Millipore®
Preparation, Separation,
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Pellicon® 3 Cassettes with Ultracel® Membrane Performance Guide

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

The life science business of Merck KGaA, Darmstadt,
Germany operates as MilliporeSigma in the U.S. and Canada.

How to use this guide

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

Results are intended as general examples and are not to be constructed 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 described. Therefore, all test results should be confirmed by the end user while using a feed stream and optimized conditions representative of their specific applications.

Note: We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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Introduction

Pellicon® 3 cassettes with Ultracel® membrane are the optimum tangential flow filtration (TFF) devices for the ultrafiltration of solutions containing monoclonal antibodies, therapeutic peptides, polysaccharides, and recombinant and non-recombinant proteins. These advanced, high-performance cassettes are ideal for today's high titer therapeutic antibodies as well as more demanding filtration processes that require high operating pressures, temperatures, concentrations, and caustic cleaning regimes. From small-scale to full-scale production, Pellicon® 3 cassettes are designed for use in research, process scale-up and scale-down, applications development, and full-scale manufacturing.

For optimal performance in a range of applications, choose a cassette with a feed channel screen that best fits your needs. The C screen option is optimal for processes that require maximum mass transfer and flux. The D screen is optimized for applications that require higher concentration and higher viscosity.

The cassettes' design and automated manufacturing process enable unbeatable performance consistency and enhanced linear scalability between cassette sizes. The materials of construction of our cassettes ensure low extractables in a range of solvents, acids, and bases. The streamlined design allows you to quickly and easily handle, install, and remove cassettes, making your process more efficient, effective, and simple.

Protein Performance Scalability

Objective

To demonstrate the scalability of cassettes across available sizes using a model protein stream.

Summary

Pellicon® 3 cassettes with Ultracel® membrane are available in four linearly scalable sizes (88 cm^2, 0.11 m^2, 0.57 m^2, 1.14 m^2) with either C or D feed channel screens. This study demonstrates the scalability of cassettes with either feed screen. Using a model protein stream, the protein challenge consisted of transmembrane pressure (TMP) excursions with varying protein concentrations to evaluate protein flux performance and mass transfer comparability across the four sizes for each screen type. The results demonstrated excellent cassette scalability with consistent limiting flux data and mass transfer coefficients across the four sizes for each screen type.

Method

Cassette scalability was assessed by determining protein flux performance and mass transfer for all sizes. **Table 1** lists the cassettes used in the experiments and their respective feed conditions. Three or six cassettes of each type were used for the experiments. **Figure 1** depicts the system setup used during the experiment.

1. Protein solutions were recirculated in the TFF system from highest to lowest protein concentration.
2. Process parameters (solution temperature; inlet, outlet, and permeate pressures; and permeate and retentate flow rates) were recorded periodically while the retentate pressure was varied.
3. Process parameters measured during each flux excursion were used to characterize limiting flux (Flux vs TMP curves) and mass transfer performance (calculation of mass transfer coefficient).
4. For cassettes with C screen, Flux vs TMP curves were generated for 10, 20, and 40 g/L of sulfhydryl modified bovine serum albumin (BSA) in phosphate buffered saline pH 7.1 at an average feed flow rate of 5 L/min/m^2.
5. For cassettes with D screen, Flux vs TMP curves were generated for 25, 75, 150, and 200 g/L of human gamma globulin (HgG) in acetate buffer at an average feed flow rate of 6 L/min/m^2.

Table 1. Cassettes used in experiments and their feed conditions.

Catalog No.	Area	Cutoff	Screen	Feed Conditions
P3C010C00	88 cm^2	10 kDa	C	40, 20, 10 g/L BSA at 5 L/min/m^2
P3C010C01	0.11 m^2			
P3C010C05	0.57 m^2			
P3C010C10	1.14 m^2			
P3C030D00	88 cm^2	30 kDa	D	200, 150, 75, 25 g/L HgG at 6 L/min/m^2
P3C030D01	0.11 m^2			
P3C030D05	0.57 m^2			
P3C030D10	1.14 m^2			

Results

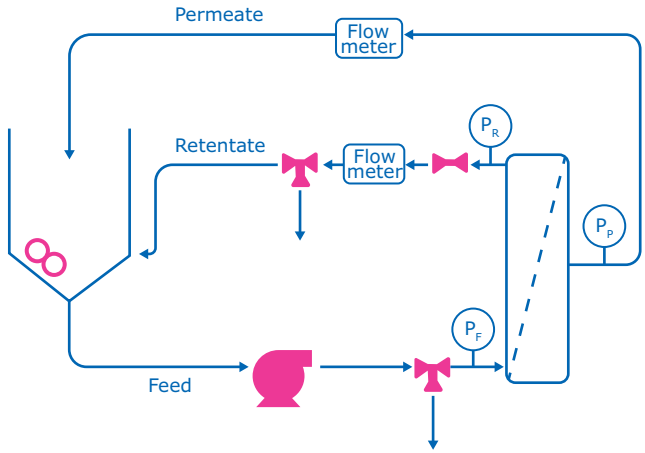

Figure 1. Schematic of TFF system setup used to run each process.

Flux Performance Analysis

At a given average feed flow rate, scalable cassettes must offer equivalent performance. To assess scalability in terms of flux, the protein challenge of cassettes consisted of a series of TMP excursions at a constant feed flow rate. The acceptance criterion for scalability requires cassettes size 88 cm^2, 0.57 m^2, and 1.14 m^2 to have mean fluxes within 20% of that of the 0.11 m^2 cassette.

The BSA flux performance of cassettes with C screen is presented for 20 g/L BSA in **Figure 2**. This representative result of protein performance of cassettes with C screen showed good agreement across all four sizes, with close distribution of data points within 20% of the average 0.11 m^2 cassette flux in both the polarized and non-polarized regions of the curve, demonstrating excellent cassette scalability. The data for BSA feed solutions of 10 g/L and 40 g/L BSA presented similar trends (not shown).

The protein flux performance of high viscosity cassettes with D screen is shown for protein concentrations 25 g/L and 200 g/L in **Figures 3** and **4**, respectively. The flux performance of cassettes with D screen showed good agreement across all sizes, with close distribution of data points within 20% of the average 0.11 m^2 cassette flux at each concentration evaluated, demonstrating the scalability of the D screen cassettes. The data for feed solutions 75 g/L and 150 g/L HgG demonstrated similar trends (not shown), further showing that scalability is maintained throughout a wide range of protein concentrations and viscosities.

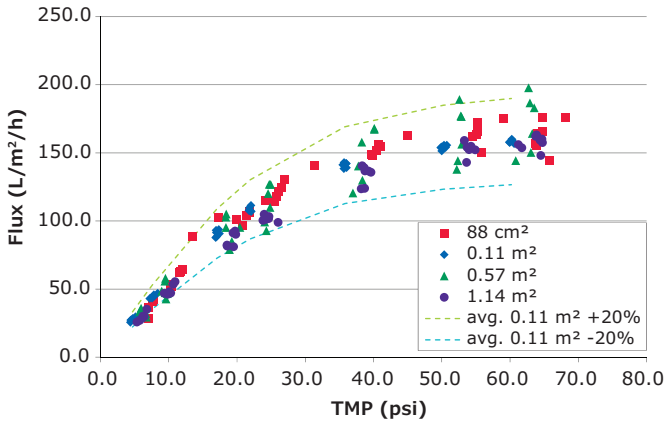

Figure 2. Permeate Flux vs TMP of Pellicon® 3 cassettes with 10 kDa Ultracel® membrane and C screen processing a 20 g/L BSA solution.

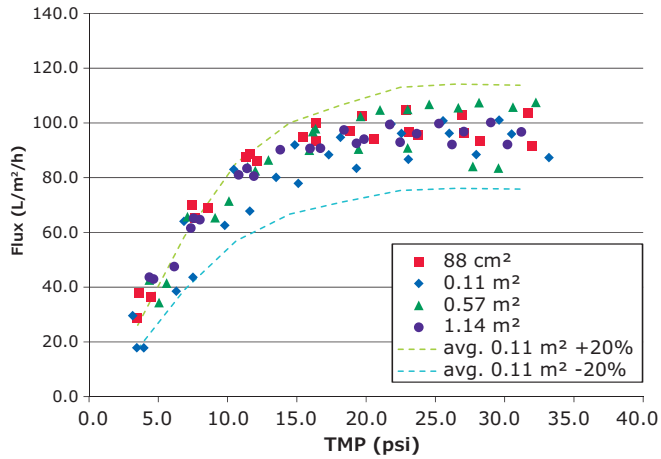

Figure 3. Permeate Flux vs TMP of Pellicon® 3 cassettes with 30 kDa Ultracel® membrane and D screen processing a 25 g/L HgG solution.

To further demonstrate the capabilities of high viscosity Pellicon® 3 cassettes with D screen, **Figure 4** shows the flux excursion of a highly concentrated protein solution demonstrating linear scalability across all sizes.

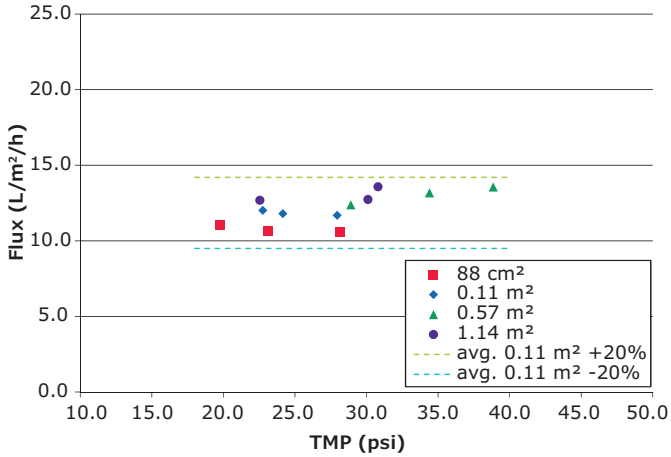

Figure 4. Permeate Flux vs TMP of Pellicon® 3 cassettes with 30 kDa Ultracel® membrane and D screen processing a 200 g/L HgG solution.

Mass Transfer Analysis

While analysis of protein flux performance reflects on the overall performance of the cassette, the mass transfer coefficient considers performance at the protein level. As the transmembrane pressure promotes a buildup of concentration on the membrane wall, osmotic pressure arising from differences in concentration lead the protein to return to the bulk fluid. The mass transfer coefficient represents this phenomenon, indicating the efficiency on buffer transfer from the bulk solution into the permeate stream and thus, the overall performance of the cassette. Accordingly, comparable and scalable TFF cassettes should have similar mass transfer coefficients under the same conditions (the mass transfer coefficient is dependent on feed flow rate and may vary with wall concentration).

The mass transfer coefficients can be determined using the limiting flux data from the TMP excursion studies by using one flux point per concentration at optimum TMP. Because the permeate flux (J) is related to both protein concentration (C_b) and mass transfer coefficient (k) through the stagnant film model (**Equation 1**), by plotting the permeate flux (J) versus the natural log scale of protein concentration, the mass transfer coefficient (k) can be determined from the slope of the linear curve for each cassette when a constant feed flow rate is maintained.

The flux versus natural log of protein concentration plot is shown in **Figure 5** for cassettes with D screen. The flux decreases linearly with the natural log of concentration as the concentration of HgG increases, as shown in the graph. Best fitted regression lines were determined for each cassette size to obtain the mean mass transfer coefficients from their slopes, listed in **Table 2**.

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

Where:

J = permeate flux (L/m²/h [LMH])

k = mass transfer coefficient (LMH)

C_w = wall protein concentration (g/L)

C_b = bulk protein concentration (g/L)

C_p = 0, assuming a fully retentive membrane

Equation 1: Simplified stagnant film model.

The mean mass transfer coefficients were also determined for cassettes with C screen, as listed in **Table 2**. Overall, the mean mass transfer coefficients of all sizes and both screens are within 20% of that of the 0.11 m² cassettes, further demonstrating the linear scalability of Pellicon® 3 cassettes.

Table 2. Mean mass transfer coefficients.

Screen	Cutoff	Membrane Area	Mass Transfer Coefficient (L/m²/h)
D Screen	30 kDa	88 cm²	43.2
		0.11 m²	41.4
		0.57 m²	45.3
		1.14 m²	38.3
C Screen	10 kDa	88 cm²	50.9
		0.11 m²	50.4
		0.57 m²	49.1
		1.14 m²	47.8

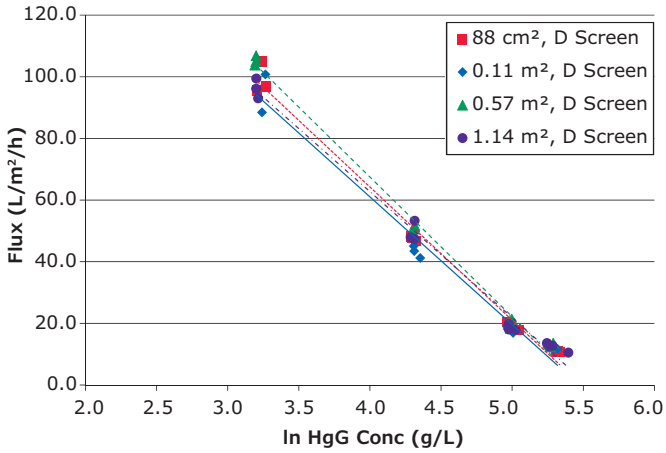

Figure 5. Flux vs ln HgG Concentration at 6 L/min/m² trendline fit for mass transfer calculation (slope) of Pellicon® 3 cassettes with Ultracel® membrane and D screen.

Conclusions

Pellicon® 3 cassettes with Ultracel® membrane demonstrated excellent protein performance scalability across all sizes while processing a model protein feed. The cassettes met scalability targets by exhibiting permeate fluxes that are within 20% of the average flux of the 0.11 m² cassette and less than 20% difference in protein mass transfer. Notably, linear scalability was maintained while processing highly concentrated solutions, a capability offered by cassettes with the high viscosity D screen. Highlighted by the results, the C screen option is optimal for processes that require high flux and mass transfer, and the D screen is optimal for applications that require higher concentrations and higher viscosities.

Competitive Performance and Process Impact Analysis

Objective

To compare the performance between Pellicon® 3 cassettes and competitor cassettes using a model protein stream.

Summary

The performance of Pellicon® 3 cassettes with Ultracel® membrane and C screen was compared to that of competitor cassettes available on the market using bovine serum albumin (BSA) as the model protein stream. The results from this study showed that Pellicon® 3 cassettes provide superior flux performance and higher mass transfer than those of the tested competitor cassettes. The results of this study were further used to assess the effect of these flux performance differences on processing efficiency and costs by applying the data to a typical manufacturing process. This analysis illustrated the benefits offered by Pellicon® 3 cassettes (e.g., membrane costs, pump size, product recovery) over the tested competitor cassettes.

Method

The performance of Pellicon® 3 cassettes was compared against that of competitor cassettes using permeate flux versus TMP excursions for three different concentrations of bovine serum albumin (BSA) in phosphate buffered saline, pH 7.1 at three different feed flow rates. The cassettes used in this study are listed in **Table 1**. Two or three cassettes of each type were used.

Table 1. Cassettes used in competitive experiments.

Cassettes	Membrane	Cutoff	Area	Screen
Pellicon® 3 cassette	Ultracel®	10 kDa	0.11 m^2	C screen
Competitor Cassette 1	Cellulose	10 kDa	0.10 m^2	Standard channel
Competitor Cassette 2	Cellulose	10 kDa	0.14 m^2	Thin channel
Competitor Cassette 3	Cellulose	10 kDa	0.10 m^2	Disposable format

1. To generate Flux vs TMP data, three BSA solutions (10, 50, and 100 g/L) were recirculated at three different feed flow rates (8, 5, and 3 L/min/m^2 [LMM]). TMP excursions were performed with increments of 5 psi until a maximum retentate pressure of 40 psi was reached. Process parameters (solution temperature; inlet, outlet, and permeate pressures; and permeate and retentate flow rates) were recorded after 10 minutes of recirculation at each TMP.
2. Process parameters measured during each flux excursion were used to characterize limiting flux (Flux vs TMP curves) and mass transfer performance (calculation of mass transfer coefficient).
3. Permeate samples at the end of each TMP excursion were taken and analyzed for BSA concentration via UV spectrophotometry to evaluate the effect of protein concentration on flux and membrane permeability.

Results

Protein Flux Performance

Protein flux performance experiments were carried out at three different feed flow rates (8, 5, and 3 LMM) for three different BSA feed concentrations (10, 50, and 100 g/L). The mean fluxes of the cassettes at 30 psi TMP for the three different protein concentrations are summarized in **Figures 1, 2,** and **3**. The results show that Pellicon® 3 cassettes exhibit higher fluxes than those of the tested competitor cassettes at the same protein concentration and feed flow rate.

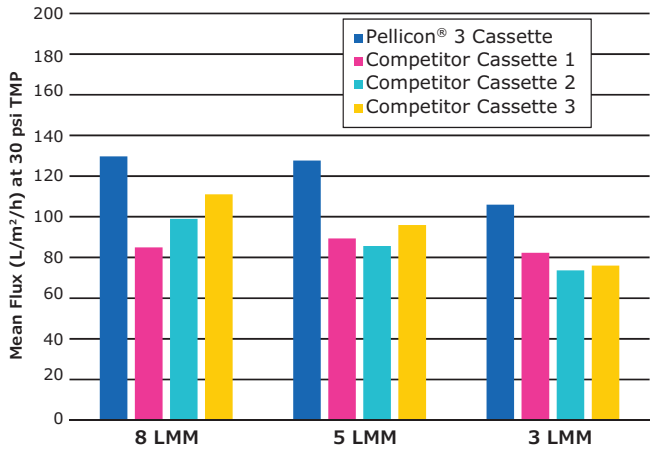

Figure 1. Mean protein fluxes of cassettes at 30 psi TMP for 10 g/L BSA.

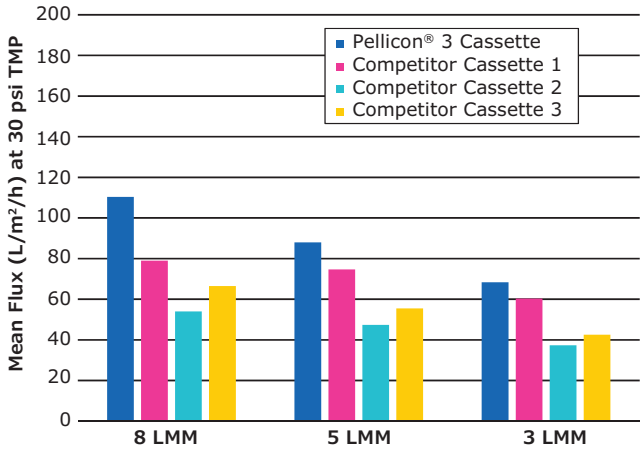

Figure 2. Mean protein fluxes of cassettes at 30 psi TMP for 50 g/L BSA.

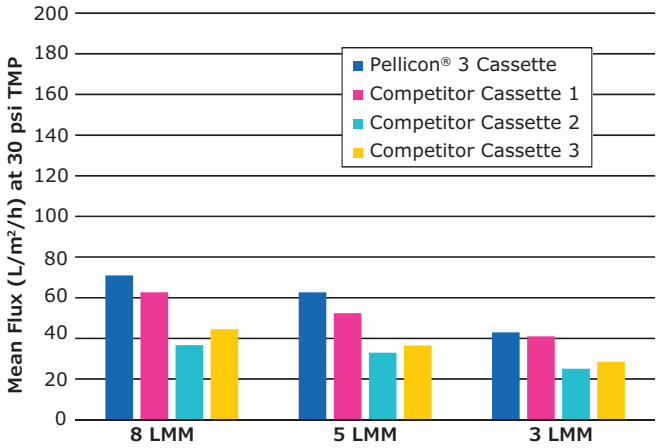

Figure 3. Mean protein fluxes of cassettes at 30 psi TMP for 100 g/L BSA.

Furthermore, **Figure 4** illustrates the effect of BSA concentration at 5 LMM on the permeate flux in the TFF process for Pellicon® 3 cassette and the three tested competitor cassettes. As the concentration of BSA increases, the limiting flux decreases, as expected by TFF theory; however, the Pellicon® 3 cassette with C screen outperformed the competitor cassettes by showing the highest flux throughout the concentration range.

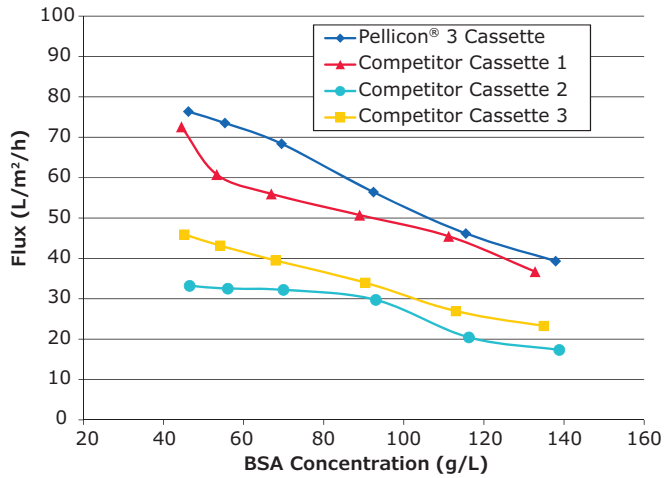

Figure 4. Comparison of Protein Flux vs Protein Concentration at 5 LMM of tested cassettes.

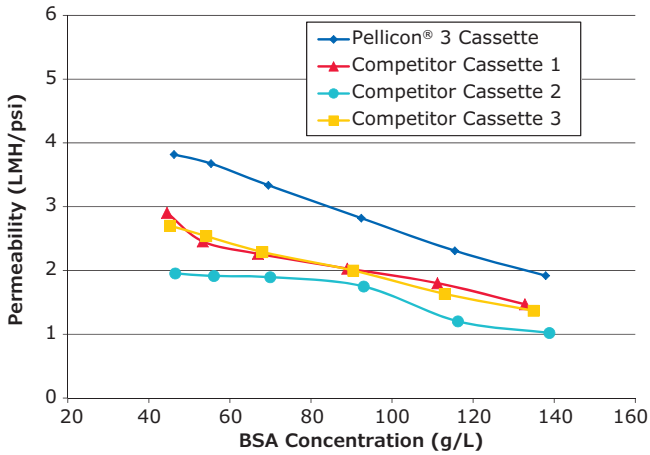

Figure 5. Comparison of Protein Permeability vs BSA Concentration at 5 LMM of tested cassettes.

Figure 5 displays the effect of protein concentration on membrane permeability during BSA processing. With increasing protein concentration, permeability through the membrane decreases, as shown in the graph; yet, the Pellicon® 3 cassette maintained the highest permeability throughout the BSA concentration range, thus surpassing the competitor cassettes.

Mass Transfer Analysis

To assess the overall performance of the cassettes at the protein level, protein mass transfer was analyzed by calculating the mass transfer coefficient. The mass transfer coefficient indicates the efficiency on buffer transfer from the bulk solution into the permeate stream, thus indicating the overall performance of the cassette. Accordingly, comparable TFF cassettes should have similar mass transfer coefficients under the same conditions.

The protein flux performance data obtained from the TMP excursion studies were used to determine the optimum flux point of each cassette (at the knee of the curve). Assuming a fully retentive membrane, the wall protein concentration (C_w) can be calculated from the osmotic pressure (π) model expressed in **Equation 1**. Next, C_w can be inserted into the stagnant film model (**Equation 2**) to calculate the mass transfer coefficient (k).

$$J = L_{fm}(TMP - \Delta\pi)$$

Equation 1: Osmotic pressure model.

$$J = k \ln(C_w / C_b)$$

Equation 2: Simplified stagnant film model.

Where:

J = permeate flux (L/m²/h [LMH])

L_{fm} = fouled membrane permeability (LMH/psi)

TMP = transmembrane pressure (psi)

π = osmotic pressure (psi) = $\alpha C_w + \beta C_w^2$, where α and β are constants

k = mass transfer coefficient (LMH)

C_w = wall protein concentration (g/L)

C_b = bulk protein concentration (g/L)

A summary of the average mass transfer coefficient for each feed flow rate is presented in **Figure 6**.

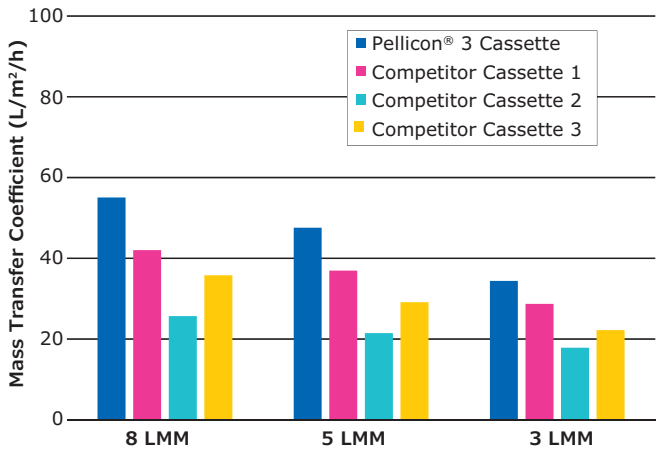

Figure 6. Comparison of mass transfer coefficients of tested cassettes at 8, 5, and 3 LMM.

In this evaluation, the competitor cassettes exhibited lower mass transfer than those of Pellicon® 3 cassette (i.e., 30% lower for competitor cassette 1), suggesting that a higher feed flow rate is required when using the competitor cassettes to achieve the same flux provided by Pellicon® 3 cassettes. The next section illustrates the impact of these differences in flux performance on process efficiency.

Impact on Membrane Area

A typical manufacturing process requires concentration of a 2000-L total volume in 4 hours at a constant feed flow rate of 5 L/min/m². Using the data gathered from the competitive studies and **Equation 3,** the required membrane area for this process was calculated for Pellicon® 3 cassette and competitor cassette 1.

Table 2 illustrates how the lower flux exhibited by competitor cassette 1 leads to a required membrane area that is greater than that needed with the Pellicon® 3 cassette, which would then result in a larger system size, thus increasing capital costs.

$$\text{Membrane Area } (m^2) = \frac{\frac{Filtrate\ Vol_1\ (L)}{Flux_1\ (L\ m^{-2}\ h^{-1})} + \frac{Filtrate\ Vol_2\ (L)}{Flux_2\ (L\ m^{-2}\ h^{-1})}}{Process\ Time\ (h)}$$

Equation 3. Calculation of required membrane area.

Table 2. Membrane area required to concentrate 2000 L of product in 4 hours at 5 L/min/m².

Cassette	Average Flux (L/m²/h)	Membrane Area (m²)
Pellicon® 3 cassette	47	11
Competitor Cassette 1	37	14

Impact on Feed Flow Rate and Product Recovery

In an alternative scenario, a comparable membrane area (11 m² of Pellicon® 3 cassette vs 12 m² of competitor cassette) may be used by adjusting the feed flow rates. However, to concentrate 2000 L of product in 4 hours, the feed flow rate needs to be increased by approximately 60% for competitor cassette 1 to achieve a comparable average flux to that provided by Pellicon® 3 cassette (**Table 3**). The higher feed flow rate needed for competitor cassette 1 would then require a larger pump and piping size than that required by Pellicon® 3 cassette, thus leading to a higher system cost and lower product recovery.

Table 3. Impact of using comparable membrane area on process and system requirements.

Parameter	Pellicon® 3 Cassette	Competitor Cassette 1
Membrane area (m²)	11	12
Average flux (L/m²/h)	47	42
Feed flow rate (L/min/m²)	5	8
Pump capacity (L/h)	3300	5760
Piping (in)	1	1½
Min. recirculation vol. (L)	12	25
Undrainable hold-up vol. (L)	0.4	0.8

Conclusion

A major processing challenge is concentration polarization (the amount of protein concentrated at the membrane surface). This polarization layer controls process flux and determines the required membrane area and/or process time. The C feed channel screen is optimized for higher mass transfer capacity, resulting in higher process flux and thus balancing membrane area costs and production time. The data generated in this study illustrates the processing and economic benefits of the higher flux offered by Pellicon® 3 cassettes with Ultracel® membrane and C screen over several competitor cassettes available on the market.

Cassette Compatibility to DMAc and DMSO

Objective

To demonstrate the compatibility of Pellicon® 3 cassettes with Ultracel® membrane to dimethylacetamide (DMAc) and dimethyl sulfoxide (DMSO).

Summary

Cassettes were subjected to diafiltration of 20% DMAc and 20% DMSO and evaluated for their hydraulic and protein performance before and after solvent clearance. Pressure drop, air integrity, normalized water permeability (NWP), and protein flux performance were maintained stable after diafiltration, demonstrating the compatibility of the cassettes to DMAc and DMSO.

Method

Clearance of 20% DMAc and 20% DMSO by constant-volume diafiltration was performed using cassettes with 30 kDa Ultracel® membrane and C screen. The cassettes were evaluated before and after diafiltration to assess solvent compatibility.

1. Each cassette was flushed of storage solution and cleaned according to the user guide.
2. Pressure drop and NWP at average feed flow rate of 6 L/min/m^2 and air integrity at 30 psi were measured.
3. Transmembrane pressure (TMP) excursions with 20 g/L bovine gamma globulin (BgG) were performed to measure protein retention at low, optimal, and high TMP conditions.
4. After BgG evaluation, the cassettes were cleaned with 0.1 M NaOH and flushed with reverse osmosis (RO) water.
5. Model feed solutions 20% v/v DMAc/RO water and 20% v/v DMSO/RO water were prepared (~40 L/m^2). RO water was used as diafiltration buffer (~10-12× feed solution volume).
6. The model solutions were recirculated for 10-20 min in total recycle mode at a feed flow rate of 6 L/min/m^2 and retentate pressure of 10 psi. Process parameters (feed, retentate, and permeate pressure; retentate and permeate flow rate; time and temperature) were recorded at the start and end of recirculation.
7. A sample from the feed tank was collected for concentration analysis.
8. The system was configured to run in diafiltration mode (buffer feed and retentate lines to the tank; permeate line to collection vessel).
9. Retentate and permeate pressures were adjusted to achieve permeate flow rate of ~55 L/m^2/h, and the buffer flow rate was adjusted to be equivalent to the permeate flow rate.
10. For each 5 L/m^2 of permeate volume collected, feed tank samples were collected for concentration analysis and process parameters (mentioned above) were recorded until the diafiltration was completed.
11. After diafiltration, steps 2-4 were repeated.

Results

For each diafiltration experiment, the concentration of DMAc and DMSO in the feed tank was analyzed throughout the process and used to plot solvent removal at various diafiltration volumes (diavolumes). The clearance of DMAc and DMSO is shown in **Figure 1**, which details the remaining concentration of solvent normalized to the original concentration (20% DMAc or 20% DMSO) throughout the diafiltration.

Ten diavolumes efficiently reduced DMAc concentration by a factor of over 25,000, and twelve diavolumes efficiently reduced the concentration of DMSO by a factor of over 10,000. The experimental data were closely aligned to those of the theoretical process (which assumes a non-retentive membrane), demonstrating a strong and consistent diafiltration performance of the cassettes through the clearance of DMSO and DMAc.

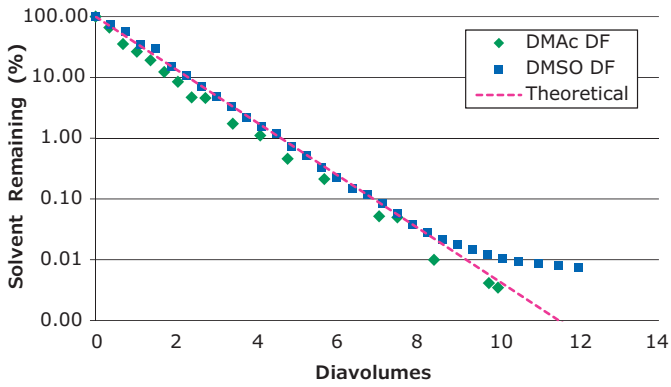

Figure 1. DMSO and DMAc clearance by Pellicon® 3 cassettes with Ultracel® membrane.

The protein flux performance of the cassettes before and after diafiltration is shown in **Figure 2**. The permeate flux of the cassettes over a 20 g/L BgG challenge is comparable pre- and post-solvent exposure. Moreover, BgG retention at optimal TMP was maintained at 99.94% before and after DMAc diafiltration and at 99.91% before and after DMSO diafiltration (data not shown), further demonstrating the compatibility of the cassettes to 20% DMSO and 20% DMAc.

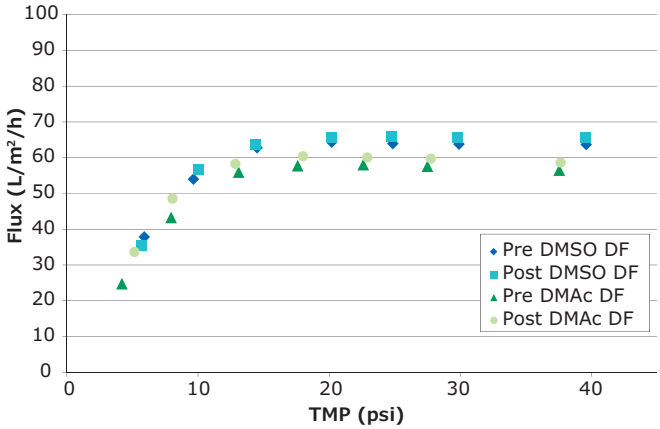

Figure 2. Flux vs TMP of Pellicon® 3 cassettes with 30 kDa Ultracel® membrane at 20 g/L BgG solution before and after DMSO and DMAc diafiltration.

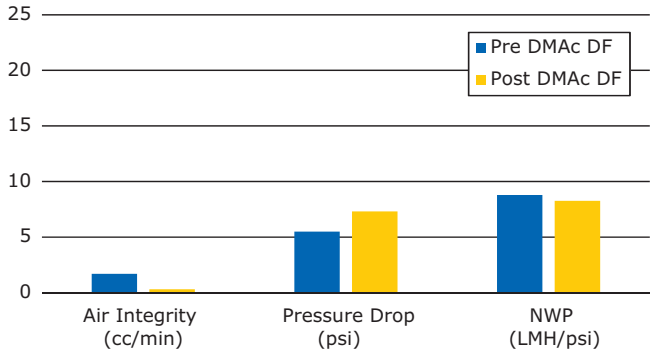

Figure 3. Air integrity, pressure drop, and NWP evaluation before and after DMAc diafiltration.

The results of air diffusion, pressure drop, and NWP of cassettes before and after diafiltration, are summarized in **Figures 3** and **4** for DMAc and DMSO clearance, respectively. Pressure drop and air diffusion were maintained within Certificate of Quality test specifications before and after diafiltration, and NWP was also maintained stable, demonstrating cassette compatibility to 20% DMAc and 20% DMSO.

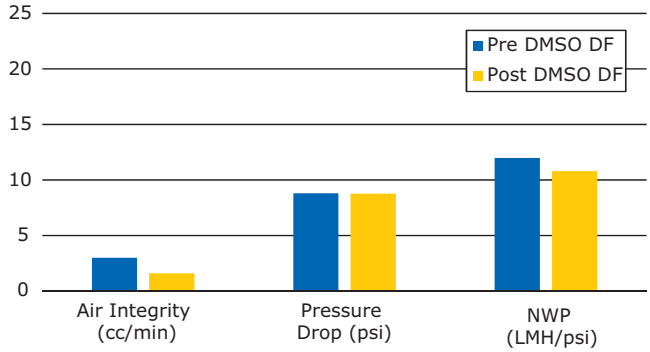

Figure 4. Air integrity, pressure drop, and NWP evaluation before and after DMSO diafiltration.

Conclusion

No adverse effects were observed on the pressure drop, air integrity, membrane permeability, and flux performance of Pellicon® 3 cassettes with Ultracel® membrane after exposure to DMSO and DMAc. Consistent pressure drop, air diffusion, and NWP values indicate the compatibility of the feed channel screen, seals, and membrane to 20% DMAc and 20% DMSO. Consistent protein flux performance further confirms the robustness of the cassettes. Overall, this study demonstrates compatibility of the cassettes to processes that require use of DMAc and DMSO.

Cassette Compatibility to Acetone and Acetonitrile

Objective

To demonstrate the compatibility of Pellicon® 3 cassettes with Ultracel® membrane to concentrated acetone and acetonitrile.

Summary

Cassettes with 3 kDa Ultracel® membrane were evaluated for acetone and acetonitrile compatibility. The cassettes were exposed to 100% acetonitrile and 100% acetone for more than 700 hours and assessed for changes in pressure drop, air integrity, and normalized water permeability (NWP). The pressure drop, air integrity, and NWP were maintained stable throughout the 700 hours of solvent exposure, demonstrating the compatibility of the cassettes to acetone and acetonitrile.

Method

The method consisted of exposing Pellicon® 3 cassettes with 3 kDa Ultracel® membrane and C screen to 100% acetone and 100% acetonitrile for over 700 hours. Pressure drop, air integrity, and NWP were evaluated before and after solvent exposure to assess cassette compatibility.

1. Each cassette was flushed of storage solution and cleaned according to the user guide.
2. Pressure drop and NWP at average feed flow rate of 6 L/min/m^2 and air integrity at 30 psi were measured.
3. The cassettes were flushed with approximately 2 L of solvent (100% acetone or 100% acetonitrile) at a vessel pressure of 5 psi to remove all air from feed and permeate channels.
4. The retentate and permeate valves were closed to increase vessel pressure to 50 psi and the cassettes were allowed exposure to solvent for >700 hours.
5. After solvent exposure, each cassette was flushed of solvent with reverse osmosis (RO) water and evaluated for pressure drop and NWP at 6 L/min/m^2 and air integrity at 30 psi.

Results

The results of air diffusion, pressure drop, and NWP before and after cassette exposure to acetone and acetonitrile are summarized in **Figures 1** and **2**. The pressure drop, air integrity, and water permeability of the cassettes were maintained stable throughout 700 hours of solvent exposure. Marginal variations of approximately ±1.5 or less demonstrate the cassette's resistance to 100% acetone and 100% acetonitrile for at least 700 hours.

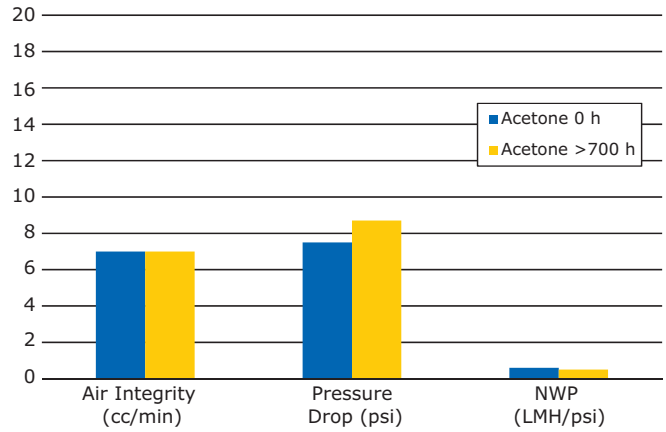

Figure 1. Air integrity, pressure drop, and NWP evaluation before and after acetone exposure.

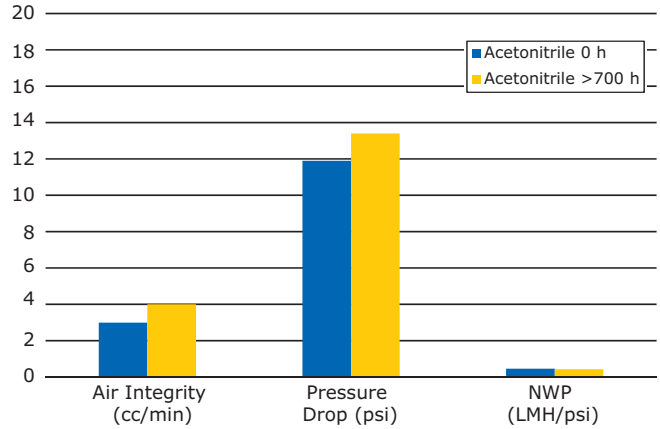

Figure 2. Air integrity, pressure drop, and NWP evaluation before and after acetonitrile exposure.

Conclusion

No adverse effects were observed on the pressure drop, air integrity, and water permeability of Pellicon® 3 cassettes with 3 kDa Ultracel® membrane after 700 hours of 100% acetone and 100% acetonitrile exposure. Thus, the cassettes are compatible for use in processes using acetone or acetonitrile solutions, such as in the processing of small biological molecules, including therapeutic peptides, oligonucleotides, and antibody fragments. Compatibility of the Ultracel® membrane to concentrated acetone or acetonitrile can be inferred from these results but performance should be confirmed with the specific molecule and process.

Multiple-Run Performance and Cleanability

Objective

To demonstrate the consistency of cassettes for high viscosity processing over multiple runs and cleaning cycles.

Summary

Process consistency and cleanability over multiple cassette uses were evaluated. Cassettes with C and D screens were subjected to cycles of protein processing and cleaning. The results show flux, pressure drop, and protein yield were consistently maintained over multiple process runs (up to 15 cycles). Permeability of the membrane was successfully restored after each cycle using sodium hydroxide (NaOH). The results further demonstrate that cassettes with D screen achieved 1.4-fold higher viscosity than that of cassettes with C screen without significant drop in mass transfer.

Methods

The method consisted of challenging cassettes to cycles of protein concentration and subsequent cleaning to evaluate performance consistency over multiple runs. **Table 1** lists the cassettes used in this study.

Table 1. Cassettes used in experiments.

Cat. No.	Area	Cutoff	Screen
P3C030C01	0.11 m^2	30 kDa	C
P3C030D01	0.11 m^2	30 kDa	D
P2C030V01	0.1 m^2	30 kDa	V

1. Each cassette was challenged with 10 g/L bovine gamma globulin (BgG) solution formulated in 10 mM phosphate buffered saline (PBS) at pH 7.2. The feed flow rate was set to 6 L/min/m^2 and the retentate pressure was kept at 10 psi or above. The feed flow was gradually decreased toward the end of the process run to avoid exceeding the maximum pressure rating of the system due to increasing viscosity of the protein solution. The BgG solution was concentrated to a maximum achievable concentration.
2. After protein processing and product recovery, the cassettes were cleaned by flushing and recirculating 0.5 N or 0.1 N NaOH for 1 hour at room temperature, along with appropriate water flush. The feed flow rate for the cleaning cycle was set to 6 L/min/m^2 and the retentate pressure was set to approximately 5 psi. The cassettes were stored in 0.1 N NaOH between process runs.

The cassettes were subjected to 3, 10, or 15 cycles of protein runs and cleaning. The acceptance criterion for water permeability recovery was set to 80% or above. The values of water permeability after cleaning were compared to the permeabilities of the new cassettes after flushing and sanitization.

Results

Multiple-run Process Performance

Figure 1 shows the process flux as a function of protein concentration for the tested cassettes. All cassettes demonstrated consistency over multiple process runs. The flux was comparable for cassettes with C and D feed channel screens (within 10%), and the cassette with V screen exhibited a much lower flux, as expected.

Feed channel pressure drop depends on the cassette screen type and increases with increasing viscosity and concentration of the protein solution, as illustrated in **Figure 2**. A significantly lower pressure profile was observed for the more open D screen cassette than that of the C screen cassette, thus enabling the D

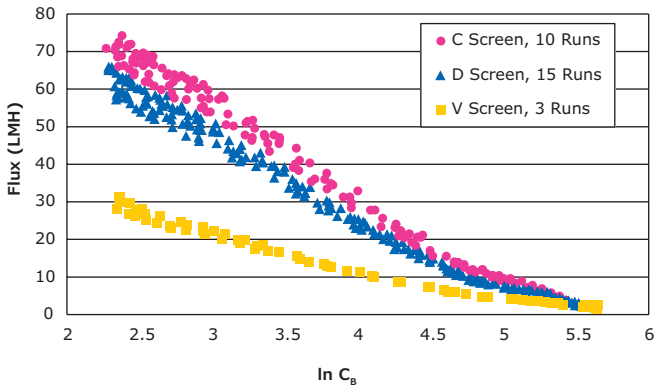

Figure 1. Flux vs Protein Concentration for 30 kDa Ultracel® membrane cassettes. C screen: 10 cycles; D screen: 15 cycles; V screen: 3 cycles.

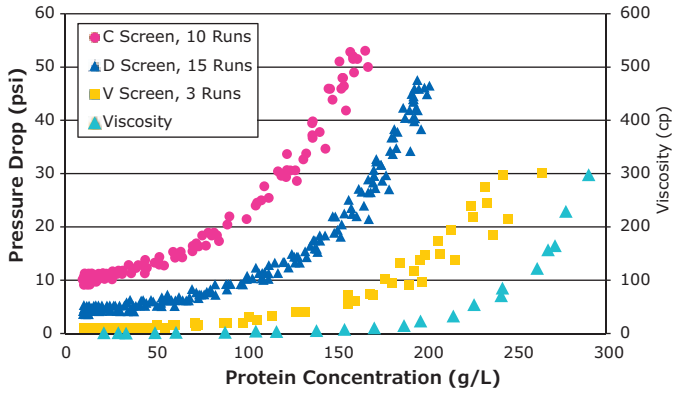

Figure 2. Feed Channel Pressure Drop vs Protein Concentration and Viscosity for Pellicon® cassettes with 30 kDa Ultracel® membrane with C, D, and V screens.

screen cassette to reach higher viscosities and protein concentrations.

Process results from all runs were averaged for each cassette and are summarized in **Table 2**. Excellent protein yields were observed for all cassettes over multiple uses. The more open D screen cassettes achieved higher final protein concentration and final viscosity than the C screen cassettes with no significant loss in mass transfer.

Table 2. Summary of process results.

Catalog No.	Screen	Mass Transfer Coefficient ($L/m^2/h$)	Final Protein Concentration (g/L)	Final Viscosity (cP)	Yield (%)
P3C030C01	C	24	226	54	103
P3C030D01	D	21	242	78	101
P2C030V01	V	10	277	230	102

Cleanability

Pellicon® cassettes with Ultracel® membranes maintained their cleanability and reusability throughout multiple processing and cleaning cycles. Water permeability was consistently restored to pre-process values (≥ 80%) after each 60-minute cleaning cycle with NaOH at room temperature (**Figure 3**).

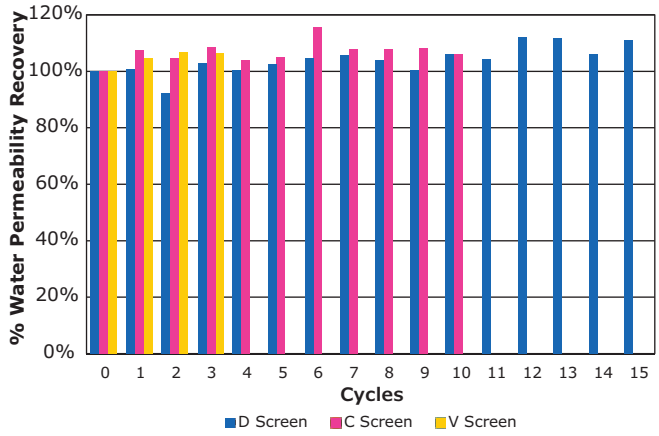

Figure 3. Water permeability recovery of 30 kDa Ultracel® membranes after cleaning with NaOH.

Conclusions

Water permeability and process performance of Pellicon® 3 cassettes with C and D screens and Pellicon® 2 cassette with V screen were successfully and consistently restored after multiple application runs. Cleanability was achieved using sodium hydroxide at room temperature and a constant feed flow rate. Flux, pressure drop, and protein yield were consistent over all process runs for the tested cassettes. The feed channel D screen, designed for high viscosity applications, is optimized to concentrate protein solutions to higher viscosities; in this study, a 1.4-fold increase of viscosity was achieved with the D screen cassette as compared to the C screen cassette.

New Cassette Flushing

Objective

To evaluate residuals from the storage solution used in Pellicon® 3 cassettes with Ultracel® membrane after initial flushing and cleaning.

Summary

Pellicon® 3 cassettes with Ultracel® membrane are packaged and shipped in a storage solution containing 3.9% benzyl alcohol and 20% glycerin in water. Experiments were performed to evaluate the levels of storage solution residuals from new cassettes after initial flushing and cleaning. Upon completion of the flushing and cleaning procedure, the total organic carbon (TOC) levels were approximately 1 ppm in both the retentate and permeate samples collected from all tested cassettes.

Method

The method consisted of subjecting new cassettes to flushing and cleaning cycles. During flushing, the system was configured in single-pass mode to remove bulk components, and cleaning was done in total recycle mode to allow time for residual components to diffuse from the filter into the bulk fluid flow. The cassettes used in this study are listed in **Table 1**.

Table 1. Cassettes used in flushing experiments.

Cat. No.	Area	Cutoff
P3C010C05	0.57 m^2	10 kDa
P3C030C05	0.57 m^2	30 kDa
P3C010C10	1.14 m^2	10 kDa
P3C030C10	1.14 m^2	30 kDa

1. The cassettes were flushed with 20 L/m^2 reverse osmosis (RO) water in single-pass mode at 25 °C. The feed flow was set to 5.5 L/min/m^2 and the retentate pressure to 5 psi (for 30 kDa cassettes) or 10 psi (for 10 kDa cassettes) to target a conversion ratio of feed-to-permeate flow of 25% to 50%.
2. Cleaning was done with 5 L/m^2 RO water in total recirculation mode for 30 minutes at a feed flow rate of 5.5 L/min/m^2 and retentate pressure of 5 psi (for 30 kDa cassettes) or 10 psi (for 10 kDa cassettes) to target a conversion ratio of feed-to-permeate flow of 25% to 50%.
3. The flush and cleaning cycle (steps 1 and 2) was repeated and retentate and permeate samples (40 mL each) were taken after each flush of 10 L/m^2 for TOC analysis. In total, four flushes (20 L/m^2 ×4) and three total recirculation cleanings (5 L/m^2 ×3) were completed, for a total consumed volume of 95 L/m^2.

Results

Figure 1 shows the levels of storage solution residuals as a function of total flush volume. The solid lines display the residual profile of the retentate (R), and the dashed lines represent that of the permeate (P) for each cassette. The permeate shows a more gradual flush of residuals than that of the retentate, which is expected as the graph was plotted as a function of total flush volume rather than individual volumes per cycle.

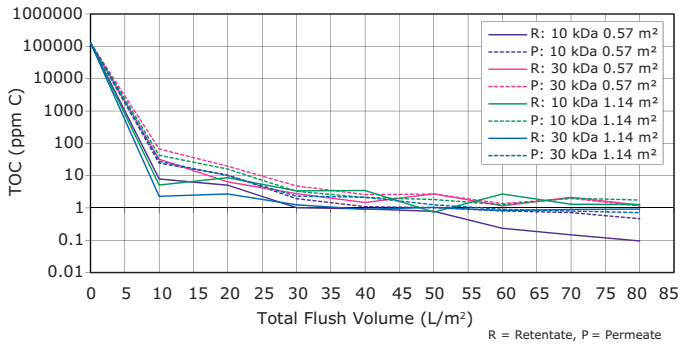

Figure 1. Evaluation of TOC flushing in new Pellicon® 3 cassettes with Ultracel® membrane.

Because recirculations of 5 L/m^2 were performed after each of the first three flushes (at 20, 40, and 60 L/m^2 total flush volume), an additional cumulative volume of 15 L/m^2 was used by the time the curves reached the 80 L/m^2 flush volume point in **Figure 1**. Thus, the total water consumption to obtain retentate and permeate samples approaching a TOC of 1 ppm is 95 L/m^2.

Conclusion

TOC levels in all tested cassettes were approximately 1 ppm upon completion of the flushing and cleaning procedures with RO water using a total volume of 95 L/m^2. The results showed that all tested cassettes followed similar flushing trends for storage solution removal in terms of total flush volume per meter square of membrane area, which suggests that the cassettes have a scalable flushing performance.

This study considered the presence of residuals by flush volume only. Please refer to the Extractables section for an assessment of residuals that may diffuse from the cassette during processing.

Extractables

Objective

To evaluate extractables from Pellicon® 3 cassettes with Ultracel® membrane after exposure to selected model stream solutions.

Summary

Extractables were assessed upon cassette exposure to the following model stream solutions: water, 25% denatured ethanol/75% water v/v, 0.1 N sodium hydroxide (NaOH), and hydrochloric acid (HCl) solution pH 2.0 for at least 24 hours at a controlled temperature (15-30 °C). Non-volatile residues (NVR) and total organic carbon (TOC) levels were found to be low. Glycerol and benzyl alcohol were identified as the main extractables, which are components of the storage solution used in the cassettes.

Method

Pellicon® 3 cassettes with 30 kDa Ultracel® membrane and C screen, size 0.57 m^2 were selected as a representative sample of the cassette family. Three cassettes were used per model solution.

Flushing Procedure

1. Each cassette was installed into the holder and filled with Milli-Q® water.
2. The retentate was drained with the retentate valve fully open. Note: For all flushing described in this protocol, the permeate valve was fully open.
3. The feed flow rate was set to 5 L/min/m^2.
4. Flushing continued until 1-2.5 L of water were drained.
5. The retentate pressure was adjusted to 10-15 psi.
6. A total of 5-10 L were pumped through the system before it was completely drained.

Cleaning Procedure

7. Four liters of 0.1 N NaOH were recirculated for 30 minutes at a feed flow rate of 5 L/min/m^2 and retentate pressure of 5-10 psi.
8. The system was drained, and the Flushing Procedure was repeated before proceeding to the Extraction Procedure.

Extraction Procedure

9. Approximately seven liters of each model stream solution (water, 25% denatured ethanol, 0.1 N NaOH, and HCl pH 2.0) were prepared. One liter (or minimum system hold-up volume) of the model solution was used as control. The remaining volume was split between three cassettes.
10. Approximately one liter (or minimum system hold-up volume) of the model solution was recirculated through the cassettes for approximately 5 minutes to equilibrate the system. The system was drained, and the solution was discarded.
11. Another liter (or minimum system hold-up volume) of model solution was recirculated through the cassette for approximately 5 minutes.
12. The cassette was left undisturbed for at least 24 hours at a controlled temperature (15-30 °C).
13. After the extraction time completed, the solution was recirculated through the cassette for approximately 5 minutes to ensure homogeneity of the extraction solution.
14. The extraction solution was collected by draining the system completely and transferred into clean glass beakers for gravimetric analysis to measure NVR or into appropriate test tubes for TOC, Fourier-transform infrared (FTIR) spectroscopy, and reverse-phase high-performance liquid chromatography (RP-HPLC) analyses.

Results

The average amounts of non-volatile extractables and the average total organic carbon, obtained for three cassettes per model solution, are shown in **Table 1**. All extractables data were based on a one-liter extraction volume. Due to the presence of high salt levels in the 0.1 N NaOH solution, the detection of low-level extractables was not achieved for that extract. Further, TOC analysis of the 25% denatured ethanol extract was not applicable due to interference by the carbon-based solvent.

Table 1. Average extractables.

Extract	NVR mg/0.57 m^2	TOC mg C/0.57 m^2
Water	59.5	111.4
25% Denatured EtOH	74.3	N/A
HCl pH 2.0	99.4	73.7
0.1 N NaOH	N/A	129.3

FTIR analysis of the NVR samples from water, 25% denatured ethanol, and HCl pH 2.0 extracts indicated the presence of glycerol. **Figure 1** shows the stacked FTIR spectra of the analyzed NVR samples and that of glycerol for comparison. The presence of glycerol is expected as it is a component in the storage solution of the cassettes.

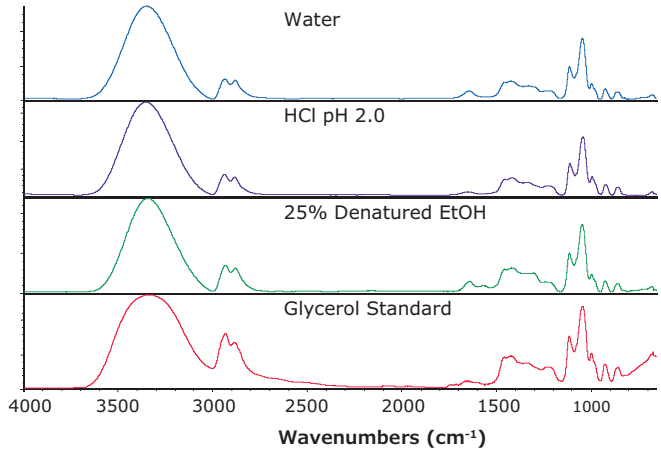

Figure 1. FTIR spectra of extracts from Pellicon® 3 cassettes show the presence of glycerol.

Extraction solutions were analyzed by RP-HPLC to account for potential volatile or semi-volatile extractables not present in the NVR samples. From this analysis, one significant peak was found at 8.1 min retention time, when compared to the control solution. This peak was identified as benzyl alcohol. **Figure 2** shows a representative chromatogram of the 0.1 N NaOH extract solution compared with that of benzyl alcohol standard solution. The data for solutions 25% denatured ethanol, HCl pH 2.0, and water are not shown but showed the same peak at 8.1 min, confirming the presence of benzyl alcohol, a component in the storage solution used for new cassettes.

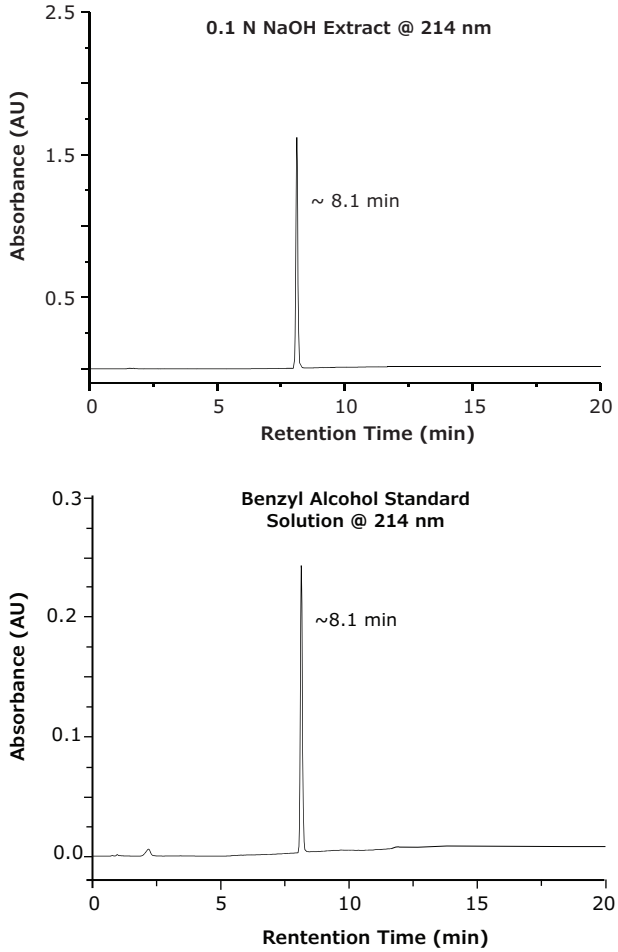

Figure 2. RP-HPLC chromatograms of the 0.1 N NaOH extract solution (top) and benzyl alcohol standard solution (bottom).

Conclusion

Pellicon® 3 cassettes with Ultracel® membrane have been assessed for extractables after mock processing with water, 25% denatured ethanol, 0.1 N NaOH, and HCl pH 2.0 at room temperature for 24 hours. The levels of extractables determined by TOC and NVR analyses are considered low. HPLC and FTIR analyses showed that the main extractables from cassettes during processing are components used in the storage solution of new cassettes. If the levels of storage agents (glycerol and benzyl alcohol) need to be reduced for a particular application, additional flushing can be performed before product processing.

Hold-Up Volume of Cassettes

Objective

To determine the hold-up volume of the feed and permeate channels of cassettes.

Summary

Hold-up volumes in the feed and permeate channels of cassettes were measured to help the user determine the minimum working volumes for their systems.

Method

1. Each cassette was flushed with a minimum feed volume of 60 L/m^2. The feed flow rate was set to approximately 5 L/min/m^2 and the retentate flow was restricted to achieve a conversion rate of 30% to 40% (permeate flow/feed flow*100).
2. 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.
3. The cassette was removed from the holder and waterproof tape was placed over the end cap seals (feed, retentate, and permeate) on one side of the cassette.
4. The cassette was returned to the holder with the taped side against the holder end plate.
5. Reverse Osmosis (RO) water was recirculated through the cassette for 10 minutes at a feed pressure of 20 psi, retentate pressure of 15 psi, and permeate pressure of 10 psi.
6. The pump was turned off and the cassette was removed from the holder, with care so that the water inside the cassette was not lost. The cassette was weighed, and the cassette weight was recorded as *Initial Wet Cassette Weight.*
7. The cassette was returned to the holder with the taped side against the holder end plate, with care so that the water inside the cassette was not lost.
8. The 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.
9. The cassette was removed from the holder with care so that the water inside the cassette was not lost. The cassette was weighed, and the cassette weight was recorded as *Post Feed Channel Blow Down Weight.*
10. The cassette was returned to the holder with the taped side of cassette against the holder end plate, taking care to orient the cassette and holder so that the water inside the cassette was not lost.
11. The permeate valve was opened. Compressed air was blown down the permeate channel at 10 psi for 3 minutes.
12. The cassette was removed from the holder with care so that the water inside the cassette was not lost. The cassette was weighed, and the cassette weight was recorded as *Post Permeate Channel Blow Down Weight.*
13. The cassette was returned to the holder with the taped side of the cassette against the holder end plate. The feed, retentate, and permeate valves were opened, and compressed air was blown through the cassette at 10 psi for ≥ 12 hours.
14. The cassette was removed from the holder. The cassette was weighed, and the cassette weight was recorded as *Final Dry Cassette Weight.* The Initial Wet Cassette and Final Dry Cassette Weights were compared to ensure that the cassette was completely dry.

Results

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

1 g H_20 = 1 mL H_20

The *post feed channel blow down weight* was subtracted from the *initial wet cassette weight* to calculate the hold-up volume of the feed channel.

Feed Channel Hold-up Volume = Initial Wet Cassette Weight — Post Feed Channel Blow Down Weight

Similarly, the *post permeate channel blow down weight* was subtracted from the *post feed channel blow down weight* to calculate the hold-up volume of the permeate channel.

Permeate Channel Hold-up Volume = Post Feed Channel Blow Down Weight — Post Permeate Channel Blow Down Weight

The average hold-up volumes for feed and permeate channels of each cassette size and screen are shown in **Table 1**. As expected, the feed channel hold-up volumes of cassettes with D screen are greater than those of cassettes with C screen due to the more open feed channel spacer of the D screen. The permeate channel hold-up volumes of cassettes are comparable for cassettes of the same size with either screen option due to comparable permeate path dimensions.

Table 1. Average hold-up volumes of feed and permeate channels.

	C Screen		D Screen	
Membrane Area	Feed Channel (mL)	Permeate Channel (mL)	Feed Channel (mL)	Permeate Channel (mL)
88 cm^2	1.5	2.4	3.6	2.0
0.11 m^2	18	15	23	17
0.57 m^2	85	68	118	75
1.14 m^2	170	127	227	138

Conclusion

Hold-up volumes in the feed and permeate channels of Pellicon® 3 cassettes with Ultracel® membrane, C and D feed channel screens, are presented in **Table 1** to help the user determine the minimum working volumes for their systems.

Cassette Materials of Construction Chemical Resistance

Objective

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

Summary

Individual materials of construction of Pellicon® 3 cassettes, excluding the Ultracel® membrane, were selected according to their compatibility with a wide range of chemicals. Certain materials of construction were evaluated for changes in hardness, mass, and physical appearance upon exposure to a variety of chemical solutions. The overall changes in mass and hardness were negligible and should not affect cassette performance.

Method

The method consisted of soaking individual materials of construction of the cassettes, excluding the membrane, in selected chemical solutions. Changes in hardness, mass, and physical appearance were recorded to assess the chemical compatibility of the materials of construction of cassettes. **Table 1** outlines the materials evaluated in this study. **Table 2** outlines the chemicals and conditions used for all soaks. Four samples of each material per solution were tested.

Table 1. Materials of construction soaked in chemical solutions.

Material Sample	Material Description
Feed and permeate border/seal	Linear low density polyethylene (LLPE)
End Cap	Polypropylene
Jacket	Polypropylene
End Cap Seal Material	Thermoplastic elastomer (TPE)

Table 2. Chemical solutions and conditions used in experiments.

Soak Solution	Time	Temperature
Open to Air (control)	1200 h	Ambient
70% Isopropanol/H_2O	1200 h	Ambient
40% Ethanol/H_2O	1200 h	Ambient
1% Triton®X-100/H_2O	1200 h	45 °C
30% Acetone/H_2O	1200 h	Ambient
30% Acetonitrile/H_2O	1200 h	Ambient
4% Benzyl Alcohol/H_2O	1200 h	Ambient

1. The mass, hardness (durometer*), and physical appearance of each material sample (four of each type) were recorded before all chemical soaks.
2. Each material sample was soaked in a chemical solution for 1200 hours.
3. After the soaks, each sample was removed from the soak solution, rinsed, and allowed to completely air dry before the mass, hardness, and physical appearance 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, and the thermoplastic elastomer sample was assessed using the Shore A Scale.*

Calculations

Change in Mass

The change in mass for each material sample (four samples in total) exposed to each chemical soak was calculated as follows:

$$\Delta m = \frac{m_f - m_i}{m_i} \times 100\%$$

Where:
Δm = change in sample mass (%)
m_f = mass of the sample after chemical soak
m_i = mass of the sample before chemical soak

Change in Hardness

The change in hardness (durometer) for each material sample (four samples in total) exposed to each chemical soak was calculated as follows:

$$\Delta h = \frac{h_f - h_i}{h_i} \times 100\%$$

Where:
Δh = change in sample hardness (%)
hf = hardness of sample after chemical soak
hi = hardness of sample before chemical soak

Results

Mass Analysis

Changes in mass of each material soaked in selected chemical solutions are shown in **Figure 1**. A negative value indicates loss of mass during chemical exposure, whereas a positive value indicates gain in mass.

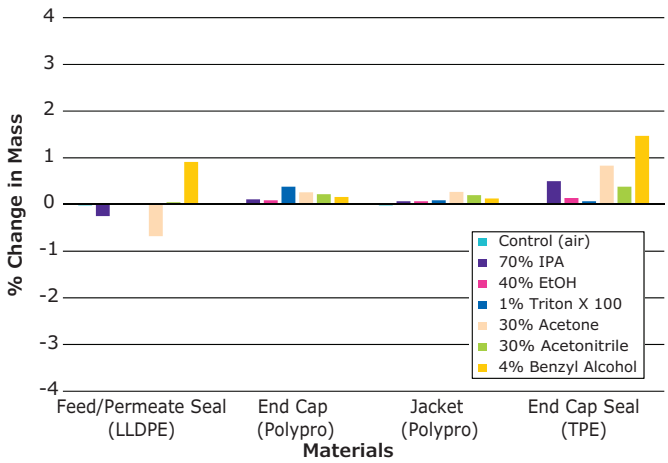

Figure 1. Change in mass of tested materials of construction after chemical soaks.

Physical Appearance Analysis

No changes in color or texture were observed for any of the cassette materials tested after soaking in the selected solutions.

Hardness Analysis

Figure 2 illustrates the results of change in hardness for each tested material after the chemical soaks. A negative value indicates that the material got softer during chemical exposure, whereas a positive value indicates that the material got harder.

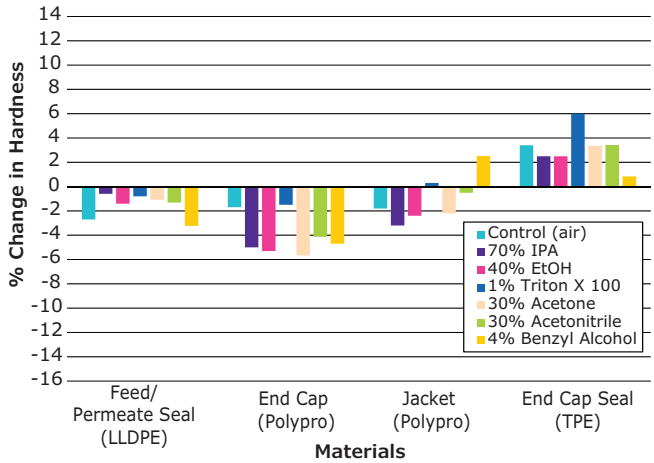

Figure 2. Change in hardness of tested materials of construction after chemical soaks.

Conclusion

All tested cassette materials of construction, which excluded the Ultracel® membrane, displayed a change in mass of less than 1.5% and change in hardness of 6% or less, in all cases. However, it is important to note that the control sample demonstrated approximately ±3% variability in hardness. The overall changes in mass and hardness are considered minimal and should not affect cassette performance.

Effect of Holder Compression

Objective

To determine the effects of cassette holder compression on the pressure drop and air integrity of cassettes.

Summary

This study evaluated the effect of holder compression on the pressure drop and air integrity of cassettes at selected torque or force values. The potential effect of compression lower than recommended on the cassettes is loss of internal and external sealing. At compression higher than recommended, the potential effects on cassettes are increased pressure drop, reduced permeability, and eventual membrane damage. In this study, the cassettes were subjected to holder torque or forces that are within or outside of the recommended compression for Pellicon® 3 cassettes. Both pressure drop and air integrity were maintained throughout all applied holder compressions, demonstrating the robustness of the cassettes.

Method

Method for Cassettes with D Screen

1. Three Pellicon® 3 cassettes with D screen of each size were tested across a range of holder torques, indicated in **Table 1**.
2. The cassettes were installed into a manual holder and the compression on the cassette was set by adjusting torque, starting with the lowest selected torque value.
3. Each cassette was flushed of storage solution with reverse osmosis (RO) water and assessed for pressure drop at 6 L/min/m^2 average feed flow rate.
4. The cassettes were then subjected to air integrity testing to determine the effect of holder compression on air diffusion.
5. Air flow was measured upstream of the cassette by using a mass flow transducer at test pressures of 30 psi and 100 psi. One minute for flow stabilization was allowed between readings.
6. The procedure was repeated at increased torques for a total of three tested torques: below, within, and above the recommended holder compression range.

Table 1. Torque range evaluated in experiments for cassettes with D screen.

Membrane Area	Tested Torque (in-lb)	Estimated Force (lbf)
88 cm^2, 0.11 m^2	150-250	4000-6600
0.57 m^2, 1.14 m^2	250-450	8000-14400

Method for Cassettes with C Screen

7. For cassettes with C screen, one cassette of each size and each cutoff (10 kDa and 30 kDa) was tested across a range of holder torques or holder forces, according to **Table 2**.
8. Each cassette was installed into the appropriate holder and the compression on the cassette was set by adjusting torque (manual holders) or force (hydraulic holders).
9. To determine the effect of holder compression on pressure drop, sizes 88 cm^2 and 0.11 m^2 were installed into manually torqued holders and sizes 0.57 m^2 and 1.14 m^2 were installed into hydraulic compressed holders.
10. The cassettes were flushed of storage solution with RO water. The feed and retentate flows were controlled to achieve a pressure drop of 15 psi; the permeate flow was unrestricted.
11. The cassettes were removed from their hydraulic testing stand and mounted into the holder on the Air Integrity Test Stand and compressed either manually or hydraulically, according to their size, as above.
12. The cassettes were subjected to air integrity testing to determine the effect of holder compression on air integrity.
13. Air flow was measured upstream of the cassette by using a mass flow transducer at test pressures of 30 psi and 100 psi. One minute for flow stabilization was allowed between readings.
14. The procedure was repeated at increased holder torques or forces, for a total of three tested compression values: below, within, and above the recommended operational holder compression range.

Table 2. Compression range evaluated in experiments for cassettes with C screen.

Membrane Area	Tested Compression
88 cm^2, 0.11 m^2	150-250 in-lb
0.57 m^2, 1.14 m^2	8230-13230 lbf

Results

Results for cassettes with D screen

For cassettes with D screen, the results of pressure drop at 6 L/min/m^2 and air integrity at 30 psi and 100 psi are shown in **Table 3** and **Table 4**, respectively. Three cassettes of each size were tested (#1, #2, #3). All cassettes were tested at three different torques (in-lb): below, within, and above the recommended torque compressions. Both pressure drop and air integrity values were within the Certificate

of Quality release specifications and maintained throughout all applied torques, demonstrating the robustness of the cassettes for consistent hydraulic and sealing performance.

Table 3. Effect of holder torque on pressure drop of cassettes with D screen.

Cassettes	Torque (in-lb)	Pressure Drop (psi) at 6 L/min/m²		
		#1	#2	#3
P3C030D00 (88 cm²)	150	3.4	4.4	4.0
	190	3.2	4.4	3.9
	250	3.3	4.6	4.0
Specification	**180-200**	**2-6**		
P3C030D01 (0.11 m²)	150	4.2	4.2	2.8
	190	4.4	4.6	3.4
	250	4.5	4.4	3.0
Specification	**180-200**	**2-6**		
P3C030D05 (0.57 m²)	250	4.4	4.9	5.7
	350	4.1	4.9	5.8
	450	4.1	5.1	5.6
Specification	**350-400**	**2-6**		
P3C030D10 (1.14 m²)	250	4.3	5.0	5.7
	350	4.8	5.1	5.7
	450	4.4	4.9	5.7
Specification	**350-400**	**2-6**		

Table 4. Effect of holder torque on air integrity of cassettes with D screen.

Cassettes	Torque (in-lb)	Air Diffusion (cc/min) at 30 psi			Air Diffusion (cc/min) at 100 psi		
		#1	#2	#3	#1	#2	#3
P3C030D00 (88 cm²)	150	0	0	0	11	10	2
	190	0	0	0	10	8	2
	250	0	0	0	8	5	3
Specification	**180-200**	**≤4**			**≤1950**		
P3C030D01 (0.11 m²)	150	2	0	2	30	10	10
	190	2	2	1	28	40	13
	250	3	3	2	20	40	13
Specification	**180-200**	**≤14**			**≤1950**		
P3C030D05 (0.57 m²)	250	16	14	15	94	97	101
	350	8	6	10	92	88	97
	450	20	11	12	84	93	98
Specification	**350-400**	**≤60**			**≤500**		
P3C030D10 (1.14 m²)	250	50	32	12	276	206	178
	350	38	27	26	261	198	184
	450	49	31	24	265	210	177
Specification	**350-400**	**≤117**			**≤1000**		

Results for cassettes with C screen

The results for average feed flow rate and permeability as a function of torque (in-lb) at constant pressure drop for cassettes with C screen, sizes 88 cm² and 0.11 m², are shown in **Table 5**. The results for average feed flow rate and permeability as a function of force (lbf) at constant pressure drop for cassettes with C screen, sizes 0.57 m² and 1.14 m², are shown in **Table 6**. Three different compression values were applied to the cassettes via manual holder (for sizes 88 cm² and 0.11 m²) or hydraulic holder (for sizes 0.57 m² and 1.14 m²) compression.

At a constant pressure drop of 15 psi, the average feed flow (Q_{avg}) changed by approximately 2.5% or less for sizes 0.57 m² and 1.14 m² and 7% or less for sizes 88 cm² and 0.11 m² when the applied holder compression was outside of the recommended range. In addition, the observed fluctuations on water permeability and flux were negligible, further showing that no damage occurs to the membrane when the applied force is outside of the suggested operating range.

Table 5. Effect of holder torque on cassettes with C screen, sizes 88 cm² and 0.11 m².

Cassettes	Torque (in-lb)	Q_{avg} (mL/min)	dP (psi)	TMP (psi)	Jw (L/m²/psi)
P3C010C00 (10 kDa, 88 cm²)	150	54.1	15.2	12.7	4.2
	190	53.7	14.6	12.7	5.1
	250	48.8	15.3	12.7	4.7
P3C030C00 (30 kDa, 88 cm²)	150	70.3	15.1	12.7	8.3
	190	71.8	14.8	12.7	8.6
	250	67.3	15.0	12.6	9.2
P3C010C01 (10 kDa, 0.11 m²)	150	1014.8	15.1	12.7	6.2
	190	973.5	15.3	12.7	6.0
	250	942.8	15.2	12.8	6.0
P3C030C01 (30 kDa, 0.11 m²)	150	958.2	15.2	12.6	10.0
	190	917.8	15.0	12.5	9.3
	250	895.4	15.0	12.6	9.2
Recommended Torque Compression: 180-200 in-lb					

Table 6. Effect of holder force on cassettes with C screen, sizes 0.57 m² and 1.14 m².

Cassettes	Holder Force (lbf)	Qavg (L/min)	dP (psi)	TMP (psi)	Lp (LMH/ psi)
P3C010C05 (10 kDa, 0.57 m²)	8230	3.70	15.1	14.6	3.0
	10699	3.60	15.1	15.1	3.0
	13168	3.50	15.2	15.5	3.0
P3C030C05 (30 kDa, 0.57 m²)	8230	7.10	15.1	12.8	10.0
	10699	6.90	15.2	13.2	9.9
	13168	6.80	15.2	13.5	9.8
P3C010C10 (10 kDa, 1.14 m²)	8230	3.80	15.2	14.9	11.4
	10699	3.80	15.3	15.3	11.5
	13168	3.70	15.2	15.7	11.4
P3C030C10 (30 kDa, 1.14 m²)	8230	8.50	15.0	13.5	4.4
	10699	8.40	15.1	14.2	4.3
	13168	8.20	15.3	14.8	4.3
Recommended Force Compression: 10000-11000 lbf					

The results of air integrity at 30 psi and 100 psi for cassettes with C screen are shown in **Table 7** for sizes 88 cm² and 0.11 m² and in **Table 8** for sizes 0.57 m² and 1.14 m². Air integrity of cassettes was maintained throughout the compression range applied, either as a function of torque (for sizes 88 cm² and 0.11 m²) or as a function of hydraulic force (for sizes 0.57 m² and 1.14 m²), passing the Certificate of Quality release specifications for air integrity at all compressions evaluated. Thus, the robustness of Pellicon® 3 cassettes protect the sealing integrity at the tested compressions, allowing the user flexibility with torque or force.

Table 7. Effect of holder torque on air integrity of C screen cassettes, sizes 88 cm² and 0.11 m².

Cassettes	Torque (in-lb)	Air Diffusion (cc/min) at 30 psi	Air Diffusion (cc/min) at 100 psi
P3C010C00 (10 kDa, 88 cm²)	150	1	7
	190	1	6
	250	1	7
Specification	**180-200**	**≤3**	**≤1950**
P3C030C00 (30 kDa, 88 cm²)	150	2	68
	190	4	74
	250	4	78
Specification	**180-200**	**≤4**	**≤1950**
P3C010C01 (10 kDa, 0.11 m²)	150	4	25
	190	5	27
	250	5	27
Specification	**180-200**	**≤9**	**≤1950**
P3C030C01 (30 kDa, 0.11 m²)	150	3	106
	190	11	124
	250	12	134
Specification	**180-200**	**≤14**	**≤1950**

Table 8. Effect of holder force on air integrity of cassettes with C screen, sizes 0.57 and 1.14 m².

Cassettes	Holder Force (lbf)	Air Diffusion (cc/min) at 30 psi	Air Diffusion (cc/min) at 100 psi
P3C010C05 (10 kDa, 0.57 m²)	8230	12	84
	10730	11	84
	13230	12	81
Specification	**10000-11000**	**≤32**	**≤500**
P3C030C05 (30 kDa, 0.57 m²)	8230	12	114
	10730	16	112
	13230	16	113
Specification	**10000-11000**	**≤60**	**≤500**
P3C010C10 (10 kDa, 1.14 m²)	8230	30	161
	10730	29	151
	13230	27	150
Specification	**10000-11000**	**≤60**	**≤1000**
P3C030C10 (30 kDa, 1.14 m²)	8230	49	407
	10730	64	413
	13230	67	409
Specification	**10000-11000**	**≤117**	**≤1000**

Conclusion

Both pressure drop and air integrity were maintained throughout all applied holder compressions, demonstrating the robustness of cassettes for consistent hydraulic and sealing performance. The manual and hydraulic compression results show no significant changes in air flow with increasing compression, demonstrating no membrane damage occurs within the compression range investigated. In addition, no significant changes in air flow with decreased compression were observed, demonstrating that no venting of internal or external seals occurs within the compression range investigated. Overall, the results show the cassettes' resistance to manual or hydraulic compression within the compression range that was investigated, allowing the user flexibility with holder torque or force.

Sources of Information

1. Pellicon® 3 Cassettes Installation and User Guide. Lit. No. AN1065EN00.
2. Pellicon® 3 Cassettes with Ultracel® Membrane Data Sheet. Lit. No. DS1209EN00.
3. Ultracel® Membranes Data Sheet. Lit No. PF1401EN00.
4. Pellicon® 3 Cassettes with High Viscosity Feed Screen. Lit. No. AN4425EN00.
5. Performance Evaluation and Cleanability Study using Pellicon® 3 Cassettes with 30 kDa Biomax® and Ultracel® Ultrafiltration Membranes. Lit. No. TB1241EN00.
6. A Hands-On Guide to Ultrafiltration/Diafiltration Optimization using Pellicon® Cassettes. Lit. No. AN2700EN00.

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