the feed volume that can be used for the simulation or the area of the desired filtration device. For example, if the process is to be performed on one Pellicon® 2 Mini cassette (with an area of 0.1 m²), then the required feed volume will be:

$$\text{Scale-down feed volume} = 0.1 \text{ m}^2 \times (5000 \text{ L}/62 \text{ m}^2) = 8 \text{ L}$$

Instead, if there is a specific volume of feedstock to process (example: 25 L), then the required membrane area will be:

$$\text{Scale-down membrane area} = 25 \text{ L} \times (62 \text{ m}^2/5000 \text{ L}) = 0.3 \text{ m}^2$$

The process parameters, including Pellicon® device type, should be consistent between scales, allowing the process to be completed in a similar timeframe with similar fluxes, pressures and loadings. The concentration factors, number of diavolumes and feed quality should be kept consistent at all scales to ensure robust scalability. However, to demonstrate process robustness and repeatability, the process should be tested at pilot scale before proceeding to manufacturing.

Definitions

Apparent Sieving (S_{app})

The fraction of a particular protein that passes through the membrane to the permeate stream based on the measurable protein concentrations in the feed and permeate streams. A sieving coefficient can be calculated for each protein in a feedstock.

$$S_{app} [-] = (\text{Concentration in permeate, } C_p) / (\text{Concentration in feed, } C_b)$$

Concentration Factor (CF)

The amount that the product has been concentrated in the feed stream. This depends on both the volume concentration factor and the retention. As with the VCF, for a Fed-Batch concentration process, calculate CF based only on the volume of feedstock added to the TFF application.

$$CF [-] = \text{Final product concentration} / \text{initial product concentration} = VCF(R_{app})$$

Conversion Ratio (CR)

The fraction of the feed side flow that passes through the membrane to the permeate.

$$CR [-] = Q_p / Q_F$$

Diavolume (DV or N)

A measure of the extent of washing that has been performed during a diafiltration step. It is based on the volume of diafiltration buffer introduced into the unit operation compared to the retentate volume. If a constant-volume diafiltration is being performed, where the retentate volume is held constant and diafiltration buffer enters at the same rate that permeate leaves, a diavolume is calculated as:

$$DV \text{ or } N [-] = \text{Total buffer volume introduced to the operation during diafiltration} / \text{retentate volume}$$

Intrinsic Sieving (S_i)

The fraction of a particular protein that passes through the membrane to the permeate stream. However, it is based on the protein concentration at the membrane surface. Although it cannot be directly measured, it provides a better understanding of the membrane's inherent separation characteristics.

$$S_i [-] = (\text{Concentration in permeate, } C_p) / (\text{Concentration at membrane wall, } C_w)$$

Mass Flux (J_m)

The mass flow of protein through the membrane normalized for the area of membrane (m^2) through which it is passing.

$$J_m [g \cdot m^{-2} \cdot h^{-1}] = Q_p \times C_p / \text{area}$$

Permeate Flux (J_f)

The permeate flow rate normalized for the area of membrane (m^2) through which it is passing.

Pressure Drop (ΔP)

The difference in pressure along the feed channel of the membrane from inlet to outlet.

$$\Delta P [\text{bar}] = P_F - P_R$$

Retention (R)

The fraction of a particular protein that is retained by the membrane. It can also be calculated as either apparent or intrinsic retention. Retention is often also called rejection.

$$R_{app} [-] = 1 - S_{app} \text{ or } R_i = 1 - S_i$$

Transmembrane Pressure (TMP)

The average applied pressure from the feed to the permeate side of the membrane.

$$TMP [\text{bar}] = [(P_F + P_R)/2] - P_p$$

Volume Concentration Factor (VCF or X)

The amount that the feed stream has been reduced in volume from the initial volume. For instance, if 20 L of feedstock are processed by ultrafiltration until 18 L have passed through to the permeate and 2 L are left in the retentate, a ten-fold concentration has been performed so the Volume Concentration Factor is 10. In a Fed-Batch concentration process, where the bulk feedstock is held in an external tank and added to the TFF operation continuously as permeate is removed, VCF should be calculated based only on the volume that has been added to the TFF operation.

$$VCF \text{ or } X [-] = \text{Total starting feed volume added to the operation} / \text{current retentate volume}$$

References/Footnotes

1. Total recycle means retentate and permeate lines return to feed vessel
2. If process flux is unstable, it may be necessary to allow additional time or investigate other membrane options
3. Retention samples are not required at every data point; sampling at lowest and highest TMP is typical
4. The point at which the flux levels off is defined as the point around which the slope of the flux vs. TMP curve decreases to $\leq 50\%$ of the previous slope. This point is also referred to as the “knee” of the curve.
5. These other conditions are described in more detail in Parts 2 and 3.
6. Example: 10X concentration with a final volume of 300 mL requires $(300 \text{ mL} \times 10) = 3 \text{ L}$ of feed
7. Retention samples are not required at every data point; initial and final concentration are typical. Typical data recording interval is approximately every 10–15 minutes.
8. See Guide: Pellicon® 2 Cassettes Installation, User, and Maintenance Guide (UG2996EN) or Pellicon® 3 Cassettes Installation and User Guide (AN1065EN00)
9. Ng P, Lundblad J, and Mitra G, Optimization of Solute Separation by Diafiltration, Separation Science, 11(5): 499-502, 1976.
10. See Technical Brief: Protein Concentration and Diafiltration by Tangential Flow Filtration (TB032)
11. See Technical Note: Increase Product Yield in Your UF/DF Processes (AN1026EN00)
12. Average flux can also be calculated for each step by dividing the total process volume by the total process time

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