Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Pellicon® Capsules with Ultracel® Membrane Performance Guide

Innovative, high performance single-use tangential flow filtration devices that offer ease-of-use, operational flexibility, and enhanced operator safety.

MilliporeSigma is the U.S. and Canada
Life Science business of Merck KGaA,
Darmstadt, Germany.

MilliporeSigma

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How to Use the Guide

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

Introduction

Pellicon® Capsules are innovative tangential flow filtration (TFF) devices for ultrafiltration and diafiltration of solutions that require single-use capabilities, including enhanced ease-of-use, process flexibility, fast product turnaround, and reduced operator exposure to harmful fluids. Pellicon® Capsules employ a self-contained, holderless design and come ready for processing within minutes. These single-use TFF filters are gamma sterilized with preservative-free reverse osmosis water, significantly reducing pre-use requirements. Offered with the C feed channel screen and Ultracel® membrane, Pellicon® Capsules are optimal for processes that require superior mass transfer and flux, including ultrafiltration and/or diafiltration of monoclonal antibodies, antibody drug conjugates, and recombinant and non-recombinant proteins. The capsules' design and automated manufacturing process provides performance consistency and linear scalability.

Protein Flux Performance, Scalability, and Retention

Objective

To evaluate the protein flux performance, scalability, and retention of capsules using a model protein stream.

Summary

A protein challenge that consisted of transmembrane pressure (TMP) excursions with 10 g/L bovine gamma globulin (BgG) was performed to evaluate protein flux performance and mass transfer comparability within the capsule family. The capsules demonstrated excellent scalability with consistent limiting flux data and mass transfer coefficients for all sizes, meeting the acceptance criterion for linear scalability of within 10% difference across sizes. In addition, BgG retention was evaluated, and the results demonstrated capsules exhibit excellent protein retention.

Method

Capsule performance scalability was assessed by determining protein flux performance and the mass transfer coefficient for all sizes. **Table 1** lists the capsules used in the experiments and their respective feed conditions. **Figure 1** depicts the system setup used in this study.

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

Catalog No.	Area	Feed Conditions
PCC030C01	0.1 m^2	10 g/L BgG in PBS at 6 L/min/m^2
PCC030C05	0.5 m^2	
PCC030C10G	1 m^2	
PCC030C15C	1.5 m^2	
PCC030C30G	3 m^2	
PCC030C30L & PCCO30C30E	6 m^2	
PCC030C45L & PCCO30C45E	9 m^2	

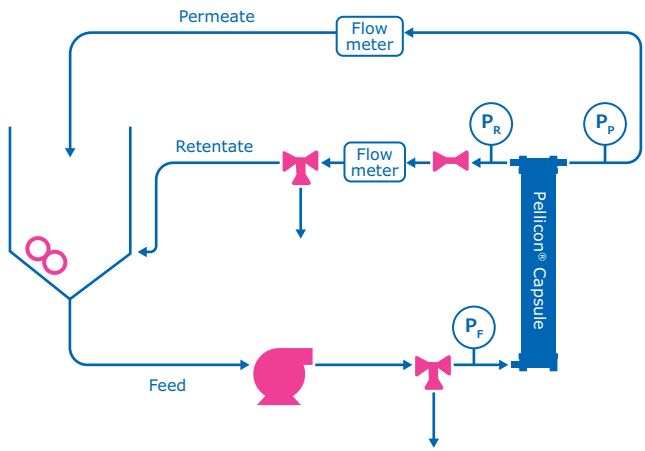

Figure 1. Schematic of TFF system setup.

1. The TFF system was conditioned by recirculating with phosphate buffered saline (PBS) buffer (10 L/m^2) for 30 min at feed flow rate of 6 L/min/m^2 with retentate flow restricted to achieve 30% conversion (permeate flow/feed flow).
2. 10% BgG in PBS buffer solution was recirculated in the TFF system at 6 L/min/m^2 and starting TMP of 5-7 psi.
3. Process parameters (solution temperature; inlet, outlet, and permeate pressures; and permeate and retentate flow rates) were recorded periodically as the retentate pressure was varied to increase TMP.
4. Process parameters measured during each flux excursion were used to characterize limiting flux and mass transfer performance (calculation of mass transfer coefficient).

Results

Flux Performance Analysis

At a given average feed flow rate, scalable capsules must offer equivalent performance. To assess scalability in terms of flux, the protein challenge of capsules consisted of a series of TMP excursions at a constant feed flow rate. The acceptance criterion for performance scalability requires all capsule sizes to have a mean flux within 10% of that of the 0.1 m^2 capsule. The study was performed at constant feed flow rate of 6 L/min/m^2 with BgG concentration of 10 g/L.

The BgG flux performance of capsules is shown in **Figure 2**. The results show average flux of all capsule sizes to be within 10% of the average flux of the 0.1 m^2 capsule in both the pressure dependent and pressure independent regions, demonstrating excellent flux performance scalability within the Pellicon® Capsule family.

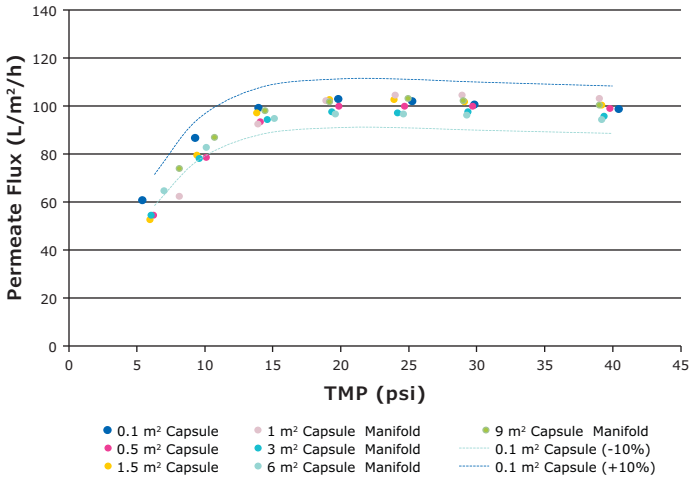

Figure 2. Permeate flux vs TMP of capsules processing a 10 g/L BgG solution.

Mass Transfer Analysis

While analysis of protein flux performance reflects on the performance of the capsules, the mass transfer coefficient considers performance at the protein level. As the transmembrane pressure promotes 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 capsule.

Accordingly, comparable and scalable TFF devices 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). Mass transfer coefficients can be determined using the limiting flux data from 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 J versus the natural log scale (ln) of C_b, k can be determined from the slope of the linear curve for each device when a constant feed flow rate is maintained.

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

Where:

J = *permeate flux (L/m^2/h [LMH])*

k = *mass transfer coefficent (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.

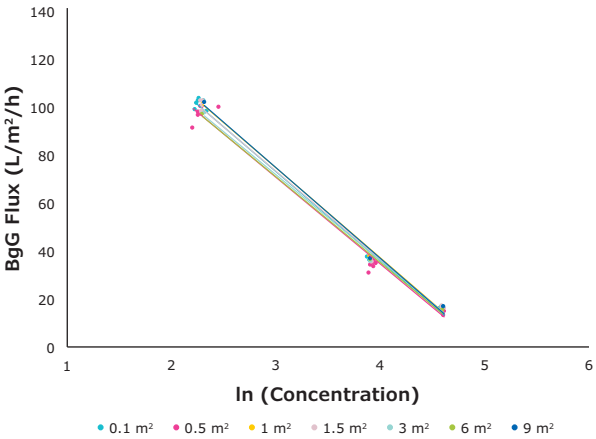

Figure 3. Flux vs ln BgG concentration trendline fit for mass transfer (slope) calculation of capsules.

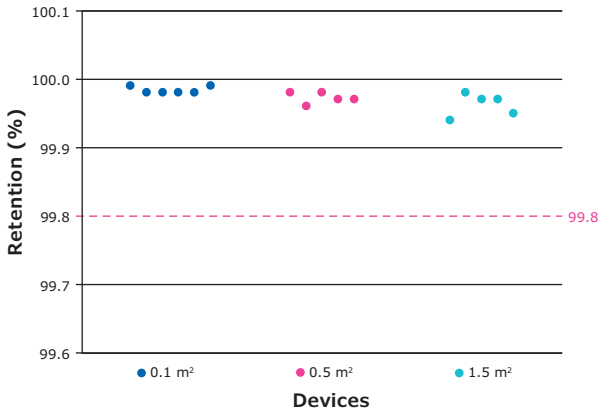

Figure 4. Protein retention analysis of capsules processing a 10 g/L BgG solution.

The flux versus natural log of protein concentration plot of capsules is shown in **Figure 3**. The flux decreases linearly with the natural log of concentration as the concentration of BgG increases, as shown in the graph. Best fitted regression lines were determined for each capsule size to obtain the mean mass transfer coefficients from their slopes, shown in **Table 2**.

Table 2. Average mass transfer coeffcients for Pellicon® Capsules.

Device Area	Mass Transfer Coefficient
0.1 m²	37.3
0.5 m²	36.1
1 m²	37.6
1.5 m²	36.3
3 m²	35.9
6 m²	35.7
9 m²	37.8

The results show the average mass transfer of all sizes to be well within 10% of the average mass transfer of the 0.1 m² capsule, demonstrating excellent scalability between all capsule sizes.

Protein Retention Analysis

Capsule retention was evaluated using 10 g/L BgG in PBS buffer at optimal TMP, determined during the flux excursion experiments. The results shown in **Figure 4** demonstrate capsules exhibit excellent protein retention of ≥99.8% using the model feed stream.

Conclusion

Pellicon® Capsules demonstrated excellent scalability across sizes while challenged with a model BgG protein stream. Scalability was evaluated by comparing BgG flux performance and mass transfer of capsules. The capsules met scalability targets by exhibiting permeate fluxes and mass transfer coefficients that are within 10% difference across sizes while at constant feed flow rate. In addition, the capsules exhibited protein retention greater than or equal to 99.8%.

Capsule Flushing and Leachables

Objective

To evaluate and characterize flushing and leachables content of capsules.

Summary

Pellicon® Capsules are supplied gamma sterilized and with preservative-free reverse osmosis (RO) water, enabling reduced device preparation requirements; sanitization of capsules is not needed, and the storage water can be flushed out immediately after installation. Experiments were performed to evaluate flushing and leachables of capsules through measurement of Total Organic Carbon (TOC). After dynamic flushing with 20 L/m² RO water, the capsules exhibited ≤5 ppm TOC. Leachables were quantified after a subsequent mock product concentration process and 1-hour hold of the resultant product pool.

Method

Capsule flushing and leachables content were evaluated through measurement of TOC. The experimental design is summarized in **Table 1**.

Table 1. Capsules and conditions used in this study.

Catalog No.	Flushing	Leachables
PCC030C01	Dynamic flushing with 20 L/m² RO water	10× mock UF processing, then 1-hour hold
PCC030C05		
PCC030C10G		
PCC030C15C		
PCC030C30G		
PCC030C30L & PCC030C30E		
PCC030C45L & PCC030C45E		

Flushing

1. Capsules were flushed with 20 L/m² MilliQ® water with the retentate and permeate lines directed into collection vessels. The feed flow rate was set to 2 L/min/m² and retentate pressure to 1-2 psi.
2. After 1-minute flow stabilization, retentate and permeate samples were collected. Additional samples were collected at ~1-minute intervals until the tank was empty.
3. Samples were analyzed for TOC content.

Leachables

1. After capsule flushing was completed, 20 L/m² MilliQ® water were recirculated at a feed flow rate of 6 L/min/m² and retentate pressure at 1-2 psi to achieve a 10× feed volume reduction.
2. Once the 2-liter feed volume was achieved, the feed was recirculated in total recycle mode at feed flow rate of 2 L/min/m² and retentate pressure of 1-2 psi.
3. After 5 minutes, a sample from the tank was taken and MilliQ® water of equivalent volume was added to the tank. A final sample was taken after 1 hour of total recirculation.
4. Both samples were analyzed for TOC content.

Results

During the flushing procedure, samples were collected from the retentate effluent and then analyzed for TOC and plotted against feed flow volume. For capsule size 0.1 m² (**Figure 1**), the results show the average TOC at the end of the 20 L/m² flush to be ~0.5 ppm after quickly decreasing from initial TOC levels. A similar trend was observed for all capsule sizes, including the pre-assembled manifolds, by meeting the ≤5 ppm TOC target at ~5 L/m² flush volume (**Figures 2–4**).

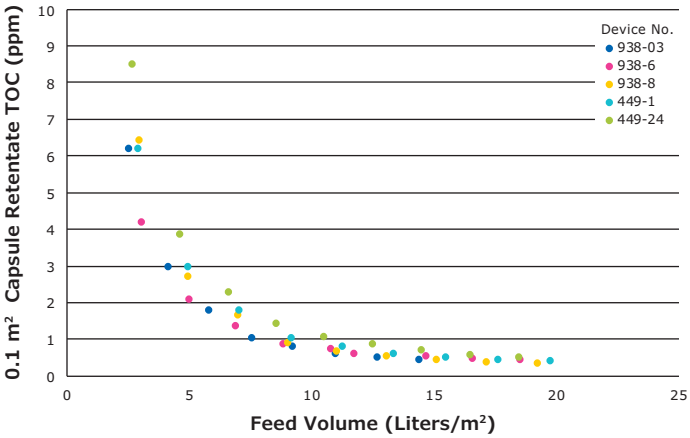

Figure 1. Evaluation of retentate TOC by feed volume for capsule size 0.1 m².

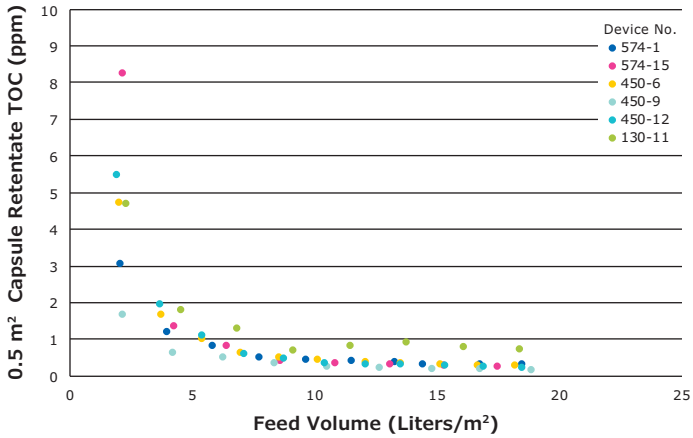

Figure 2. Evaluation of retentate TOC by feed volume for capsule size 0.5 m².

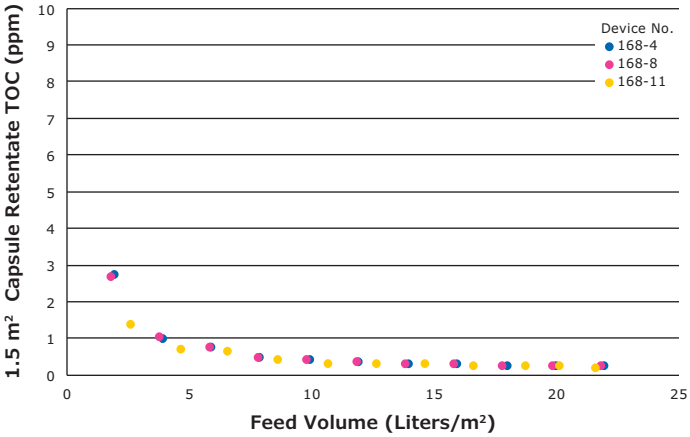

Figure 3. Evaluation of retentate TOC by feed volume for capsule size 1.5 m².

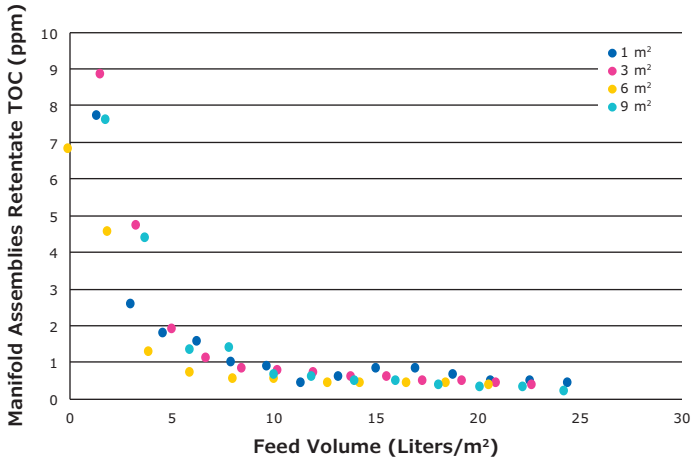

Figure 4. Evaluation of retentate TOC by feed volume for manifold sizes.

After the capsules were flushed with 20 L/m² water, a mock UF process was performed, consisting of concentrating 20 L/m² product pool of water to a final 2 L/m² product pool (10× concentration). Then, the retentate and permeate were recirculated (full recycle mode) to mimic a dynamic post-processing product hold. The product pool was sampled after the mock concentration procedure and after 1-hour total-recirculation hold for TOC analysis. The results after the 1-hour hold show TOC levels below ~22 mg/m². The results shown in **Figures 5–8** were normalized to represent a 1 L/m² product pool.

Conclusion

TOC levels for flushing and leachables in tested capsules are considered low after following the described procedures. The results support the benefit of using Pellicon® Capsules, which are supplied gamma sterilized and free of preservatives, in considerably reducing flushing volumes before product processing as well as leachables content during product processing.

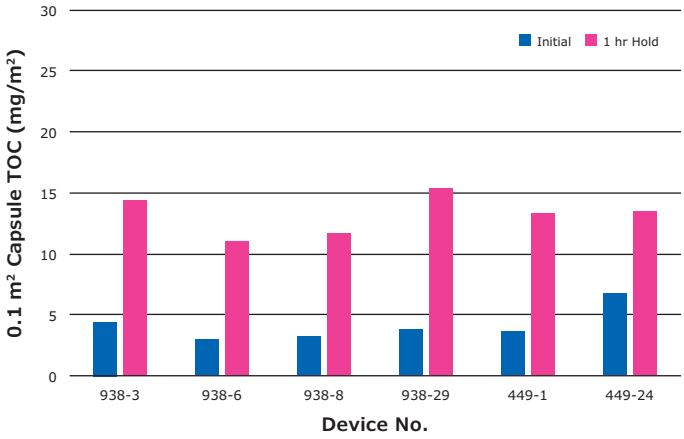

Figure 5. Evaluation of product pool TOC before and after mock product hold for capsule size 0.1 m².

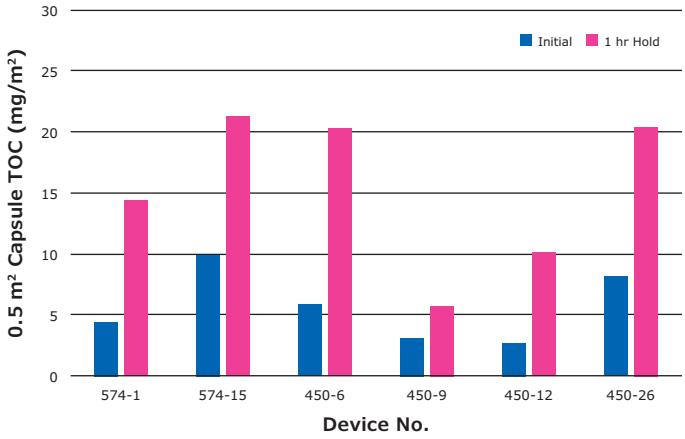

Figure 6. Evaluation of product pool TOC before and after mock product hold for capsule size 0.5 m².

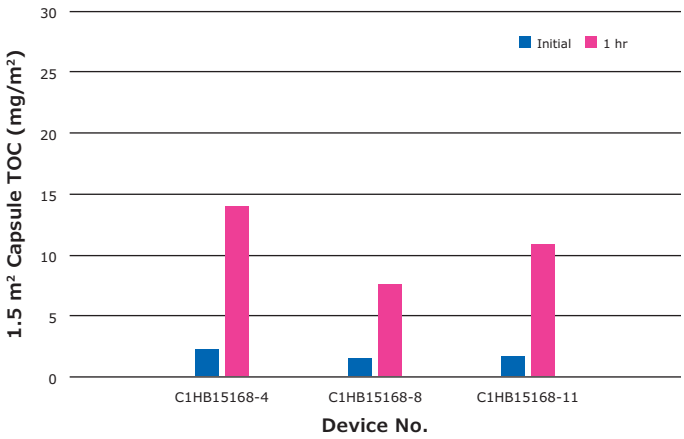

Figure 7. Evaluation of product pool TOC before and after mock product hold for capsule size 1.5 m².

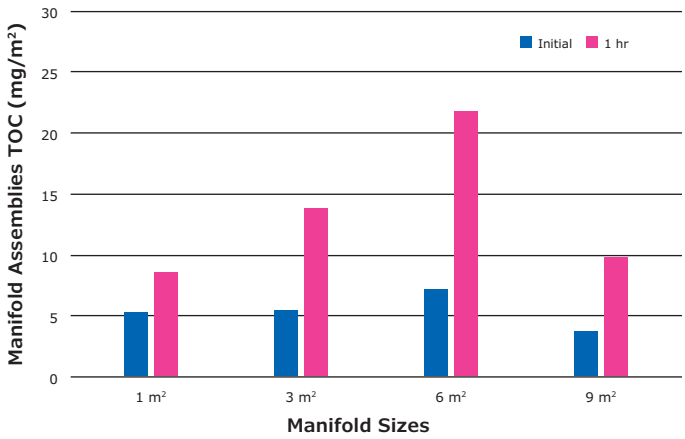

Figure 8. Evaluation of product pool TOC before and after mock product hold for manifold sizes.

Capsule Compatibility to DMAc and DMSO

Objective

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

Summary

Capsules were subjected to diafiltration of 20% DMAc and 20% DMSO and evaluated for their hydraulic and protein processing performance before and after solvent clearance. Pressure drop, air integrity, normalized water permeability (NWP), and protein flux and retention were stable after diafiltration, demonstrating the robustness of capsules when exposed to these solvents.

Method

Clearance of 20% DMAc and 20% DMSO by constant-volume diafiltration was performed using capsules with 30 kDa Ultracel® membrane. Pressure drop, air integrity, NWP, and protein flux and retention were evaluated before and after diafiltration to assess hydraulic and performance stability of the capsule upon exposure to the solvent. The experiment included evaluation of Pellicon® 3 cassettes to assess comparability during diafiltration of both filter formats. **Table 1** lists the devices used in this study.

Table 1. TFF filters used in this study.

Catalog No.	Area
PCC030C01 (capsule)	0.1 m^2
P3C030C01 (cassette)	0.11 m^2

1. Each capsule was flushed of storage water with reverse osmosis (RO) water. Cassettes were cleaned with 0.1 M NaOH and flushed with RO water before use.
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 optimal TMP.
4. After BgG evaluation, the filters were cleaned with 0.1 M NaOH and flushed with RO water to remove model protein residuals.
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. Each model solvent solution was 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 the recirculation.
7. A sample from the feed tank was collected for solvent 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 a 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.

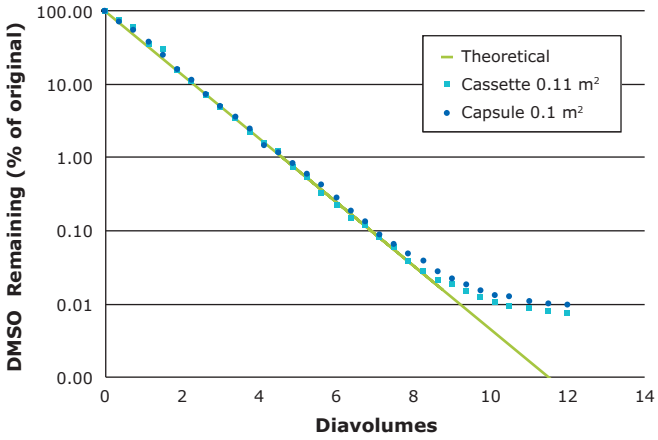

Figure 1. 20% DMSO clearance by diavolume using cassette and capsule formats.

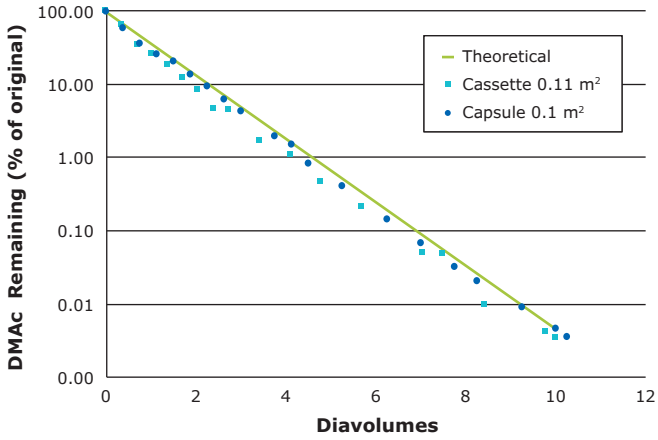

Figure 2. 20% DMAc clearance by diavolume using cassette and capsule formats.

Results

For each diafiltration experiment, the concentration of DMAc and DMSO in the feed tank was analyzed throughout the diafiltration process and used to plot solvent removal at various diafiltration volumes (diavolumes). The plots detail the remaining concentration of solvent normalized to the initial concentration (20% DMAc or 20% DMSO) throughout the diafiltration (**Figures 1 and 2**). Diafiltration performance of both capsule and cassette was compared to a theoretical process in which no solvent retention by the membrane is assumed, calculated with a sieving coefficient of 1.

The experimental data closely tracked the theoretical values, showing strong and comparable diafiltration performance of capsule and cassette through the clearance of DMSO and DMAc. Plausible explanations for the decrease in DMSO removal rate for both filter formats after ~8 diavolumes include presence of dead legs in the system and charge interactions.

To assess the effect of solvent exposure on capsules during diafiltration, pressure drop, air integrity, normalized water permeability, and protein flux and retention were evaluated before and after diafiltration of each solvent.

The protein flux performance of the capsule before and after diafiltration is shown in **Figure 3**. The permeate flux over a 20 g/L BgG challenge is comparable pre- and post-solvent exposure.

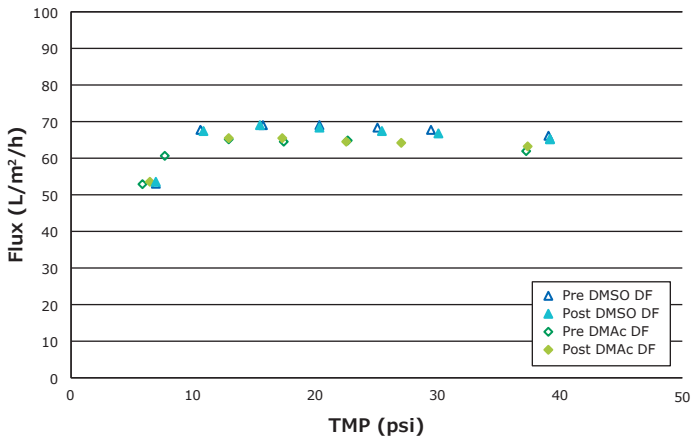

Figure 3. Flux vs TMP of capsule at 20 g/L BgG solution before and after DMSO and DMAc diafiltration.

Pressure drop and air diffusion values for the capsule are within the Certificate of Quality acceptance criteria before and after diafiltration, and fluctuations in membrane permeability are within standard test method variations. In addition, BgG retention was unaffected after solvent exposure, further demonstrating the compatibility of the device to both 20% DMAc and 20% DMSO (**Figures 4 and 5**). Overall, the data indicate no significant effects on these stability and performance parameters from exposure to either solvent.

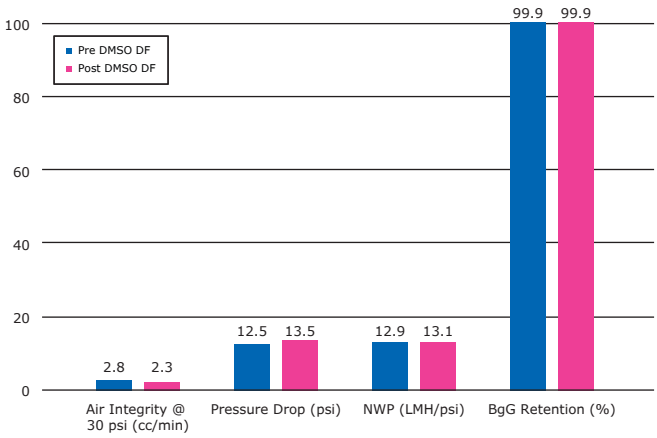

Figure 4. Air integrity, pressure drop, NWP, and protein retention analysis before and after DMSO diafiltration.

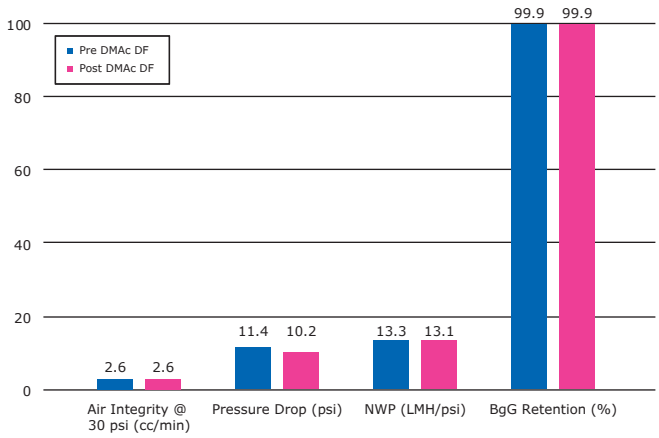

Figure 5. Air integrity, pressure drop, NWP, and protein retention analysis before and after DMAc diafiltration.

Conclusion

Diafiltration of 20% DMSO and 20% DMAc was used to mimic exposure of the capsule to the organic solvents. No adverse effects were observed on the pressure drop, air integrity, membrane permeability, and protein flux and retention of the capsule after diafiltration, indicating the compatibility of the feed channel screen, seals, and Ultracel® membrane to these solvents.

Hold-Up Volume of Capsules

Objective

To determine the hold-up volume of capsules.

Summary

Experiments were performed to characterize the hold-up volumes of capsules 0.1, 0.5, 1.5 m^2, as well as the tubesets used in the Pellicon® Capsule manifolds. Hold-up volumes in the feed channel were measured to indicate the recoverable volume within the feed channel. The total hold-up volume of capsules and manifold tubesets was measured to help the user determine the minimum working volumes required to operate their systems.

Method

Capsule Devices

Hold-up volumes for Pellicon® Capsule device sizes 0.1, 0.5, and 1.5 m^2 were evaluated according to the procedure outlined below. Three capsules of each size were used for testing.

1. Reverse osmosis water was recirculated through the capsule for 5 minutes at a feed pressure of 20 psi, retentate pressure of 15 psi, and permeate pressure of 10 psi.
2. The feed, retentate, and permeate ports of the capsule were capped, making sure water inside the capsule was not lost.
3. The Initial Wet Capsule Weight was weighed and recorded.
4. The caps from the feed and retentate ports were removed and compressed air was blown down the feed channel at 10 psi for 3 minutes.
5. The caps were placed back onto the feed and retentate ports of the capsule to weigh and record the Post Feed Channel Blow Down Weight.
6. The cap from the permeate port was removed and the capsule was inverted. The capsule was shaken to remove as much water as possible from the permeate channel.
7. The cap was placed back onto the permeate port of the capsule to weigh and record the Post Permeate Channel Blow Down Weight.
8. The caps from the feed, retentate, and permeate ports were removed and compressed air was blown down through the capsule at 10 psi for ≥ 12 hours.
9. The caps were placed back onto the feed, retentate, and permeate ports to weigh and record the Final Dry Capsule Weight. The Initial and Final Dry Capsule Weights were compared to ensure that the capsule was completely dry.

Capsule Manifolds

Following the measurement of device hold-up volumes, manifold tubeset hold-up volumes were determined according to the procedure below.

1. An empty, dry tubeset was weighed and recorded to obtain the Dry Tubeset Weight.
2. Reverse osmosis water was circulated through the tubesets. The tubeset ports were capped to ensure water was not lost and all air was removed.
3. The Wet Tubeset Weight was weighed and recorded.
4. The Dry Tubeset Weight was then subtracted from the Wet Tubeset Weight to obtain the hold-up volume of the tubeset.

After measuring the hold-up volumes of the tubesets, the hold-up volumes of fully pre-assembeled Pellicon® Capsule manifolds were calculated by adding the corresponding tubeset hold-up volumes to the hold-up volume of capsule devices used in the assembly configuration.

Results

All weights were converted to volumes, assuming one gram of water equals one milliliter of water.

1 g H_20 = 1 mL H_20

Calculations for hold-up volume of individual capsule devices were as follows:

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

Permeate Channel Hold-up Volume = Post Feed Channel Blow Down Weight — Final Dry Capsule Weight.

Total Capsule Hold-up Volume = Feed Channel Hold-up Volume + Permeate Channel Hold-up Volume

Manifold hold-up volumes were calculated by adding the hold-up volumes of each individual capsule and tubeset in an assembly unit. For example, a 3 m^2 manifold consists of two 1.5 m^2 capsule devices and three tubesets (feed, retentate, permeate). Calculations for hold-up volume of manifolds were as follows:

Tubeset Hold-up Volume = Wet Tubeset Weight - Dry Tubeset Weight

Manifold Assembly Feed Channel Hold-up Volume = (Number of Capsules in Manifold * Feed Channel Hold-up Volume of Capsule) + (2 * Tubeset Hold-up Volume)

Manifold Permeate Channel Hold-up Volume = (Number of Capsules in Manifold * Permeate Channel Hold-up Volume of Capsule) + Tubeset Hold-up Volume

All calculated volumes for device and manifold sizes are presented in **Table 1**.

Table 1. Hold-up volume results.

Catalog No.	Membrane Area (m^2)	Feed Channel Hold-up Volume (mL)	Permeate Channel Hold-up Volume (mL)	Total Device Hold-up Volume (mL)
Pellicon® Capsule Devices				
PCC030C01	0.1	26	62	88
PCC030C01C		38	68	106
PCC030C05	0.5	107	143	250
PCC030C05C		119	149	268
PCC030C15C	1.5	455	719	1174
Pellicon® Capsule Manifolds				
PCC030C10G	1	254	306	560
PCC030C30G	3	1108	1537	2645
PCC030C30L		1128	1547	2675
PCC030C30E		1288	1627	2915
PCC030C45G	4.5	1665	2307	3972
PCC030C45L		1683	2316	3999
PCC030C45E		1857	2403	4260

Conclusion

Hold-up volumes in the feed channel of Pellicon® Capsules were characterized to indicate their recoverable volume. The total capsule hold-up volume indicates the volume contained within the device during a TFF process and is provided to help the user determine the minimum working volume required to operate their systems.

Further Information

1. Pellicon® Capsule Datasheet. Lit. No. DS1285EN.
2. Pellicon® Capsule User Guide. Lit. No. UG1549EN.
3. Ultracel® Membranes Data Sheet. Lit No. PF1401EN00.

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