Millipore
Filtration, Separation
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Technical Brief

Small Molecule Processing by Tangential Flow Filtration

Considerations and strategies for optimal processing success

Due to their higher osmotic pressures and mass transfer coefficients, small molecules in the range of 3 – 10 kDa, like insulin, often require unique processing conditions as compared to those of larger molecules. Tangential flow filtration (TFF) processing strategies developed for larger molecule applications may not be appropriate and can lead to an increase in process variability and sub-optimal performance. This application note explores the key limitations and challenges typically observed with small molecule TFF processing and explains the strategies required for optimal success with your TFF step.

Introduction

Certain considerations for processing small molecules like insulin are necessary when establishing processing parameters for TFF systems. Due to the high osmotic pressures and mass transfer coefficients of small molecules coupled with the lower membrane permeability of tight membranes, small molecule processing does not exhibit the same behavior as more open molecule processing. For comparison, Table 1 shows the average permeability for tight membranes and the more open 10 and 30 kDa membranes. The process flux cannot exceed the permeability.

Table 1. Average water permeability of Ultracel® Membranes

Ultracel® Membrane MWCO (kDa)	Average Permeability (LMH/psi)	Permeability compared to Ultracel® 30 kDA
3	0.6	6%
5	0.9	8%
10	6.4	59%
30	10.9	100%

Mathematical modeling as well as bench-scale trials with recombinant insulin have been completed to help guide the decision process for working with tight membranes.

Modeling performance using a monoclonal antibody (mAb) feed

Equation 1:
Osmotic Pressure Model[1]

$$J = L_p(TMP - \Delta\pi)$$

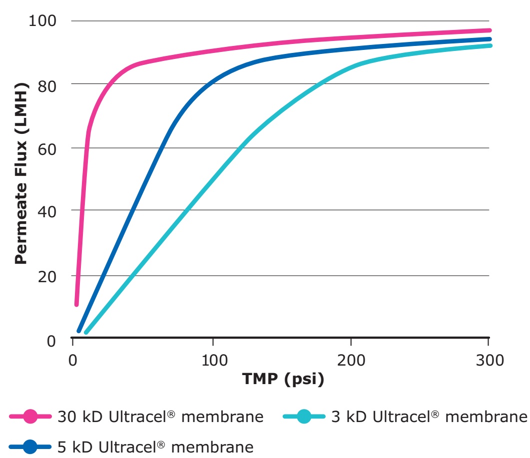


Figure 1.
TMP vs Flux for Various Pellicon® 3 Cassettes with Ultracel® Membranes

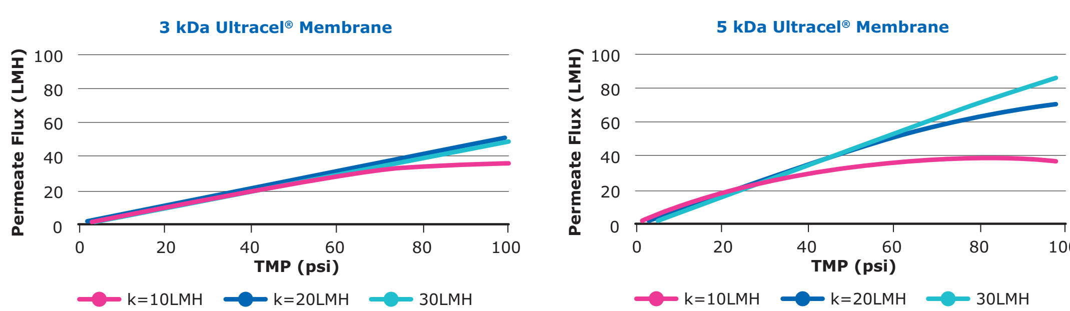
To help understand the effects of small molecule performance, mAb behavior was modeled using the osmotic pressure model shown (at left) as Equation 1.

TMP vs permeate flux curves were determined for Pellicon® 3 membranes using known mAb mass transfer coefficient (k) behavior and the osmotic pressure model. Figure 1 shows the expected behavior for various tight and more open Ultracel® membranes.

Although the fundamental performance is similar, the curves in Figure 1 suggest the transition to mass-transfer dependent flux from linear pressure dependent flux behavior of tight membranes requires much higher membrane pressures. Figures 2 and 3 take a closer look into the effect of the k value obtained as result of different feed flow rates for the 3 and 5 kDa membranes respectively.

The modeling work predicts mass transfer still factors into performance, especially for the 5 kDa membrane, where a greater effect can be seen at different feed flow rates.

Figure 2 and 3.
Effect of k on Flux vs TMP curves for 3 and 5 kDa Ultracel® Membranes



Studies with Recombinant Insulin

The performance for 3 and 5 kDa Pellicon® 3 cassettes was evaluated using recombinant Insulin to determine the relationship between retention, flux, trans-membrane pressure (TMP) and feed flow rate. A series of controlled bench-scale experiments were run under various varying conditions in order to establish this relationship. Table 2 shows the range of conditions under which the experiments were run.

Table 2.
Experimental Range for Recombinant Insulin Evaluation on 3 and 5 kDa Ultracel® Membrane

Feed Flow Rate (LMM)	Feed Pressure (psi)	Insulin Concentration (g/L)
1 – 6	30 – 100	2 - 75

[1] Lutz, Ultrafiltration for Bioprocessing

At low insulin concentrations between 2 and 10 g/L, there were no observable mass transfer effects up to 100 psi at 1 LMM and greater feed flow rates. Measured retention was greater than 99.9%. Figures 4 and 5 show the flux vs TMP curves for various flow rates for the 3 and 5 kDa membranes at 10 g/L.

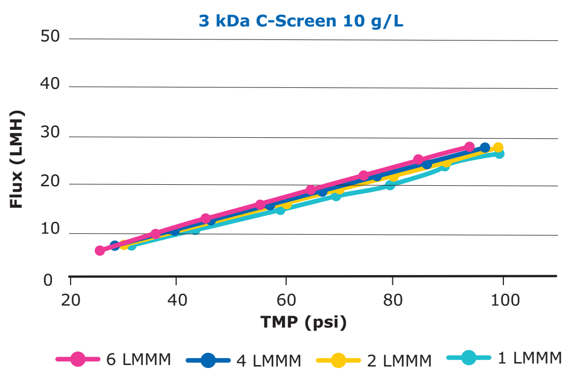


Figure 4.
Flux vs TMP for 3 kDa Ultracel® Membrane at various feed flow rates for 10g/L Insulin

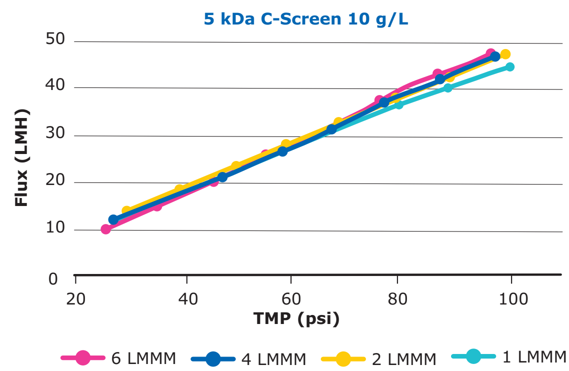


Figure 5.
Flux vs TMP for 5 kDa Ultracel® Membrane at various feed flow rates for 10 g/L insulin

As concentration increased, the effects of mass transfer became more noticeable. At 50 g/L, effects were seen for the 5 kDa membrane at greater than 50 psi, but were still not noticeable for the 3 kDa. At 76 g/L, mass transfer effects were seen for the 3 kDa membrane around 60 psi and likewise for the 5 kDa membrane near 40 psi. Retention remained consistent at greater than 99.9% throughout. Figures 6 and 7 demonstrate this relationship.

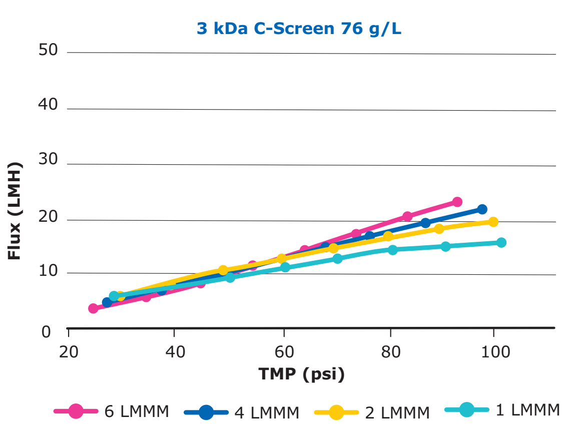


Figure 6.
Flux vs TMP for 3 kDa Ultracel® Membrane at various feed flow rates for 76 g/L insulin

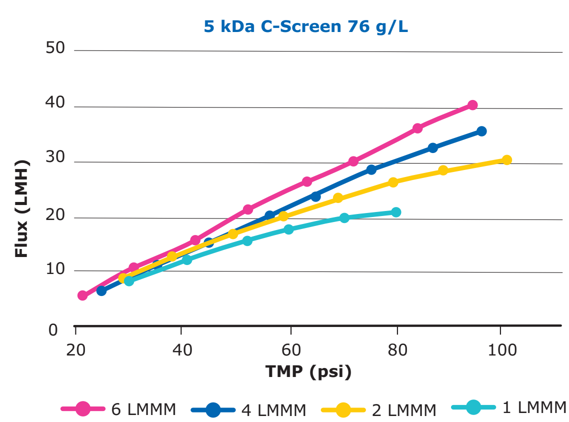


Figure 7.
Flux vs TMP for 5 kDa Ultracel® Membrane at various feed flow rates for 76 g/L insulin

Sizing estimates are provided for both 3 and 5kDa membranes for a process to concentrate 20 L insulin feed in 4 hrs from 2 to 50 g/L with an 8x diafiltration at final concentration at 100 psi for different feed flow rates. Figure 8 shows the area required for each membrane at the different feed flow rates.

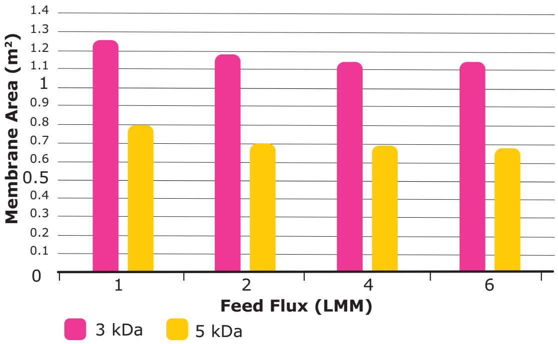


Figure 8.
Sizing Estimates for 3 and 5kDa Ultracel® Membrane for various feed flow rates

It can be seen from the estimates in Figure 8 there is a larger increase in area at the flow rates where mass transfer effects are noticed (<2LMM). Although the system is running more efficiently, requiring fewer pump passes, a slower flow rate equates to more area in order to process in the same amount of time. Area is not sacrificed at the higher flow rates (2-6 LMM), however one starts to consider the effects of multiple pump passes and size in order to reach the desired concentration. This could be an important consideration for molecules that are sensitive to shear, or where there are existing equipment limitations. You must also consider your final system size when determining whether or not the difference in area has a significant impact.

General Recommendations and Strategies for Processing Small Molecules

When working with small molecules, certain factors should be considered to customize your process. Depending on your limitations and desired outcomes, different approaches should be exercised.
The following should be used as general guidelines:

- Tight Ultracel® membranes are less likely to be mass transfer limited
  - Use lower feed flow rates (1 – 2 LMM) to minimize system, piping, pump size and number of passes
  - Use higher feed flow rates (2 – 6 LMM) to optimize for membrane area
- Understand the differences and needs between 1, 3 and 5kDa nominal molecular weight cut-offs (NMWCOs).
  - Higher TMP needed for lower membrane NMWCOs
  - More area required for lower NMWCOs
- Importance of NMWCO and TMP selection
  - Carefully select the largest NMWCO and TMP that obtains acceptable product retention
  - Compare performance with a lower NMWCO to evaluate effects of higher TMP
- Control Strategy Considerations
  - Constant TMP
    - Maximizes consistency
  - Constant feed pressure and feed flow rate
    - Maximizes efficiency of your system

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