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Application Note

Increase Product Yield in Your UF/DF Process

Understanding the key factors that can reduce yield and review the techniques to maximize recovery

Tangential Flow Filtration (TFF) processes are well established in the biopharmaceutical industry. A well-designed ultrafiltration/diafiltration (UF/DF) process should be robust, reproducible and result in high yields (> 95%). This application note will review potential sources of yield loss, and propose techniques to improve recovery.

There are five main sources of product loss; adjusting your UF/DF process to combat these dynamics will result in yield improvements.

1. **Retention Losses:** loss in the permeate due to product that has passed through the membrane
2. **Adsorption Losses:** loss due to product adsorption to the membrane
3. **Solubility Losses:** activity loss of the product because of exceeding solubility limits
4. **Unrecoverable Holdup Losses:** product that is not recovered from the system
5. **Product Degradation:** product that no longer has intended biological function

The first step is to determine current yield loss and close the mass balance. Yield losses are defined as the amount of product in the feed minus the amount of product in the final pool.

Yield loss [%] = $100^* [V_o{}^* C_o - V_r{}^* C_r]$

Once the product losses are quantified, the following approaches can be used to increase product yield.

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1. Retention Losses

Choosing a membrane with appropriate retention characteristics is critical to ensuring high product yield. If a product in the retentate is being concentrated or diafiltered, low retention could result in the product loss through the membrane into the permeate. Even highly retained products can show measurable filtrate loss when they are significantly concentrated or diafiltered. In addition, because of charge effects, the size of a molecule may change if the pH and the ionic strength of the solution changes, which may affect retention. The % product loss in permeate can be calculated using the following formula:

Product Loss in Permeate =
100* {1-exp[(R-1)(ln VCF+N)]}

To measure retention losses, samples from the permeate stream should be collected at various points during the concentration phase, for example: beginning, middle and end. If possible, a sample of the pooled permeate at the end of concentration could also be taken and further concentrated using a tighter membrane to increase the assay sensitivity. The same approach could be followed for the diafiltration process, as well.

In a UF system, it is possible the membrane selection was made for a high-yield process, but the system is not providing maximum retention. The general rule is to select a membrane that has a NMWL one-third to one-fifth of the molecular weight of the product to be retained (some mAb processes have shown protein passage with the use of Biomax® 50 membrane; therefore, lower NMWL Biomax® membrane should be evaluated). If the product must pass through the membrane to the filtrate, select a NMWL 3 to 5 times larger than the product. Generally, a minimum size difference of approximately five-fold is required between components that are being separated. Use a membrane that has sufficiently high retention to meet your yield goal. This is determined during optimization trials and will address the following important variables: impact of transmembrane pressure (TMP) and feed flow rates on process flux (the flow rate normalized for the area of membrane through which it is passing) and retention; impact of product concentration and buffer conditions on process flux and retention; impact of diavolumes (measures of the extent of washing that has been performed during a diafiltration step) on buffer exchange and contaminant removal.

Cassette systems typically use compression to seal the cassettes together, which can be checked by an integrity test or pressure hold test. It is also possible that, over time, mechanical and/or chemical degradation of the membrane can occur and retention could change over time. Trends in process yields that change over time are indicative of this type of behavior. It is important to take samples and measure increased permeate losses to confirm this is happening. A review of system sealing techniques and/or cleaning procedures may be appropriate if this is the case.

If no product is measured in the permeate, other areas of the process should be considered.

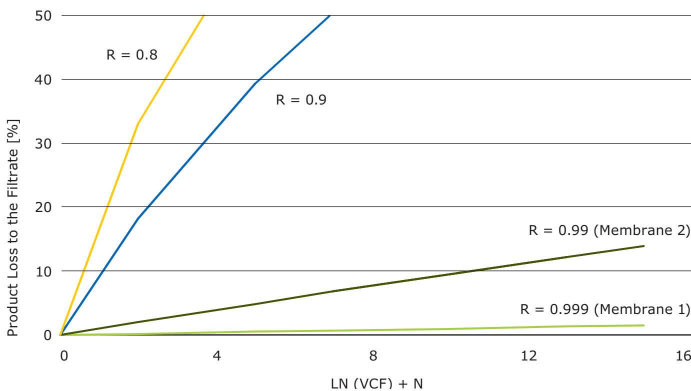

Figure 1.

Product Loss in Permeate is equal to the equation on the left, where:

N = number of diavolumes

VCF = volumetric concentration factor

R = product retention

Membrane number	Retention
Membrane 1	99.9% retention = **0.73% loss**
Membrane 2	99.0% retention = **7.0% loss**

Table 1.

Example: 20x concentration with 5 DVs. The effect of product retention on product yield during a batch ultrafiltration/constant-volume diafiltration process where the product is in the retentate and the retention is constant throughout the process.

2. Adsorption Losses

Adsorption losses occur when the product binds to a membrane and cannot be desorbed in an active form prior to recovery. For applications where the product concentration and volume is high in comparison to the membrane area used to process it, adsorption should not be a significant mode of yield loss. However, if the product concentrations and volumes are very low and/or a very large membrane area is used for processing, this loss mechanism should not be ignored. The membrane material that is chosen will affect the product adsorption. In general, hydrophilic membranes will exhibit lower protein binding than hydrophobic membranes. Adsorption losses will also be affected by other components in the feedstream, such as antifoams that may interact with both the membrane and the product.

Approximate protein adsorption values for the various membrane materials are summarized in Table 2.

As discussed previously, if there are no retention losses and the application is in the "low concentration" range, adsorptive losses may be significant. The adsorptive losses can be quantified by running the system in a recirculation mode so the permeate is being returned to the feed vessel. The system should be run with a low crossflow and low TMP to avoid generating polarization. The feed concentration can be assayed over time to look for any decrease in feed concentration due to adsorption.

Membrane material	Protein adsorption (μg/cm²)
Ultracel® regenerated cellulose membrane	1 – 35
Cellulose acetate membrane	40 – 50
Biomax® modified polyethersulfone membrane	10 – 125
Unmodified polyethersulfone membrane	150 – 200

Table 2.
Protein Adsorption Values

Sources: *A.M.Pitt J.Paren.Sci.Tech. May 1987 pp 110-114,*
C. Samy Pharm.
Tech. Intl. Nov. 1992 pp 38-48, Millipore in-house tests

3. Solubility Losses

It is recommended that the solubility limits for the protein product in both the starting and ending buffer be understood, and the UF process be designed so as to not exceed these limits in processing. Even if the final bulk concentration of a product is not beyond the solubility limit, the polarization at the membrane surface could result in areas of higher concentrations. Inadequate mixing will also create concentration differences. Product concentration in these areas could potentially exceed the solubility limit. Since higher permeate fluxes lead to higher polarization, reducing the permeate flux or increasing the crossflow could be ways to minimize solubility losses. Buffer compatibility with the product (for cases where diafiltration is performed) should also be considered.

4. Unrecoverable Holdup Losses

After the process step is complete, a certain amount of liquid still remains in the filter cassettes and the system piping. In cases when the final product concentration is high and/or when the final product volume is low, these losses can be significant. Careful design of the piping, optimization of the total membrane area, and development of an efficient product recovery step will help to minimize the product loss incurred due to unrecoverable holdup.

Examples of typical cassette holdup volumes are given in Table 3. Examples of typical system holdup losses are given in Table 4.

The following equation can be used to estimate system related holdup losses:

Holdup Loss [%] = $100*(V_{hu}*C_r \text{ or } C_{flush})/(V_o*C_o)$

Developing a product recovery scheme is important to minimize holdup losses. One recovery method is to gravity-drain the system. Low pressure (~1–2 psig) air blowdown may also be used to improve volumetric recovery. Rinsing techniques can also be considered. A buffer recirculation rinse at low crossflow and TMP is very effective. For this recovery technique, the product needs to be overconcentrated to allow the buffer rinse volume to be added into the final retentate. To minimize dilution effects, buffer displacement/plug flow with a completely full or drained system can also be used to flush the product.

These recovery techniques should be tested following a process simulation so the product is at the final concentration and buffer conditions. Prior to stopping the process, a low crossflow, low TMP recirculation of the product for 5 minutes can be effective to “depolarize” the membrane surface and provide a well-mixed product. The system should then be drained and sampled for recovery (volume/weight and concentration). At least two recirculation rinses should be performed using the minimum working volume of the system. These should be run at low crossflow and TMP for 5 minutes. The rinses can then be drained and sampled (volume/weight and concentration). From this data, the amount of recovery in rinsing can be determined.

Cassette	Area (m^2)	Approximate feed (mL)	Approximate Permeate (mL)
Pellicon® 2	0.1	18	10
Pellicon® 2	0.5	90	50
Pellicon® 2	2.5	450	250
Pellicon® 3	0.0088	1.5	2.4
Pellicon® 3	0.11	18	15
Pellicon® 3	0.57	85	68
Pellicon® 3	1.14	170	127

Table 3.
Typical Holdup Volumes of Pellicon® 2 and 3 Cassettes with C-Screen

Note: *These volumes are approximate — other documents list slightly different volumes.*

Pipe size (inch)	Flow rate (L/min)	Minimum recirculation volume (L)	Irrecoverable V_{hu} (L)
1/2	3–12	1.0	0.03
3/4	9–33	1.5	0.1
1	18–630	8.0	0.25
1 1/2	45–160	18.0	0.55

Table 4.
Typical Systems Holdup Losses

Note: *These volumes are approximate — other documents list slightly different volumes.*

5. Product Degradation

The quality of the product due to aggregation or denaturation could be affected by several factors, such as a large number of pump passes, micro-cavitations, air-liquid interfaces, high protein concentrations and temperature effects. For instance, operating at a lower temperature often minimizes product denaturation. However, lowering the temperature can increase the number of pump passes, as well as lower the solubility, leading to increased aggregation/denaturation. The potential for product degradation also depends on the susceptibility of the product. However, the damage can be minimized by designing a robust process/system.

If the product being recovered is not active (increased aggregate formation, loss of activity on activity assay), further investigation may be necessary. Feed samples can be taken during the concentration step of the process (beginning, middle, end) to see if the concentration and/or operating conditions are affecting the quality of the product. Similar samples should be taken during the diafiltration process to see if the change in buffer conditions may contribute to this activity loss.

Improper Calculations

In addition to the five sources of yield losses listed above, improperly calculated yield values can also lead to false alarms. Here are a few examples:

- Improper volumes/concentrations used for the yield calculation
- Variation of the size of the product between the beginning and the end of the process

This can be relevant, especially when the yield is calculated using the concentration in g/L and the volume of product recovered.

High Concentration Assay Methodology

Protein at very high concentrations can be very viscous and can stick to the pipette tips during sample preparation for assays. A small amount of protein adhered to the pipette tip at these high concentrations can affect the assay results significantly. Thus, a direct 100X or 200X dilution on the high protein concentration sample is not recommended for running the assay. A 2X dilution first followed by subsequent dilutions of 10X each will minimize the assay errors.

Conclusion

Yields for well-designed ultrafiltration/diafiltration process should be >95%. Product losses can be attributed to numerous sources, such as retention, adsorption, solubility, unrecovered holdup and product degradation. A thorough review of each of these areas should result in a process with stable and continuous high yields.

Summary Table

The following table summarizes various factors affecting product loss for each scenario.

Source of loss	Retention	Adsorption	Solubility	Holdup/system	Product degradation
Factors affecting loss	• Membrane cutoff • Operating parameters (concentration polarization) • Mechanical compression seal • Buffer conditions, such as pH affecting product size	• Membrane material • System materials	• Operating parameters (concentration polarization, high conversion) • Buffer selection • Mixing • Solution chemistry (pH, pI, isoelectric strength)	• System design • Recovery method • Micro leakage of a valve or sudden opening of a valve that should be closed (and consequently potential loss into the drain)	• Product susceptibility • Operating parameters, such as temp, pump passes, process time • Buffer selection • System components
Detecting the cause of product loss	• Permeate UV detection and/or by analytical study of permeate samples	• System run in total re-circ mode under low polarization conditions and the retentate assayed over time	• Sampling during the concentration and diafiltration steps	• Sampling the buffer rinses	• Sampling during the concentration and diafiltration steps
Troubleshooting/ Correction measures	• A tighter membrane cutoff • Operating under conditions causing lower polarization • Check torque, Integrity test value before each use	• For products containing hydrophobic components, such as antifoams, use more hydrophilic membranes, such as regenerated cellulose • For extremely low product concentrations in the feed, use regenerated cellulose • Use optimized product volume/ concentration to membrane area ratio	• Operating under conditions causing lower polarization, e.g., lower permeate flux • Using appropriate buffer • Good mixing	• Use good system design practices, minimizing working volumes lines sizes, etc. • Optimize product recovery, e.g., high point air blowdown, low point drain, recirculation rinse, plug flow rinse, etc.	• Optimize process parameters to minimize number of pump passes, process time • Temperature considerations • System design, e.g., in the recycle tank, the retentate stream should always be returned below the liquid surface to prevent foaming. Vortex formation should be avoided by using an off-center drain or baffles in the tank. Filling the system with buffer before introducing the product and an optimized recovery technique at the end of the process will minimize air-liquid interfaces. • Selection of appropriate pumps/valves can prevent denaturation caused by micro-cavitations (maintain a net positive suction head for the pump)

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