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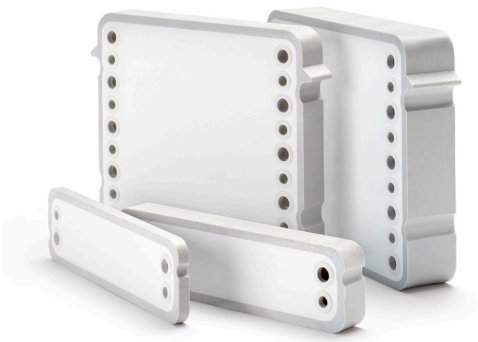
Application Note

Performance Evaluation and Cleanability Study using Pellicon® 3 Cassettes with 30 kD Biomax® and Ultracel® Ultrafiltration Membranes

Consistent, high performance ultrafiltration throughout multiple process runs

Introduction

Current trends in the bioprocessing industry are driving mAb and plasma producers to formulate at higher protein concentrations. As a result, formulating using tangential flow filtration (TFF) may be limited in reaching these concentrations due to high pressures caused by highly viscous feed streams. Filtration devices used during processing must be optimized to handle high viscosity and pressures, while maintaining high flux, excellent product recovery and cleanability. An effective cleaning protocol removes residual protein and other contaminants from the membrane surface and cassette feed channels, and restores the membrane performance to predictable and consistent levels. Normalized water permeability and process reproducibility are important parameters to monitor when assessing cleanability and both are indicators of effectiveness and consistency of the cleaning procedures.



Objective

Evaluate Pellicon® 3 filtration cassettes to characterize the impact of membrane material and channel geometry on process performance, and evaluate cleanability by demonstrating consistency over multiple process runs when working with high concentration/high viscosity feed streams.

Design Background

Flat sheet membrane cassettes are a very efficient module design for concentration and diafiltration of therapeutic proteins due to their high packing density, linear scalability and high mass transfer coefficients. Screens inserted into the cassette feed channels contribute to the high mass transfer by increasing fluid turbulence and decreasing protein polarization resulting in higher fluxes at lower crossflow requirements.

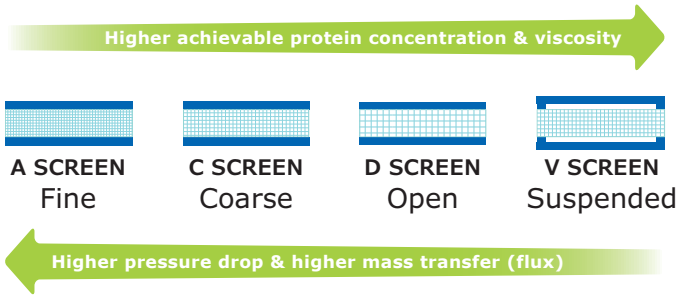


Figure 1
Types of Feed Channel Screens in Cassettes

As the concentration and viscosity of protein solution increases during ultrafiltration, feed channel pressure drop increases as well, eventually reaching a limit above which the process cannot continue. Tighter screens and channels exhibit higher pressure drops and therefore reach this process limitation at lower protein concentrations. However, very open screens and channels have much lower mass transfer, which means that fluxes are low and more membrane area is required to complete a process. Pellicon® filtration devices are available with a range of different feed channel screens, enabling the end user to find a filter that is best suited to a particular application challenge (Figure 1).

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Methods

Cassettes

Pellicon® 3 Cassettes with 30 kDa Biomax® membrane and A and D screens, and with 30 kDa Ultracel® membranes and C and D screens, were used. The membrane surface area was 0.11 m^2 per cassette. For comparison purposes, a 0.1 m^2 Pellicon® 2 cassette with 30 kDa Ultracel® membrane and V screen was also used.

Process Run

Each cassette was challenged with bovine gamma globulin solution (BgG) and cleaned multiple times to demonstrate process consistency and cleanability of 30 kD Biomax® and Ultracel® membranes (Table 1). BgG solution was concentrated from an initial 10 g/L to the maximum achievable concentration. The feed flow rate was set to 6 L min^{-1} m^{-2} and transmembrane pressure to the optimum value determined during a flux versus TMP optimization test. The following process control strategy was used as the viscosity of the protein solution increased:

- Starting feed flow rate and TMP conditions were maintained for as long as possible.
- The retentate valve was adjusted to maintain TMP until retentate pressure reached a minimum of 10 psi. The valve was not opened to allow a lower retentate pressure.
- The feed flow rate was maintained while feed pressure increased until P_{Feed} reaches 60 psig maximum.
- The feed pump was ramped down to maintain P_{Feed} at 60 psig until the feed flow rate reached a minimum of 1 L min^{-1} m^{-2} or the lowest controllable flow rate of the feed pump.

Membrane	Screen Type	Number of Process and Cleaning Cycles
Ultracel®	C	10
	D	16
	V (Pellicon® 2)	3
Biomax®	A	10
	D	10

Table 1
Total Number of Process Runs and Cleaning Cycles for Pellicon® 3 Cassettes with 30 kD Ultracel® and Biomax® Membranes

Cleaning Procedure

After processing and product recovery, the Pellicon® 3 cassettes with 30 kD Biomax® and Ultracel® membranes were cleaned by flushing and recirculating sodium hydroxide solution at room temperature. The feed flow was set to 6 L min^{-1} m^2 and the retentate pressure to approximately 5 psi for all cleaning steps. Table 2 summarizes cleaning procedures used for each membrane material.

Step	Membrane	Screen	Cycles	Solution	Volume [L/m^2]	Mode	Time [min]
1	All	All	All	Buffer	10	SPFO, drain	n/a
2	Biomax®	D	1-10	0.5N NaOH	10	SPFO	n/a
	Biomax®	A	1-7				
	Ultracel®	C, V*, D	1-10				
	Biomax®	A	8-10	1N NaOH			
	Ultracel®	D	11-15	0.1N NaOH			
3	Biomax®	D	1-10	0.5N NaOH	5	TRFO	60
	Biomax®	A	1-7				
	Ultracel®	C, V*, D	1-10				
	Biomax®	A	8-10	1N NaOH			
	Ultracel®	D	11-15	0.1N NaOH			30
4	All	All	All	Water	20	SPFO, measure NWP	n/a
5	All	All	All	0.1N NaOH	10	TRFO, store	15

* V Screen with Pellicon® 2 Cassettes
SPFO: Single-Pass Filtrate Opened
TRFO: Total Recycle Filtrate Opened

Table 2
Cleaning Procedures at ambient temperature for Pellicon® 3 Cassettes with 30 kD Ultracel® and 30 kD Biomax® Membrane

Results

Process Performance – Flux and Feed Channel Pressure Drop

Figures 2 and 3 show process flux as a function of protein concentration for Pellicon® 3 cassettes with 30 kD Biomax® and Ultracel® membranes, with A, C, D, and Pellicon® 2 cassettes with V screen. All sequential process cycles with a specific cassette are plotted using the same color. The close distribution of data points demonstrate that process flux remained very consistent over multiple process runs.

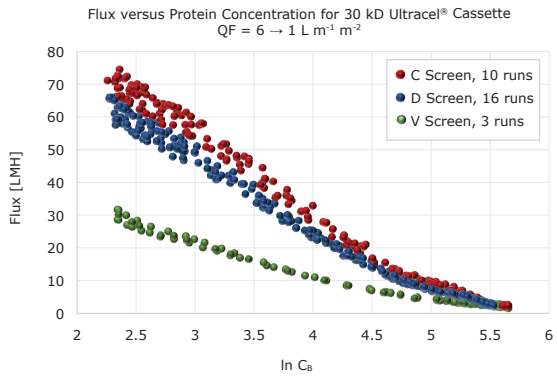

Figure 2
Flux versus Protein Concentration for 30 kD Ultracel® Cassettes with C, D and V Screen

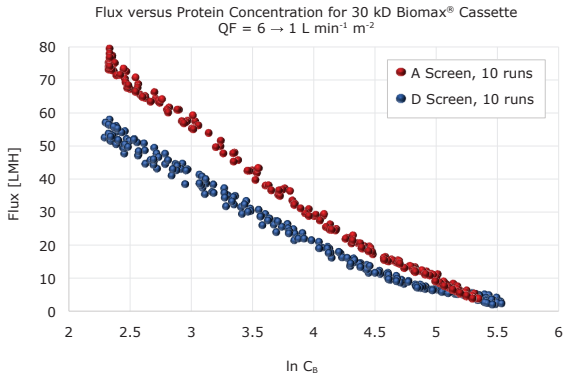

Figure 3
Flux versus Protein Concentration for 30 kD Biomax® Cassettes with A and D Screen

Since flux is related to both protein concentration and mass transfer coefficient through the stagnant film model (Equation 1), by using a natural log scale for the x-axis (protein concentration) on the above plots, we can get a measure of the cassette mass transfer coefficient from the slope of the data within the linear portion of the curve, where a constant feed flow rate was maintained.

$$Flux = k * \ln (C_w/C_b)$$
where k = mass transfer coefficient (L h^{-1} m^{-2})
C_w = protein concentration at the membrane surface
C_b = protein concentration in the bulk solution

Equation 1
Simplified stagnant film model assuming no protein passage to the filtrate

As expected, the slope and overall flux is higher for tighter screens and lower for more open screens. The flux for cassettes with Ultracel® membrane with C and D screen is very similar, within approximately 10%. When comparing process flux between cassettes with Biomax® membrane containing A and D screens, the D screen cassette demonstrates approximately 25% lower flux. As expected, the flux of a cassette with V screen is less than half compared to the flux of C and D screen cassettes with Ultracel® membrane.

Feed channel pressure drop also depends on the screen type installed in the cassette and increases with increasing viscosity and concentration of the protein solution as illustrated in Figures 4 and 5. Significantly lower pressure profiles were observed for more open D screen cassettes with both Ultracel® and Biomax® membranes, enabling them to reach higher final viscosities and protein concentrations than the A and C screen cassettes. This pressure drop difference was much more marked than the difference in mass transfer, indicating a significant window of performance advantage for the D screen devices.

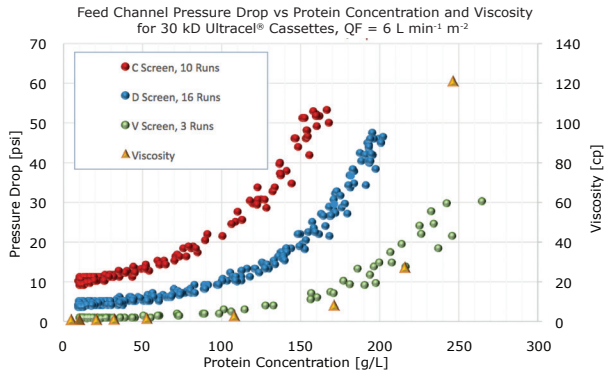

Figure 4
Feed Channel Pressure Drop versus Protein Concentration and Viscosity for Cassettes with 30 kD Ultracel® Membrane with C, D and V Screen

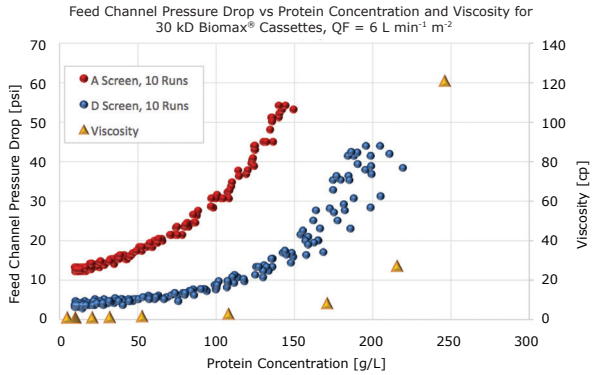

Figure 5
Feed Channel Pressure Drop versus Protein Concentration and Viscosity for Cassettes with 30 kD Biomax® Membrane with A and D Screen

The viscosity versus protein concentration is also plotted on a second y-axis of the above graphs to illustrate how the process limit of the cassettes is associated with increasing viscosity. As viscosity starts to rise sharply, the cassette pressure drop increases to the maximum operational point, triggering the end of the process. Each protein/buffer combination will exhibit a different viscosity versus concentration response, meaning that the absolute protein concentration that is achievable will vary from one protein to the next. However, the trend of increasing viscosity and concentration capability with more open screens will remain consistent.

Process Performance – Mass Transfer, Yield, Final Concentration

Process results from all runs were averaged for each device and are summarized in Table 3. As predicted from the previous graphs, the more open D screen cassettes achieved higher final protein concentration and significantly higher final viscosity as compared to the A and C screen cassettes. Mass transfer was only 10 to 25% lower, indicating that for a given process, only slightly more membrane area would be required for the D screen to achieve an equivalent process time versus the A and C screen cassettes. The Pellicon® 2 cassette with V screen cassette, while reaching the highest concentration and viscosity, had a very low mass transfer, which means that significantly more membrane area would be needed to run a given process in the same time. Excellent yield was observed for all cassettes over multiple uses.

Membrane Type	Screen Type	Mass Transfer Coefficient [LMH]	Final Protein Conc [g/L]	Final Viscosity [cP]	Yield [%]
30 kD Ultracel®	C	24	226	50	103
	D	21	242	104	101
	V	10	277	>200	102
30 kD Biomax®	A	25	200	14	100
	D	19	236	95	98

Table 3
Summary of the Results for Pellicon® 2 and 3 Cassettes with Ultracel® and Biomax® Membrane

Membrane Cleanability and Reuse

Pellicon® 3 cassettes with Biomax® and Ultracel® membranes and the newly designed D screen for high viscosity applications maintain the same cleanability and reusability as the rest of the Pellicon® 3 family. Water permeability was consistently restored to pre-process values after a 60-minute cleaning with room temperature sodium hydroxide (Figures 6 and 7). For the Pellicon® 3 cassette with Biomax® membrane A screen, the concentration of sodium hydroxide was increased from 0.5N to 1N during cleaning cycles 8-10, because at the end of cleaning cycle 7 the permeability recovery dropped below the 80% target that was set for this study. Permeability was consistently restored afterwards. For the Pellicon® 3 cassette with Ultracel® membrane D screen, an additional five runs were performed after the first 10 runs were completed. For these last runs, the cleaning cycle was reduced to a 30-minute recirculation of 0.1N sodium hydroxide. Even with this less rigorous cleaning exposure, water permeability and process performance were successfully maintained.

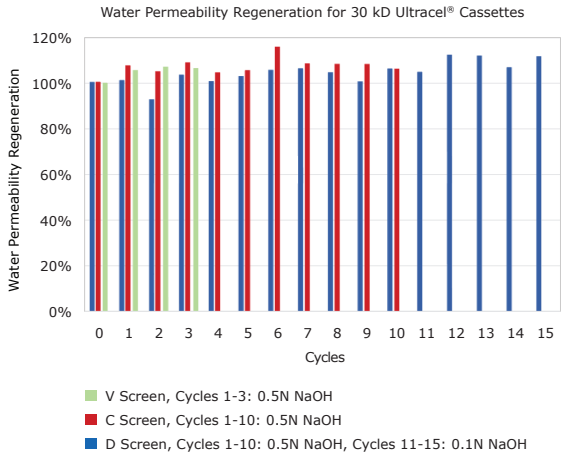

Figure 6
Water Permeability Recovery after Cleaning Cycles for Cassettes with 30 kD Ultracel® Membrane with C, D and V Screen

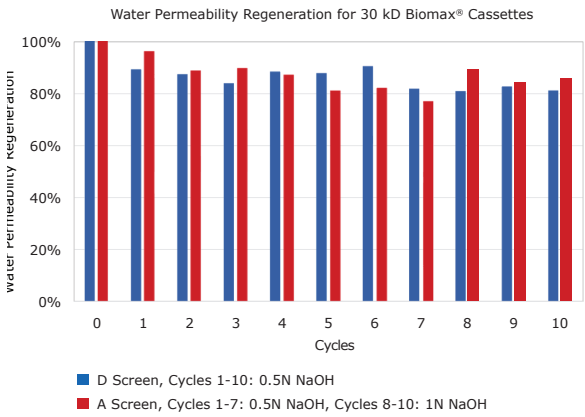

Figure 7
Water Permeability Recovery after Cleaning Cycles for Cassettes with 30 kD Biomax® Membranes with A and D Screen

Carryover Analysis

To evaluate carryover from process to process, an analysis of the TFF system was performed. A Pellicon® 3 cassette with Ultracel® membrane D screen that was previously used in 15 process run/cleaning cycles was installed into the TFF system and an additional process run was performed. Post run, the system was cleaned with 0.1N sodium hydroxide for 30 minutes at room temperature. After flushing the system with water and draining, 200 mL of RO water was added to the recycle tank and recirculated for 10 minutes prior to collecting samples from the recycle tank for TOC, bioburden, endotoxin and total protein analysis. The results are summarized in the following table.

Bioburden [CFU/ml]	Endotoxin [EU/ml]	TOC [ppm]	Total Protein [µg/ml]
< 1	0.013	0.56	below Limit of Detection (LOD) (< 2 µg/ml)

Table 4
Summary of Carryover Analysis of TFF system

The results from all assays show low values, which demonstrates that the TFF system was clean and there is no significant carryover from the cassette.

Summary

This work illustrates process consistency and cleanability of Pellicon® 3 cassettes with both 30 kD Ultracel® and Biomax® membranes over multiple uses. Novel D screen cassettes, designed for high viscosity applications are capable of concentrating protein solutions to significantly higher concentrations and viscosities compared to traditional A and C screen cassettes.

Results demonstrate that two-fold higher viscosity could be achieved with Pellicon® 3 cassettes with Ultracel® membrane D screen compared to Ultracel® C screen cassettes with less than a 10% drop in mass transfer coefficient. Almost seven-fold higher final viscosity was achieved with only 25% lower mass transfer coefficient when comparing the performance of a Pellicon® 3 cassette with 30 kD Biomax® membrane D screen to A screen.

Water permeability was easily restored using room temperature sodium hydroxide. Flux, pressure drop and protein yield were consistent across up to 16 process runs for the entire Pellicon® family.

Carryover analysis demonstrated the cleanliness of the system.

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