Millipore®

Filtration, Separation & Preparation

Pellicon® 3 Cassettes with Biomax® Membrane Performance Guide

The tangential flow filtration device of choice for demanding filtration processes requiring unbeatable performance consistency.

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How to Use the Guide

This Performance Guide is a reference document providing you with assistance in evaluating and validating Pellicon® 3 cassettes with Biomax® membrane for your ultrafiltration solutions. Included in this Performance Guide are general recommendations on various aspects of ultrafiltration to be considered and evaluated by potential users. Several performance studies have been highlighted in order to provide you with a well-rounded overview of the entire Pellicon® 3 family of cassettes with Biomax® membrane.

Results are intended as general examples and are not to be construed as product claims or specifications. The results included in this guide summarize outcomes and observations obtained in the specific application studies with the particular model stream and experimental conditions. Therefore, all test results should be confirmed by the end user while using feed stream and optimized conditions representative of the specific application.

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Introduction

Pellicon® 3 cassettes with Biomax® membrane are the optimum tangential flow filtration (TFF) devices for the ultrafiltration of solutions containing therapeutic proteins, albumin, hormones, vaccines and growth factors. These advanced, high-performance cassettes are ideal for today's higher titer therapeutic antibodies, as well as the more demanding filtration processes that require higher operating pressures, temperatures and caustic cleaning regimes. From small-scale to full-scale production, Pellicon® 3 cassettes with Biomax® membrane are designed for use in research, process scale-up and scale-down, applications development and full-scale manufacturing.
Our Pellicon® 3 cassette design provides unbeatable performance consistency between cassette sizes. Their streamlined construction allows operators to quickly and easily handle, install and remove the Pellicon® 3 cassettes, making your process more efficient, effective and simplified.

Protein Performance Scalability

Objective
The objective of this study was to demonstrate the scalability of Pellicon® 3 cassettes with Biomax® membrane using a model protein stream and an appropriate membrane molecular weight cut-off (MWCO).

Summary
The scalability of Pellicon® 3 cassettes with 30 kD Biomax® membrane and D screen was evaluated using bovine gamma globulin (BGG) in 10 mM phosphate buffered saline solution, pH 7.1. This study considered protein flux performance and mass transfer comparability, at concentrations of BGG from 10 to over 200 g/L.

Pellicon® 3 cassettes with Biomax® membrane D screen demonstrated < 20% difference in limiting flux performance and mean mass transfer coefficient amongst the four sizes, meeting the acceptance criteria for linear scalability.

Figure 1. Pellicon® 3 Cassettes with 30kD Biomax® Membrane D Screen Flux vs TMP - BGG 10 g/L

Flux versus Transmembrane Pressure for Pellicon® 3 cassettes (88 cm², 0.11 m², 0.57 m², 1.14 m²) with Biomax® 30 kD membrane D screen processing a 10 g/L BGG solution

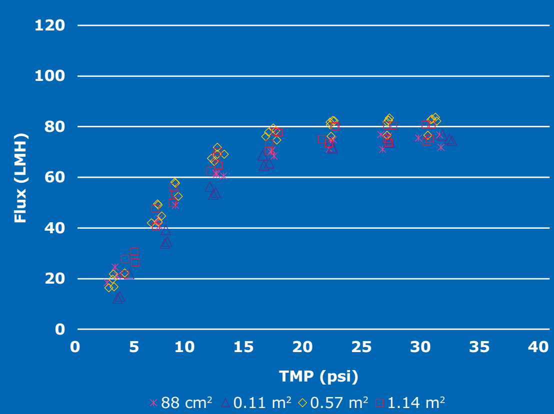
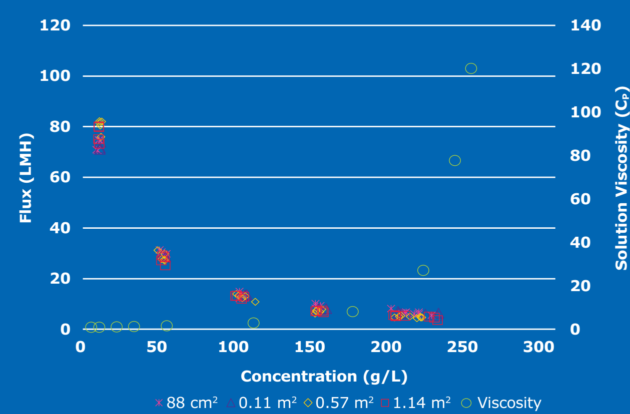


Figure 2. Pellicon® 3 cassettes with Biomax® Membrane D Screen Flux and Viscosity vs Concentration

Flux versus Protein Concentration and Viscosity for the four sizes of Pellicon® 3 cassettes with 30 kD Biomax® Membrane D screen



Method

Scalability was assessed using two methods. The model protein stream was BGG in phosphate buffered saline, pH 7.1. Three Pellicon® 3 cassettes with 30kD Biomax membrane and D screen, from each size, were randomly selected to provide a sample representation of the available cassettes (Table 1).

Catalog No.	Filtration Area	MWCO	Membrane Type	Screen Type
P3B030D00	88 cm²	30 kD	Biomax	D
P3B030D01	0.11 m²	30 kD	Biomax	D
P3B030D05	0.57 m²	30 kD	Biomax	D
P3B030D10	1.14 m²	30 kD	Biomax	D

Table 1. Pellicon® 3 cassette sizes used in experiments.

The first method included evaluation of process flux versus transmembrane pressure (TMP) excursion curves at different initial BGG feed concentrations: 10, 50, 100, 150 and 200 g/L, for each cassette size. The feed flow rate was maintained constant at 6 L/min/m² throughout the TMP excursions.

The second method included evaluation of process flux and viscosity as functions of protein concentration and analysis of mass transfer coefficient for each cassette size.

In order to verify scalability, the acceptance criteria requires the population means for the tested Pellicon® 3 cassette sizes (88 cm², 0.11 m², 0.57 m², 1.14 m²) to have less than 20% difference across scales.

Results

Figure 1 represents an exemplary performance of process flux versus TMP, with a 10 g/L BGG feed solution.

The variation throughout the TMP excursion curves among the sizes is within 20%, in both the non-polarized and polarized regions of the curves, which demonstrates excellent scalability within the Pellicon® 3 cassette family. The data for BGG feed solutions of 50, 100, 150 and 200 g/L is not shown, but demonstrates similar trends.

Figure 2 illustrates the effect of different BGG feed concentrations (10, 50, 100, 150 and 200 g/L) on the filtrate flux in the TFF process. As the concentration of the target protein increases, the limiting flux decreases, as expected by TFF theory. Protein viscosity was also included on the second y-axis in order to demonstrate the processing capability of the cassettes. The viscosity increases with increase in protein concentration in solution, and sharply increases when the filtrate flux in all four size cassettes decreases to the maximum operational limit.

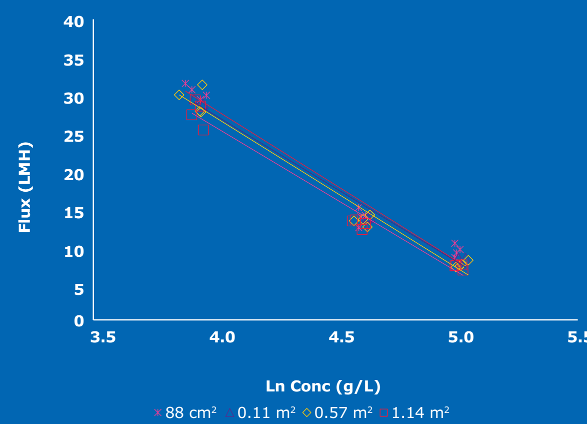
Equation 1. Simplified stagnant film model assuming no protein passage to the filtrate ($C_p = 0$)

$$J = k \ln\left(\frac{C_w - C_p}{C_b - C_p}\right) \approx k \ln\left(\frac{C_w}{C_b}\right)$$

Where k = mass transfer coefficient (L/h/m²)
C_w = protein concentration at the membrane surface
C_b = protein concentration in the bulk solution
J = filtrate flux (L/h/m²)

Figure 3. Pellicon® 3 Cassettes with 30kD Biomax® Membrane D Screen Mass Transfer Study

Flux versus natural log of protein concentration



Mass Transfer Analysis

While analysis of the overall flux performance demonstrates the overall performance of cassettes, it is necessary to consider the physics active at the protein level in order to calculate cassette mass transfer coefficients. The mass transfer coefficient reflects the protein flux from the polarized layer back into the bulk solution. As the transmembrane pressure promotes a build-up of concentration on the membrane wall, osmotic pressure arising from differences in concentration drives the protein back to the bulk fluid. Comparable and scalable TFF cassettes should have similar mass transfer coefficients. The acceptance criteria require the population means for Pellicon® 3 cassettes to have less than 20% difference across device scales.

The Pellicon® 3 cassettes mass transfer coefficients can be determined using the stagnant film model. Given that the filtrate flux (J) is related to both protein concentration (C_b) and mass transfer coefficient (k) through the stagnant film model, by plotting the filtrate flux (J) versus the natural log scale of the protein concentration, the mass transfer coefficient (k) can be empirically measured from the slope of the data within the linear portion of the curve in Figure 2 (i.e., at 50 g/L, 100 g/L and 150 g/L), where a constant feed flow rate was maintained.

The flux vs. the natural log of BGG concentration in solution is shown in Figure 3 for each size; 88 cm², 0.11 m², 0.57 m² and 1.14 m². As the concentration of the target protein increases, the flux decreases linearly with the natural log of the concentration. A best fitted regression line was determined for each cassette. The calculated slopes of the linear curves was then averaged between the number of cassettes tested for each size, where the slope of the linear curves represents the mean mass transfer coefficient (Table 2).

Cassette Type	Cassette Size	Ave Mass Transfer [LMH]
Pellicon® 3 30 kD Biomax® Membrane, D Screen	88 cm²	20.2
	0.11 m²	19.5
	0.57 m²	20.1
	1.14 m²	19.3

Table 2. Mass Transfer Coefficients for each size Pellicon® 3 cassette

Individual cassettes of the same size show very comparable performance. In addition, the mass transfers across the full family of cassettes sizes are well within the 20% target for linear scalability.

Conclusion

Pellicon® 3 cassettes with Biomax® membrane D screen demonstrated exceptional scalability with bovine gamma globulin as a model protein feed stream. Consistent mass transfer coefficients and limiting flux were observed throughout the experiments performed with four different Pellicon® 3 cassette sizes and the data variability was within the acceptance criteria of 20%.

Cleaning Analysis of Pellicon® 3 Cassettes

Objective
The objective of this study was to demonstrate cleanability and evaluate the performance consistency of Pellicon® 3 cassettes with Biomax® membrane throughout a multiple-run application.

Summary
Pellicon® 3 cassettes with 30 kD Biomax® 0.11m² membrane A and D screens were tested to demonstrate cleanability and performance consistency during multiple process runs. Room temperature sodium hydroxide solution was effective at restoring water permeability, providing consistent process flux, yield and pressure drop throughout ten runs.

Figure 1. System Schematic

Schematic of System used in each process run

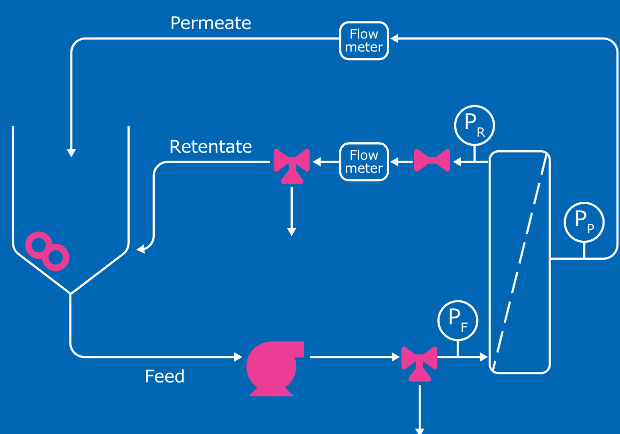
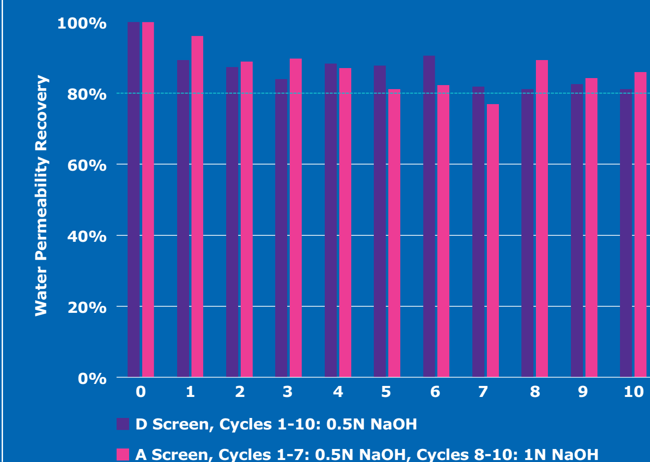


Figure 2. Water Permeability Recovery for Pellicon® 3 with 30kD Biomax® Membrane A and D Screens

Water Permeability Recovery before (cycle 0) and after Cleaning (cycles 1-10), comparing Pellicon® 3 Cassettes with 30 kD Biomax® Membrane with A and D Screen



Method

A Pellicon® 3 cassette with 30 kD Biomax® 0.11m² membrane containing the A screen and an additional Pellicon® 3 cassette containing the D screen were used in this study. Both Pellicon® 3 cassettes were challenged with bovine gamma globulin (BGG) solution formulated in 10 mM phosphate buffered saline, pH 7.2. The BGG was concentrated from the initial 10 g/L to the maximum achievable concentration for each cassette. The feed flow rate was set to 6 L/min/m² and the retentate pressure was maintained at 10 psi or above to avoid backflow from the permeate side. The feed flow was gradually decreased towards the end of the process run to avoid exceeding the maximum pressure rating of the system due to increasing viscosity of the protein solution. Ten sequential process cycles were performed for each cassette. Figure 1 illustrates the system setup used in each process run for each cassette.

A cleaning cycle was performed in between each of the ten process cycles by initially recirculating 0.5N sodium hydroxide at room temperature for 1 hour, along with appropriate water flushes. For the cleaning cycle, the feed flow rate was set to 6 L/min/m² and the retentate pressure to approximately 5 psi. The Normalized Water Permeability (NWP) of the cassettes was calculated after each cleaning. The cassettes were then stored in 0.1N NaOH after determining the NWP and prior to the subsequent process run.

For the purpose of this test, the target for water permeability recovery was set to 80% or above. In any application a comparison of NWP and process flux can be made to determine an appropriate NWP% recovery which will be indicative of cleanliness and ensure consistent process performance. The values of water permeability after cleaning were compared to the initial water permeability of the cassette, which is the permeability measured after flushing and sanitization but prior to use with protein.

The performance consistency of the cassettes was evaluated throughout the ten process runs by evaluating the mass transfer coefficient, the maximum achievable concentration of BGG and yield.

Figure 3. Flux versus Natural Log of Protein Concentration for Pellicon® 3 Cassettes Biomax® 30kD Membranes, A and D Screens

Process Flux Consistency for Pellicon® 3 Cassettes with Biomax® Membrane Containing A and D Screen

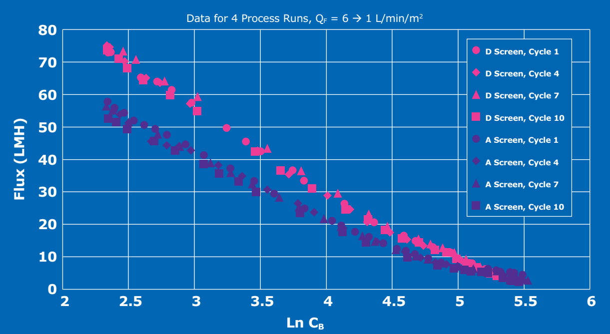
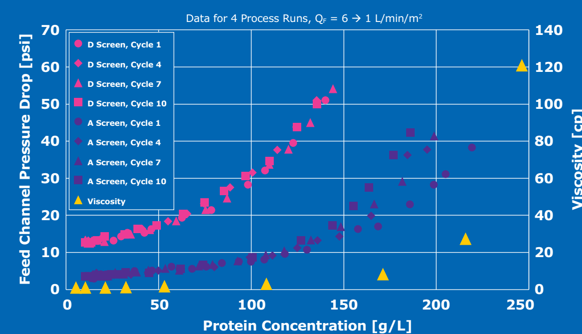


Figure 4. Feed Channel Pressure Drop versus Protein Concentration for Pellicon® 3 Cassettes Biomax® 30kD Membranes, A and D Screens

Feed Channel Pressure Drop versus Protein Concentration in Pellicon® 3 Cassettes with Biomax® 30 kD Membrane Containing A and D Screen



Results

Figure 2 illustrates the water permeability recovery of A and D screen containing Pellicon® 3 cassettes after each cleaning.

The Pellicon® 3 cassette containing D screen was cleaned using 0.5N sodium hydroxide at room temperature for 1 hour for each of the 10 cycles. Figure 2 shows that the water permeability dropped to 90% of the initial water permeability after the first process run and was consistently restored to within the of 80-90% of the initial water permeability during 10 cleaning cycles.

The Pellicon® 3 cassette containing A screen was cleaned by initially recirculating 0.5N sodium hydroxide at room temperature for 1 hour during the first 7 cycles. At the end of the seventh cleaning cycle, the water permeability was recovered to 77% of the initial water permeability, which is below the targeted 80% minimum acceptable limit. Despite the fact that water permeability increased to 83% during overnight storage in 0.1N sodium hydroxide, the concentration of sodium hydroxide was increased to 1N for cleaning cycles 8 through 10. Overall, water permeability restoration throughout the 10 cycles was in the range of 80-96% of the initial water permeability.

The performance consistency of the cassettes was evaluated throughout the ten process runs concentrating a BGG solution from 10 g/L to the maximum achievable concentration for each screen. Figure 3 illustrates flux results as a function of the natural log of protein concentration in solution for cycles 1, 4, 7 and 10, for clarify of the results. The cassettes demonstrated excellent process reproducibility over multiple uses. Cycles 2, 3, 5, 6, 8 and 9 are not shown but demonstrate a similar trend.

Feed channel pressure drop depends on the screen type installed in the cassette. It increases as the feed flow rate, viscosity and protein concentration increase in solution.

Results of process runs 1, 4, 7 and 10 in Figure 4 illustrate that the maximum solution viscosity that can be reached at a feed flow of 6 L/min/m² is approximately 6 cP, which equates to a protein concentration of approximately 140 g/L of BGG in solution. Higher protein concentration can be achieved without exceeding the pressure limits of the system by decreasing the feed flow rate. The flow rate was gradually reduced from 6 L/min/m² to a minimum of 1 L/min/m² before ending the process to achieve the data shown in Table 1.

Mass Transfer Coefficient, final achievable BGG concentration, and protein yield for each of the ten process runs and screen types are listed in Table 1, confirming that the Pellicon® 3 cassette with 30kD Biomax® 0.11m² membrane, containing either A or D screen can be effectively cleaned to provide consistent process performance.

The Pellicon® 3 cassette containing D screen, designed for high viscosity applications, achieved final BGG concentration >200 g/L, demonstrating the D screen's ability of concentrating protein solutions to higher concentrations compared to the A screen cassette. Excellent yield was observed for both cassettes over multiple uses.

Cycle	Mass Transfer Coefficient, k [LMH]		Final BGG Concentration [g/L]		Yield [%]	
	D Screen	A Screen	D Screen	A Screen	D Screen	A Screen
1	20	25	237.2	204.2	90.40%	101.10%
2	18	26	224.9	203.5	91.02%	98.33%
3	20	26	245.8	203.9	99.89%	100.54%
4	18	25	246.8	199.3	95.41%	101.19%
5	18	25	253.0	195.5	100.69%	99.10%
6	17	25	240.8	200.5	97.31%	99.12%
7	19	25	249.4	205.2	100.06%	100.66%
8	19	25	228.2	197.9	95.64%	99.73%
9	20	25	232.4	195.9	91.51%	99.42%
10	19	25	229.5	198.5	97.08%	99.34%

Table 1. Mass Transfer Coefficient, Final Protein Concentration and Protein Yield of Pellicon® 3 cassette with Biomax® 30 kD membrane A and D screen, throughout the ten process runs

Conclusions

This work illustrates process consistency and cleanability of Pellicon® 3 cassettes with Biomax® 30 kD membrane 0.11m², containing A and D screen over multiple uses. Water permeability was consistently restored to pre-process conditions using room temperature sodium hydroxide. Flux, pressure drop, mass transfer and protein yield were consistent throughout 10 process runs.

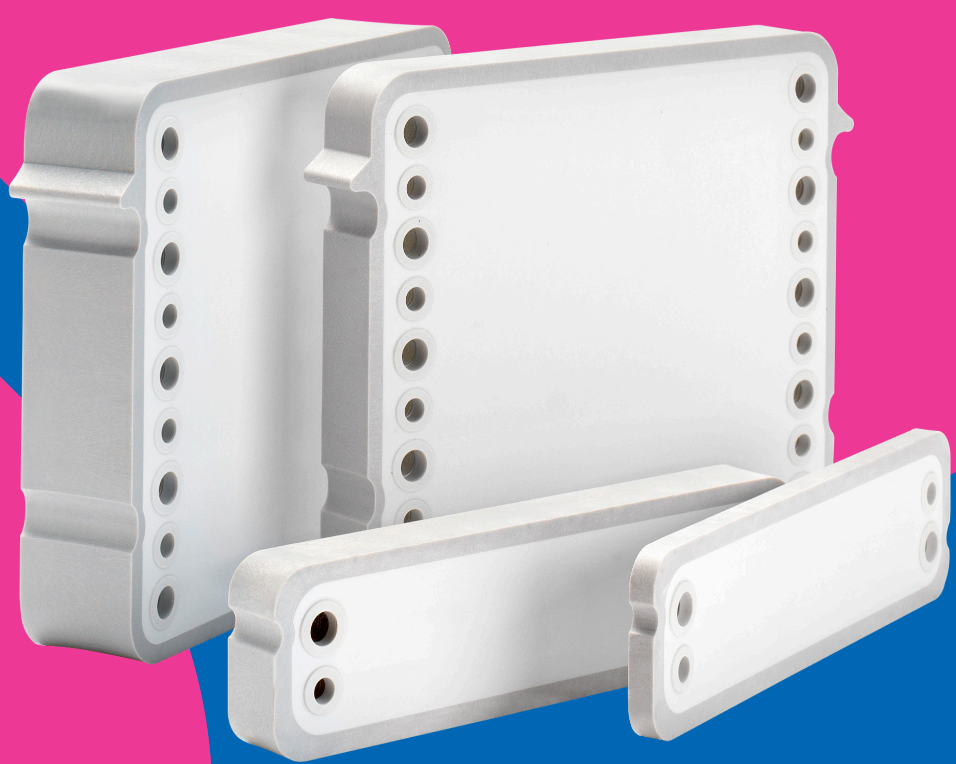
Hold Up Volume of Cassettes

Objective

Determine the hold up volumes of the A and D screen, feed and permeate channels in Pellicon® 3 cassettes with Biomax® membrane.

Summary

Hold up volume was measured on Pellicon® 3 cassettes with 30 kD Biomax® membrane A and D feed screens. Four cassettes containing A screen of each size, 88 cm^2, 0.11 m^2, 0.57 m^2 and 1.14 m^2, as well as four cassettes containing D screen of each size, 88 cm^2, 0.11 m^2, 0.57 m^2 and 1.14 m^2, were evaluated.



Method

Pellicon® 3 cassette holders were used for the experiments.

Method for Flushing Cassettes

1. Each tested cassette was torqued in the appropriate holders according to our specifications; 190 in-lb for the 88 cm^2 and 0.11 m^2 sizes and 350 in-lb for the 0.57 m^2 and 1.14 m^2 sizes.
2. The system was configured to run in Single Pass Mode with retentate and permeate control valves fully open and directed to drain in order to flush out the storage solution.
3. Water was introduced into the feed port of the cassette assembly and flow was increased to achieve a feed flow rate of approximately 5 $L/min/m^2$.
4. While feed flow rate was maintained, retentate flow was restricted to achieve a conversion rate (Permeate Flow/Feed Flow*100) of 30% to 40%.
5. The cassette was flushed with a minimum feed volume of 60 L/m^2.

Method for Determining Hold Up Volumes

6. After the cassette was properly flushed, the retentate valve was opened to reduce the pressure and the water flow to the feed port was stopped.
7. The cassette was removed from the holder and water proof tape was placed over the end cap seals (feed, retentate and permeate) on one side of the cassette.
8. The cassette was returned to the holder with taped side against the holder end plate.
9. RO water was circulated through the cassette for 10 minutes at feed pressure of 20 psi, retentate pressure of 15 psi and permeate pressure of 10 psi.
10. The pump was turned off and the cassette was removed from the holder, being careful to orient the cassette and holder so that water inside device was not lost. The cassette was weighed and the cassette weight was recorded as Initial Wet Cassette Weight.
11. The cassette was returned to the holder with taped side of cassette against the holder end plate, being careful to orient cassette and holder so that water inside device was not lost.
12. Feed and retentate valves were opened and the permeate valve was closed. Compressed air was blown down the feed channel at 10 psi for 3 minutes.
13. The cassette was removed from the holder, being careful to orient the cassette and holder so that water inside device was not lost. The cassette was weighed and the cassette weight was recorded as Post Feed Channel Blow Down Weight.
14. The cassette was returned to the holder with taped side of cassette against the holder end plate, being careful to orient cassette and holder so that water inside device was not lost.
15. The permeate valve was opened. Compressed air was blown down the permeate channel at 10 psi for 3 minutes.
16. The cassette was removed from the holder, being careful to orient cassette and holder so that water inside device was not lost. The cassette was weighed and the cassette weight was recorded as Post Permeate Channel Blow Down Weight.
17. The cassette was returned to the holder with taped side of cassette against the holder end plate. Feed, retentate and permeate valves were opened, and compressed air was blown through the cassette at 10 psi for ≥ 12 hours.
18. The cassette was removed from the holder. The cassette was weighed and the cassette weight was recorded as Final Dry Device Weight. The final blow down procedure was repeated and the weight of the cassette at Initial and Final Dry Device Weight was compared in order to ensure that the cassette was totally dry.

Results

All weights were converted to volumes as shown in Table 1, assuming that one gram of water equals one milliliter of water.

1 g H20 = 1 mL H20

The weight of the post feed channel blow down cassette was subtracted from the weight of the completely filled initial wet weight cassette to calculate the hold up volume of the feed channel. Similarly, the weight of the post permeate channel blow down cassette was subtracted from the weight of the post feed channel blow cassette to calculate the hold up volume of the permeate channel.

Hold Up Volume of the Feed Channel = Initial Wet Cassette Weight - Post Feed Channel Blow Down Weight

Hold Up Volume of Permeate Channel= Post Feed Channel Blow Down Weight - Post Permeate Channel Blow Down Weight

The volumes for all measurements for each cassette size and screen were averaged and are presented below in Table 1.

Area	Biomax® A Screen Feed Channel (ml)	Biomax® D Screen Feed Channel (ml)	Biomax® Approximate Permeate Channel (ml)
88 cm^2	1.8	3	2.8
0.11 m^2	9	23	7
0.57 m^2	69	127	39
1.14 m^2	134	229	88

Table 1. Hold Up Volumes of Feed and Permeate Channels of Pellicon® 3 cassettes with Biomax® membrane A and D screens

Conclusion

Hold up volumes in the A and D screen feed and permeate channels of Pellicon® 3 cassettes with Biomax® membrane are presented in Table 1 in order to help the user determine minimum working volumes for their systems.

Pellicon® 3 Cassette Material Compatibility

Objective

To characterize the change in hardness and weight of certain materials of construction used in Pellicon® 3 cassettes, excluding the Biomax® membrane, after exposure to various chemical solutions that could be used in TFF processes.

Summary

Certain materials of construction of Pellicon® 3 cassettes, excluding the Biomax® membrane, were selected according to their compatibility with a wide range of chemicals. The materials were evaluated for hardness, weight and color change upon exposure to a variety of chemicals.

Method

Table 1 outlines the selected materials within Pellicon® 3 cassettes used in this chemical compatibility study. Four samples of each material were injection molded at identical molding conditions that are used in the fabrication of the Pellicon® 3 cassettes.

Item	Material Description
Feed and Permeate border/seal	Linear low density polyethylene (LLPE)
End Cap	Polypropylene
Jacket	Polypropylene
End Cap Seal Material	Thermoplastic elastomer (TPE)
Feed Channel Screens	Polypropylene

Table 1. Components of the Pellicon® 3 cassette and material description

Table 2 outlines the chemicals and conditions used for all soaks.

Soak Solution	Time	Temperature
1% Acetic Acid/1.2% Phosphoric Acid/ $H_2$0	1200 hrs	45°C
1% Triton® X-100/ $H_2$0	1200 hrs	45°C
1 N NaOH	1200 hrs	45°C
1000 ppm NaOCl/ H20	1200 hrs	45°C
70% IPA/ H20	1200 hrs	Ambient
40% EtOH/ H20	1200 hrs	Ambient
Control-open to air	1200 hrs	Ambient

Table 2. Chemical solution and conditions

Each sample was weighed, visually assessed for color and appearance and tested for durometer hardness before each chemical exposure. After completing every chemical soak, each sample was removed, rinsed, and then allowed to completely air dry before the hardness, mass and color analysis was repeated.

Durometer hardness was assessed using a Shore Hardness gage that measures the depth of an indentation in the material created by a given force on a standardized presser foot. This depth is dependent on the hardness of the material, its viscoelastic properties, the shape of the presser foot and the duration of the test. The polypropylene and polyethylene samples were assessed using the Shore D Scale; the thermoplastic elastomer sample was assessed using the Shore A Scale.

*Calculations

Change in Mass

The change in mass for the materials exposed to each chemical soak was calculated as follows:

1. For each individual sample:

$$\Delta m = \left(\frac{m_f - m_i}{m_i}\right) * 100$$

Where: Δm: change in sample mass [%]
m_f: weight of sample after the chemical soak
m_i: weight of sample before the chemical soak

2. Then the four samples in each material were assessed for a given chemical.

Change in Hardness

The average durometer change (hardness) of the samples tested was calculated as follows:

1. For each individual sample:

$$\Delta h = \left(\frac{h_f - h_i}{h_i}\right) * 100$$

Where: Δh: change in sample mass [%]
h_f: hardness of sample after the chemical soak
h_i: hardness of sample before the chemical soak

2. Then the four samples in each material were assessed for a given chemical.

Figure 1. Cassette Material Change in Mass after Chemical Soaks

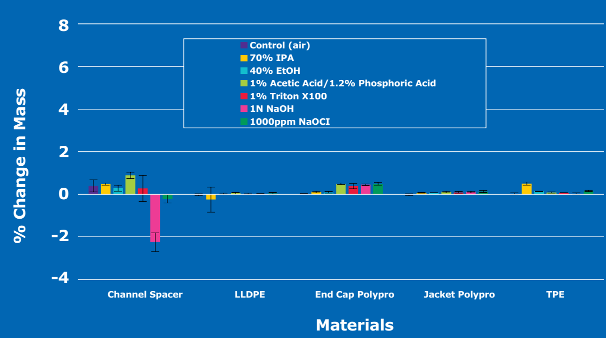
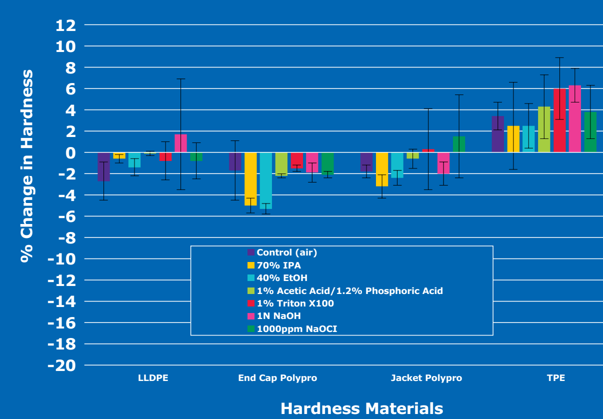


Figure 2. Cassette Material Change in Hardness after Chemical Soaks



Results

Mass

Figure 1 displays the results for the change in mass of material after the chemical soaks. A negative value indicates loss of mass during chemical exposure, while a positive value indicates gain in mass.

Hardness

Figure 2 displays the change in hardness for each material. A negative value indicates that the material got softer during chemical exposure, while a positive value indicates that the material got harder.

* Hardness assessment could not be performed on the Feed Channel Screens as the Durometer test method requires a solid material sample.

Color and Physical Appearance

Table 3 illustrates visual changes that were observed in some of the tested materials of construction. The table lists the results only for materials/chemicals combinations for which a change in appearance was observed.

Materials	Chemicals	
	1N NaOH	1000 ppm NaOCl
Feed Channel Screen Polypropylene	Translucent, curl	Translucent, curled
LLDPE	Yellow tint after soak	Yellow tint and dull after soak
End Cap Propylene	Dull surface after soak	none
Jacket Propylene	Shiny surface after soak	none
TPE	Yellow tint on edges after soak	Yellow tint on edges after soak

Table 3. Color and physical changes in materials observed after chemical soaks

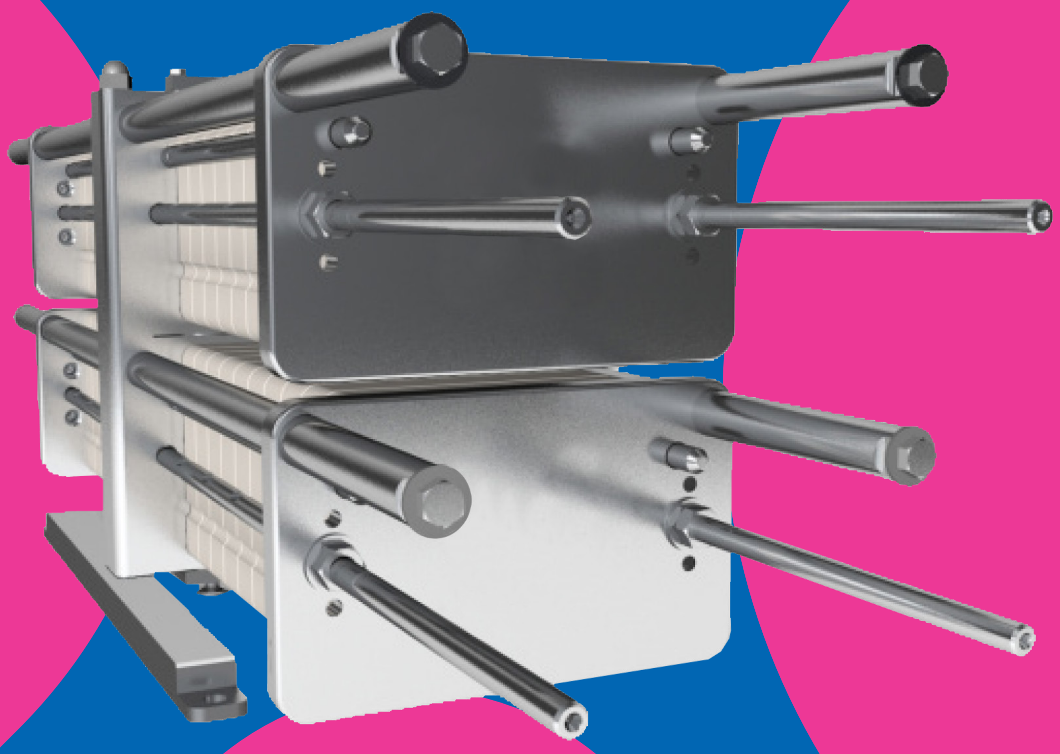
Conclusion

All tested Pellicon® 3 cassette materials, excluding the Biomax® membrane, displayed a change in mass that was 1% or less. The change in hardness was less than 6.5% in all cases. However, it is important to note that the control sample demonstrated approximately ±3% variability in hardness. Most of the color/physical changes were seen in sodium hydroxide (NaOH) and hypochlorite (NaOCl) soaks. In the case of Pellicon® 3 cassette materials, either the samples were left with a yellowish tint, or the surface changed from a shiny to dull or vice versa. These overall changes in mass and hardness are considered minimal and should not affect cassette performance.

Effect of Holder Compression Sensitivity on Air Integrity

Objective

Determine the effect of holder compression on the integrity of Pellicon® 3 Cassettes with Biomax® membrane A and D screens.



Introduction

In this study, the effect of holder compression was measured on the Pellicon® 3 cassettes, listed in Table 1.

Screen Type	Membrane Area	Catalog Number
A	88 cm^2	P3B030A00
	0.11 m^2	P3B030A01
	0.57 m^2	P3B030A05
	1.14 m^2	P3B030A10
D	88 cm^2	P3B030D00
	0.11 m^2	P3B030D01
	0.57 m^2	P3B030D05
	1.14 m^2	P3B030D10

Table 1. Pellicon® 3 cassettes with Biomax® Membrane A and D screens

Table 2 outlines the recommended torque range for each size Pellicon® 3 cassette, along with the torque values which were evaluated in this study. The tested torque values ranged below and above the recommended range for each cassette size to evaluate the integrity of the system (cassette and holder) under a wide range of compression. Table 2 also outlines the corresponding force for each torque value, which was calculated using the equation below:

$$\text{Force} = \frac{\text{Torque}}{(\text{Coefficient of friction})(\text{nominal major thread diameter})}$$

Equation 1. Equation for calculating the force for every torque value tested in this study

Potential effects of compression force on cassettes are:

- Loss of internal and external sealing at lower compression than recommended
- Increased pressure drop, reduced permeability, and eventual membrane damage at compression higher than recommended

88 cm^2 Cassette	
Recommended Torque = 180-200 in-lbs	
Holder Torque (in-lb)	Total Equivalent Force (lbf)
100	2667
190	5067
285	7600
0.11 m^2 Cassette	
Recommended Torque = 180-200 in-lbs	
Holder Torque (in-lb)	**Total Force (lbf)**
150	4000
200	5334
250	6667
0.57 m^2 Cassette	
Recommended Torque = 350-400 in-lbs	
Holder Torque (in-lb)	**Total Force (lbf)**
175	5600
350	11200
525	16800
1.14 m^2 Cassette	
Recommended Torque = 350-400 in-lbs	
Holder Torque (in-lb)	**Total Force (lbf)**
265	8480
350	11200
525	16800

Table 2. Torque Recommendations and Measurements for Pellicon® 3 devices

Method

Method for evaluating the effect of torque measuring on Pellicon® 3 cassettes with Biomax® membrane D Screen:

1. Three of each cassette size were tested across a range of holder torques.
2. Cassettes were installed into manually torqued holders and the storage solution was flushed out with RO water.
3. Cassettes were subjected to air integrity testing to determine the effect of holder compression on air integrity.
4. With the permeate side open to drain, air flow was measured on the upstream side of a pressurized cassette, using an air flow transducer. Test pressures of 30 and 100 psi were evaluated with air flow rate recorded at each pressure, after allowing one minute for flow stabilization.
5. The experimental procedure was repeated twice more at increasing torque to test the effect of different torque values on air integrity.

Method for evaluating the effects of torque measuring on Pellicon® 3 cassettes with Biomax® membrane A Screen:

1. Two of each cassette size were tested across a range of holder torques.
2. Pellicon® 3 cassettes sizes 88 cm^2 and 0.11 m^2 were installed in a manual holder, where the holder compression was measured in torque. Pellicon® 3 cassettes sizes 0.57 and 1.14 m^2 were installed in a hydraulic holder, where the compression was measured in force. Storage solution was flushed out with RO water.
3. Cassettes were subjected to air integrity testing to determine the effect of holder compression on seal integrity.
4. With the permeate side open to drain, air flow was measured on the upstream side of a pressurized cassette, using an air flow transducer. A test pressure of 30 and 100 psi were evaluated, with air flow rate recorded at the noted pressure, after allowing one minute for flow stabilization.
5. The experimental procedure was repeated twice more at increasing torque values for cassette sizes 88 cm^2 and 0.11 m^2, and at increasing force values for cassette sizes 0.57 m^2 and 1.14 m^2.

Results

The results of integrity by air diffusion as a function of manual torque for the Pellicon® 3 cassettes with Biomax® membrane D screen are shown in Table 3. Three cassettes were air integrity tested at 30 psi and 100 psi at three different holder torque values (in-lb). Air integrity is consistent across the three tested cassettes (#1, #2, #3) well within passing specifications for all tested torque values.

	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi			Air Integrity (cc/min) at 100 psi		
P3B030D00 88 cm^2		#1	#2	#3	#1	#2	#3
	100	0.0	0.4	0.4	0.0	0.8	1.2
	190	0.1	1.5	0.1	0.5	3.0	1.5
	285	0.1	0.5	0.4	0.5	1.4	2.4
Recommended Specification	180-200	≤7			≤500		
	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi			Air Integrity (cc/min) at 100 psi		
P3B030D01 0.11 m^2		#1	#2	#3	#1	#2	#3
	100	2.0	2.0	2.0	5.0	6.0	6.0
	190	1.0	1.0	1.0	5.0	6.0	7.0
	285	2.0	2.0	1.0	5.0	6.0	6.0
Recommended Specification	180-200	≤20			≤133		
	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi			Air Integrity (cc/min) at 100 psi		
P3B030D05 0.57 m^2		#1	#2	#3	#1	#2	#3
	175	7.6	8.0	8.0	36.0	46.0	46.0
	350	6.6	7.0	8.0	36.0	40.0	46.0
	525	7.0	6.0	8.0	36.0	46.0	46.0
Recommended Specification	350-400	≤50			≤333		
	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi			Air Integrity (cc/min) at 100 psi		
P3B030D10 1.14 m^2		#1	#2	#3	#1	#2	#3
	265	18.0	15.0	18.0	96.0	88.0	100.0
	350	12.0	11.0	10.0	96.0	86.0	100.0
	525	15.0	15.0	14.0	90.0	84.0	100.0
Recommended Specification	350-400	≤75			≤500		

Table 3 Effects of holder compression on air integrity at 30 and 100 psi, for Pellicon® 3 Cassettes with Biomax® Membrane D Screen

The results of air integrity as a function of manual torque with the 88 cm² and 0.11 m² sizes and the results of air integrity as a function of hydraulic force with the 0.57 m² and 1.14 m² sizes of Pellicon® 3 cassettes with Biomax® A screen are shown in Table 4. Two cassettes of each size were air integrity tested at pressures of 30 and 100 psi and at three different holder compression values (in-lb and lbf). Air integrity was also consistent across the tested cassettes (# 1, # 2) and well within passing specifications.

	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi		Air Integrity (cc/min) at 100 psi	
P3B030A00 88 cm²		#1	#2	#1	#2
	150	0.0	0.1	n/a	n/a
	190	0.1	0.2	n/a	n/a
	230	0.1	0.1	n/a	n/a
Recommended Specification	180-200	≤7		≤500	
	Holder Torque (in-lb)	Air Integrity (cc/min) at 30 psi		Air Integrity (cc/min) at 100 psi	
P3B030A01 0.11 m²		#1	#2	#1	#2
	140	2.0	1.4	11.0	7.0
	170	2.7	1.9	11.0	7.1
	00	1.6	1.4	9.4	7.1
Recommended Specification	180-200	≤20		≤133	
	Holder Torque (lbf)	Air Integrity (cc/min) at 30 psi		Air Integrity (cc/min) at 100 psi	
P3B030A05 0.57 m²		#1	#2	#1	#2
	8230	4.0	5.0	18.0	25.0
	10730	8.0	7.0	29.0	31.0
	13230	8.0	7.0	29.0	30.0
Recommended Specification	10,000	≤50		≤333	
	Holder Torque (lbf)	Air Integrity (cc/min) at 30 psi		Air Integrity (cc/min) at 100 psi	
P3B030A10 1.14 m²		#1	#2	#1	#2
	8230	18.8	15.8	75.0	55.0
	10730	19.9	17.0	76.0	57.0
	13230	16.1	18.4	75.0	55.0
Recommended Specification	10,000	≤75		≤500	

Table 4. Effects of holder compression on air integrity at 30 and 100 psi, for Pellicon® 3 Cassettes with Biomax® Membrane A Screen.

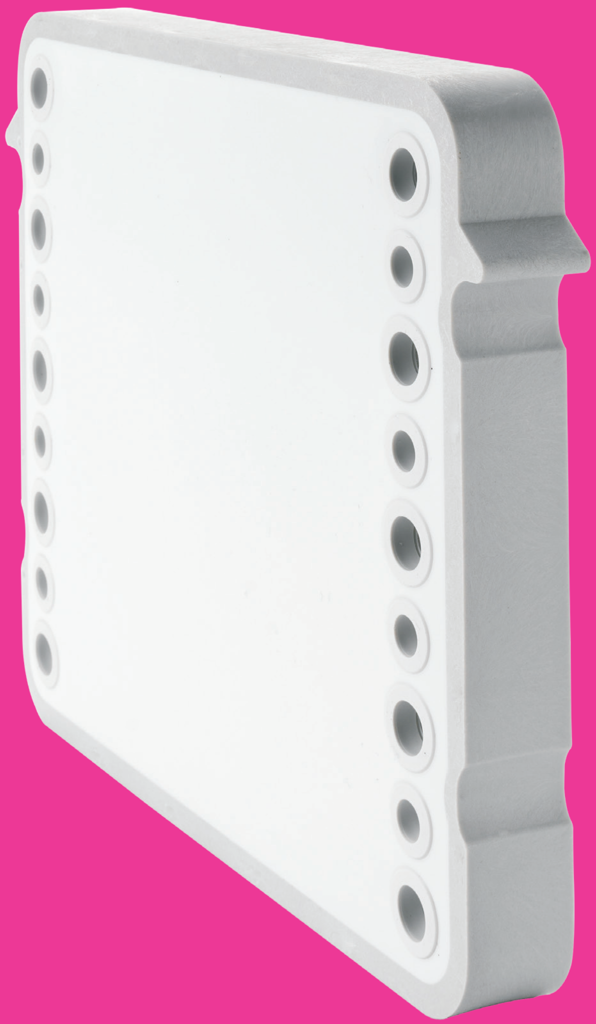
Conclusions

The manual and hydraulic compression results show insignificant change in integrity air flow with increasing compression, demonstrating no membrane damage occurring within the compression range that was investigated during the experiments. In addition, there was no significant change in air flow with decreased compression, demonstrating no venting of internal or external seals occurring within the compression range that was investigated during testing.

Pellicon® 3 cassettes passed the Certificate of Quality release specifications for all cassettes tested at all compressions. Pellicon® 3 cassettes with Biomax® membrane D screen are insensitive to manual compression on the cassettes within the compression range that was investigated. Pellicon® 3 cassettes with Biomax® membrane A screen are insensitive to manual and hydraulic compression on the devices within the compression range that was investigated.

Flushing

Objective
To determine storage solution residuals in Pellicon® 3 cassettes with Biomax® membrane shipped in acetic/phosphoric acid solution after new cassette flushing and cleaning.



Summary
Pellicon® 3 cassettes with Biomax® membrane A and D screens are packaged in a storage solution containing 1.1% acetic acid/1.6% phosphoric acid, 20% glycerin and RO Water. Experiments were performed to evaluate levels of storage solution residuals from cassettes, after cleaning and processing.

The procedures used in this evaluation are intended to imitate standard industry TFF processing conditions and include evaluation of residuals after new cassette flushing and cleaning, as well as evaluation of residuals after diafiltration and ultrafiltration processing. Evaluations are based on measurement of remaining storage solution residuals during an extended soak of mock final product pool. Results from this study are applicable to Pellicon® 3 cassettes with Biomax® membrane A and D Screens.

Method

Flushing experiments were run on four Pellicon® 3 (0.57 m^2) cassettes with Biomax® 10 kD membrane A screen (catalog number P3B010A05). Total membrane area of the four cassettes was 2.28 m^2.

Prior to initiating the new cassette preparation procedure, the system without a cassette was cleaned with 0.1 N NaOH to establish a system cleanliness baseline. Analysis of the system flush was recorded in the results section in Table 1.

New Cassette Preparation Procedure:

1. Initial FLUSH with 20 L/m^2 of RO water single pass
2. CLEANING with 10 L/m^2 of 0.1 N NaOH total recirculation for 30 minutes
3. FLUSH 0.1 N NaOH with 20 L/m^2 of RO water single pass
4. SANITIZATION with 10 L/m^2 of 1.4% Minncare total recirculation for 30 minutes
5. FLUSH 1.4% Minncare with 20 L/m^2 of RO water single pass

After flushing, cleaning, sanitization and prior to starting the mock process, 2 L/m^2 of RO was added to the system and run in total recirculation for 30 minutes. At this point a sample was taken from the recycle tank to determine the residual levels post new cassette preparation. Analysis of the post new cassette preparation was recorded in the results section of Table 1.

A mock process was performed using water as the starting pool and water as the diafiltration "buffer." The process was designed to mimic an industry-relevant extent of processing as well as a realistic overall process time, so that any storage solution residuals remaining in the cassettes would diffuse out into the mock pool and be washed through to the permeate (or remain in the pool) over a similar time and volume span as would occur during industrial usage.

Following the diafiltration/ultrafiltration, the final mock product pool was held in the system for up to 4 hours, with a 10 minute dynamic recirculation with open permeate, at 1 hour, 2 hours, and 4 hours followed by sampling from the recycle tank to evaluate whether any residual storage solution continued to diffuse out of the cassettes.

The diafiltration/ultrafiltration (DF/UF) process included:

Process Volumes
Diafiltration: 109L buffer into 22.8L retentate = 4.8 DV
Ultrafiltration: Concentrate from 22.8L to 4.0L = 5.7X VCF
Extent of processing = lnVCF + DV= 10.5 (5.7 + 4.8 = 10.5)
VCF is Volume Concentration Factor
DV is the # of Diavolumes
Volume of Mock Product Pool
4.0 L or ~1.75 L/m^2 of membrane area

Table 1. Outlining the DF/UF Process

For comparison, a second set of cassettes (4 x P3B010A05) were flushed, cleaned, and sanitized using the same new cassette preparation procedure. This was then followed directly by a static hold/recirculation of 4 L (~ 2 L/m^2) water for up to 4 hours. No diafiltration/ultrafiltration process was performed.

Samples for storage solution residual analysis were collected at the end of the initial cassette water flush, end of cleaning (new cassette preparation), end of the diafiltration, and end of ultrafiltration, as well as at 1, 2 and 4 hours of mock product pool hold/recirculation. Samples were analyzed for Total Organic Carbon (TOC), acetic acid, phosphoric acid and glycerin concentration.

Results

Table 2 is a summary of results for TOC, glycerin, acetic acid and phosphoric acid residuals from the Pellicon® 3 cassettes with Biomax® membrane storage solution flushing study. This work compares product pool residuals from cassettes with no DF/UF Processing and cassettes with mock DF/UF Processing.

Results show maximum levels of acid residuals in the product pool to be approximately 8 ppm in cassettes where the DF/UF process was performed and approximately 20 ppm in cassettes without a DF/UF process.

Results show maximum levels of glycerin residuals in product pool to be approximately 24 ppm in cassettes evaluated with DF/UF processing and approximately 140 ppm in cassettes evaluated with no DF/UF processing.

Results show maximum levels of TOC residuals in product pool to be approximately 14 ppm in cassettes evaluated with DF/UF processing and approximately 64 ppm in cassettes evaluated with no DF/UF processing.

Conclusions

Comparison of residuals from cassettes evaluated with no DF/UF Processing and cassettes with DF/UF processing show that the potential range of residuals is dependent on the extent of processing and the static hold time in the system prior to sampling. Residuals from cassettes evaluated with DF/UF processing show approximately 70 to 80 % reduction in glycerin residuals and 60 to 70 % reduction in acid residuals when compared to cassettes evaluated with no DF/UF Processing. In addition, residuals increase approximately 2 to 10-fold between 0 and 4 hours of hold time.

Catalog Number	Experiment	Sample	Vol/Mem Area	TOC	Glycerin	Acetate	Phosphate
			(L/m²)	(ppm)	(ppm)	(ppm)	(ppm)
					DL = 0.43	DL = 0.07	DL = 0.05
P3B010A05	Flush Evaluation	Post initial water Flush	n/a	0.09	< DL	0.05	< DL
		Post NCP	2	10.0	13.2	5.56	1.0
		PP-T= 0	2	6.51	11.0	2.37	< DL
		PP-T= 1	2	23.40	54.1	5.92	1.0
		PP-T= 2	2	55.90	121.0	10.22	9.5
		PP-T= 4	2	63.90	140.0	11.70	10.9
P3B010A05	Flush Evaluation with mock DF/UF	Post initial water Flush	n/a	0.09	0.00	0.06	0.00
		Post NCP	2	11.55	15.20	6.70	1.08
		Post DF	2	8.70	8.70	4.30	2.30
		Post UF	2	3.84	5.20	1.76	1.55
		PP-T= 0	2	4.80	7.60	2.13	1.74
		PP-T= 1	2	7.38	12.50	2.89	2.20
		PP-T= 2	2	10.90	16.80	3.57	2.65
		PP-T= 4	2	14.30	23.80	4.65	3.34

Table 2. Residual Analysis Storage Solution Flushing Study from Pellicon® 3 Cassettes with 10kD Biomax® Membrane 0.57m²

Legend
NCP = New Cassette Preparation
DF = Diafiltration
UF = Ultrafiltration
PP = Product Pool
T = Time (Hrs)
DL = Limit of assay detection

‘Results are intended as general examples and are not to be construed as product claims or specifications. These results included in this guide summarize outcomes and observations obtained in the specific application studies with the particular model stream and experimental conditions. Therefore, all test results should be confirmed by the end user while using feed stream and optimized conditions representative of the specific applications. In this case a change in the number of recirculations followed by flush to drain steps would change the measured residual storage solution, as would a change in the volumetric concentration factor, number of diavolumes, and/or final pool volume to membrane area ratio.

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