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Application Note

Process Optimization and Scalability Evaluation of Pellicon® Capsules for Single-Pass Tangential Flow Filtration of mAb-Based Biomolecules

Introduction

Single-pass tangential flow filtration (SPTFF) is a method of ultrafiltration for the purposes of either volume reduction or protein concentration. This versatile method has been successfully implemented between unit operations throughout the downstream production process of biopharmaceuticals. Its value lies in the simplicity of operation as well as its small footprint and equipment requirement. This document describes the principles of SPTFF and how to determine operating conditions to achieve the target concentration factor with single-use Pellicon® Capsules.

SPTFF differs from traditional batch tangential flow filtration (TFF) in several ways. Traditional batch TFF requires recirculation of the feed material through the membrane module multiple times to achieve a higher concentration with each passage as the filtrate is removed. SPTFF uses just one pass through multiple membrane modules connected in series—increasing the path length—to achieve the same volume reduction over time as the traditional batch mode. Due to the single-pass operation, there is no need to return the feed to the original tank, which in many cases can eliminate tankage altogether. Further, SPTFF is typically run at lower feed flow rates to increase conversion of feed to permeate; this allows for smaller pumps and pipe sizes, which reduce hold-up volume within the system and increases product recovery compared to batch TFF. SPTFF is also a gentler option for shear sensitive molecules.

Pellicon® Capsules are ideally suited for single-use SPTFF operation:

- Easily arranged in series for SPTFF operation by connecting the retentate of the first device to the feed of the next, and so forth (Figure 1).
- Gamma-sterilized and preservative-free: no sanitization step is needed; the SPTFF assembly can be used for product processing after a small conditioning flush.

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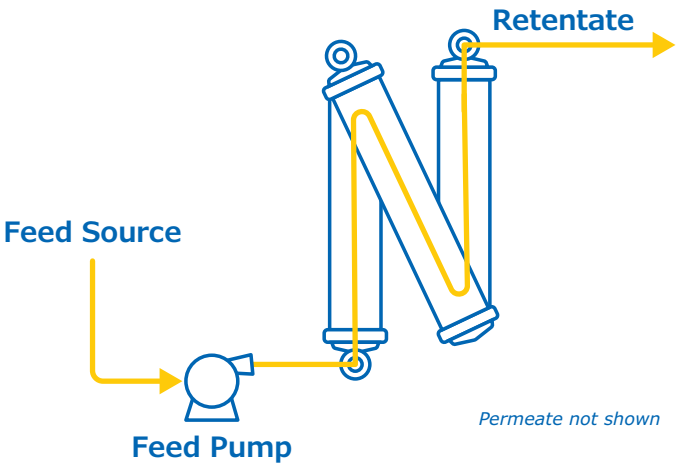

Figure 1. Three capsules in series setup.

SPTFF Operation

Conversion is the permeate flow rate divided by the feed flow rate, or equivalently the permeate flux divided by the feed flux, where flux is flow rate divided by membrane area. The permeate flux increases with transmembrane pressure (TMP) until the maximum—based on the gel model (Equation 1)—is reached (Figure 2), after which TMP is no longer a factor. For SPTFF, TMP is adjusted by increasing the retentate pressure.

The gel model in Equations 1 and 2 shows that the maximum flux will decrease as the feed flux slows down, but that the conversion, and hence the concentration factor, will increase. Therefore, the retentate concentration can be increased by decreasing the feed flow rate in an SPTFF operation. Choosing the optimal pressure, one which maximizes permeate flux without risk of fouling and allows good control, will result in long-duration operation with stable output.

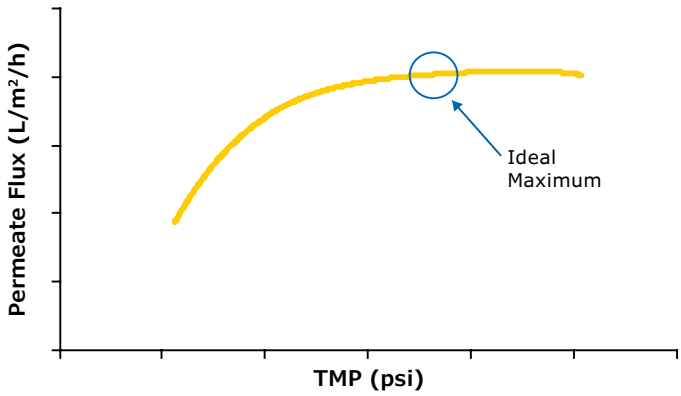

Figure 2. Typical batch TMP excursion showing example of ideal operating point for SPTFF.

$$J_P = k_o J_F^n \ln\left(\frac{C_{gel}}{C}\right)$$

Equation 1: Gel model, flux.

Equation 1 applies at any point along the feed path in the device: where J_P = permeate flux; J_F = feed flux; k_o = mass transfer coefficient at the reference feed flux (J_{Fo}); n = flow exponent 0.33 – 0.8; C_{gel} = constant, maximum wall concentration; C = concentration.

$$Y = \frac{k_o}{J_F^{1-n}} \ln\left(\frac{C_{gel}}{C}\right)$$ where Y = conversion, $\frac{J_P}{J_F}$, for any point along the feed path.

Equation 2: Gel model, conversion.

Since n < 1, Y is inversely related to the feed flux, J_F. The conversion for the whole SPTFF assembly is the average permeate flux for the assembly divided by the inlet feed flux.

Conversion and concentration factor are related as follows: Y = 1 – 1/X, and X = 1/(1 – Y) where X is volumetric concentration factor, Q_F/Q_R.

An SPTFF evaluation consists of these basic steps:

1. Set-up the filter assembly by installing the Pellicon® Capsules in series
2. Establish operating conditions:
   - determine optimal retentate pressure at a baseline test flow rate
   - conduct feed flux excursions to obtain target conversion
3. Confirm stability by conducting a single-pass process simulation at the target conversion

These steps are explained further below. Additional details can also be found in references 1 and 2.

Installing Pellicon® Capsules

The Pellicon® Capsules are first configured with the target number of sections (capsules installed in series), typically three sections, where each section must be of equal area, for a full exploration of the process window. It is recommended that the system is configured in total recycle mode (retentate and permeates returned to feed tank) to minimize feed volume required during extended run time when setting operating conditions. The retentate of the first capsule in series will be connected directly to the feed of the second and subsequently alternated for each additional capsule in series, as shown in Figure 1. This configuration will create the elongated feed channel.

Establishing Operating Conditions

To find the optimal retentate pressure, the feed flux is typically set to 1 LMM (liters/min per m^2) and the conversion is monitored starting from lowest retentate pressure (e.g. retentate valve fully open), which is then increased incrementally until the maximum desirable conversion is reached, the system becomes hard to control or unstable, or the flux stops increasing. The endpoint will depend on your application. Before setting final operating pressure conditions, it is important to allow sufficient time for the polarization to fully develop and flux to stabilize—stabilization time range is generally 1–30 minutes, depending on protein concentration and other process conditions and is indicated by stable pressure readings.

The retentate pressure is increased in small increments (e.g. 1–2 psi) until an inflection in the curve is observed (Figure 3). If the retentate valve is completely shut off or TMP extends too far into the plateau region, the membrane will foul. An example of flow ratio of feed to retentate (concentration factor) vs retentate pressure curve can be seen in Figure 3. In this example the ideal pressure setpoint is around 2 psi for the 0.1 m^2 Pellicon® Capsule and 5 psi for the 0.11 m^2 Pellicon® 3 cassette. At this setpoint combination there is an equivalent conversion between the device formats and tolerance for small changes in retentate pressure.

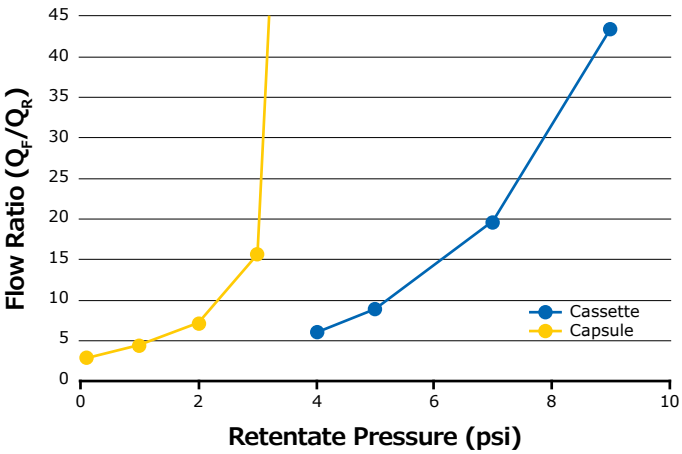

Figure 3. Example of optimal pressure curves for 1 g/L feed at 1 L/min/m^2.

Once an optimal retentate pressure is found, it must be kept consistent throughout the experiments but can be confirmed once the desired feed flow rate is established to ensure a robust process. Note that more dilute feeds generally require lower retentate pressure for a given conversion.

Feed flux excursions are then conducted using the established optimal retentate pressure and starting at 1 LMM (depending on desired conversion, the operator may choose to run at higher feed flow rates if conversion is too high at 1 LMM). For a 3-section series, the whole assembly is run, and individual permeate flows are recorded to calculate conversion for each section: 1 section, 2 sections (sections 1 and 2), and 3 sections (sections 1 through 3), at the different feed flow rates. The feed flow rate for the process is determined based on the required conversion and number of sections. This will vary from process to process depending on the desired outcome. This data is plotted to reveal the optimal feed flow rate for operation at the desired conversion.

Confirmation of Stability

Using the selected operating conditions and number of sections, stability is confirmed by conducting a single-pass process simulation. At this point, it is recommended to start with fresh feed material.

After equilibration, the feed is pumped through the capsule SPTFF assembly at the determined optimal conditions until the conversion and pressure profile become steady. This can be done in single-pass mode or in total recycle mode if there is insufficient feed volume. Stabilization time will depend on feed conditions and chosen feed flow rates. Note that initial dilution may be needed if steady state operation is achieved via single-pass mode. This process should take between 1 and 30 minutes but can vary outside this range. Once stable, the retentate is collected in single-pass mode and the conversion is monitored over the target run time.

Scaling

Scaling to or from a 0.1 m^2 Pellicon® Capsule to either another capsule size or a Pellicon® Cassette can be easily achieved by keeping the feed flux (set by the pump) and pressure drop across the feed channel (set by retentate valve at the given feed flux) the same between scales. This will result in a new optimal retentate pressure setpoint. Alternatively, the optimal retentate pressure can be re-established from scratch at the new scale.

For ease-of-use, the former method is recommended, where the same feed channel pressure drop is maintained across scales. By maintaining pressure drop and feed flux, the conversion will remain consistent. The capsule will scale within its own family or to cassettes without the need to re-establish the optimal retentate pressure.

Case Study 1: Pellicon® Capsule vs Pellicon® 3 Cassette on Bovine gamma Globulin (BgG)

Bench-scale studies were carried out to evaluate the effectiveness of Pellicon® Capsules 0.1 m² for use in SPTFF operation compared to Pellicon® 3 cassettes 0.11 m². A bovine gamma globulin (BgG) solution was used as a model antibody feed. The capsules and cassettes were run side-by-side in SPTFF mode. In each case, the optimal retentate pressure was determined before running feed flux excursions and a final conversion stability study was conducted.

Optimal retentate pressure determinations were carried out on both the Pellicon® Capsule and Pellicon® 3 cassette SPTFF setups for 1 g/L and 25 g/L BgG feeds using the methodology described above. The ideal retentate pressure setting is lower for the lower feed concentration. This is an important note as feed conditions have a direct impact on the performance of the SPTFF operation and should be checked if there is a change in feed composition. In addition to the change associated with feed, it can also be seen from the data in Figure 4 that there is a different retentate pressure requirement between the capsule and cassette. Yet, as described in Scaling, maintaining pressure drop and feed flux will allow the conversion to remain consistent between both filter formats.

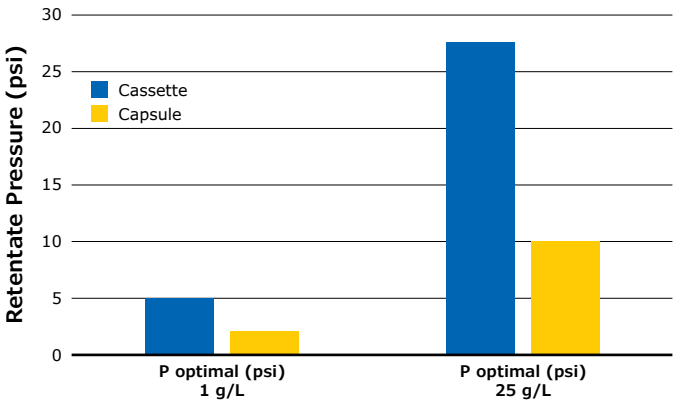

Figure 4. Optimal retentate pressure for SPTFF setups, 3 in series.

Feed flux excursions were completed for the 25 g/L feed solution at the established optimal retentate pressures starting at a feed flux of 1 LMM and decreasing at desired intervals to 0.14 LMM. The feed flux curves for the 25 g/L feed solution are compared in Figure 5. Although the highest conversion was achieved at 0.14 LMM by the third capsule in series ●, the plotted data points of both cassette and capsule formats depict comparable performance over the course of the feed flux excursions.

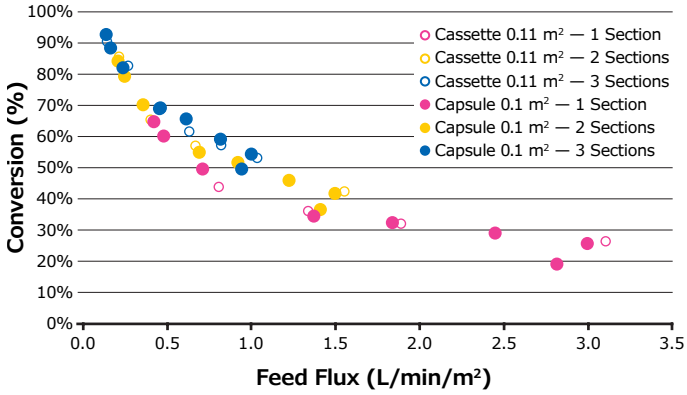

Figure 5. Feed flux excursion for 25 g/L BgG feed solution.

Following the feed flux evaluation, a stability study was completed to demonstrate process stability and overall conversion for each system. For this run, the flow rate that gave the highest conversion was chosen (0.14 LMM) to demonstrate robustness at the most challenging setpoint. As seen in the feed flux curves in Figure 5, it was anticipated that the capsule would produce a slightly higher overall conversion than the cassette. This was confirmed by the stability study; graph shown in Figure 6.

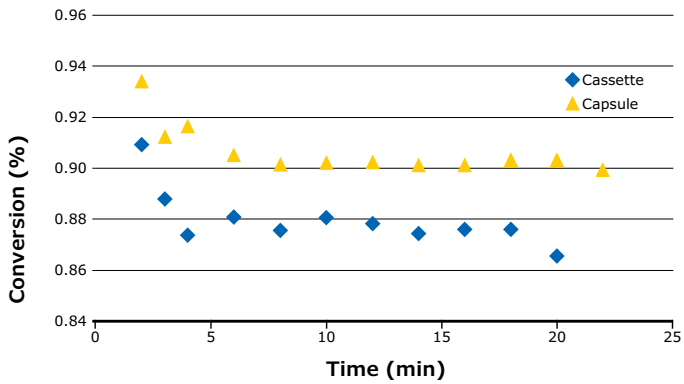

Figure 6. Process stability for SPTFF of 25 g/L BgG run at 0.14 LMM.

Based on the experimental results and findings, similar performance can be expected when switching between Pellicon® Capsule and Pellicon® 3 cassette for SPTFF operations. Feed conditions do play a significant role, especially at the low feed concentrations; therefore, it is important to check for optimal retentate pressure, particularly if switching between cassette and capsule formats.

Case Study 2: Pellicon® Capsule Scaling Study on mAb from 0.1 m² to 0.5 m²

Scale-Down Study

Scaling studies from 0.1 m² to 0.5 m² Pellicon® Capsule were carried out using clarified harvest fluid from a in-house mAb expressing CHO cell culture at a starting mAb concentration of approximately 1.3 g/L. On the day of the harvest, 2 liters of clarified feed were run at 0.1 m² scale in a 3-section (3 × 0.1 m²) SPTFF capsule system. The purpose of the experiment was to collect optimal operating conditions for the pilot scale operation.

Using the methodology described above, the optimal retentate pressure was established for the mAb feed solution at a feed flux of 1 LMM. The retentate pressure setpoint was determined to be 4 psi and was held constant throughout the subsequent feed flux excursions.

Starting at approximately 1 LMM and continuing at decreasing intervals, a series of feed flux excursions were carried out. The goal was to determine the conditions for a 90% conversion (10× concentration factor). Turbidity data and measured concentration were plotted alongside the predicted concentration curves. The plot in Figure 7 shows the output for the series of experiments as a function of concentration, predicted mathematically by the conversion obtained (reference 1) and confirmed with PrA HPLC assay measurement.

The measured values marked as "×" show agreement between predicted and actual measured data. For a target conversion of 90% (final concentration ~13 g/L), the required feed flux with 3 capsules in series was determined by simply identifying the resultant x axis value at the target concentration. Hence, a feed flux of 0.84 LMM was chosen for processing the full volume of approximately 70 L.

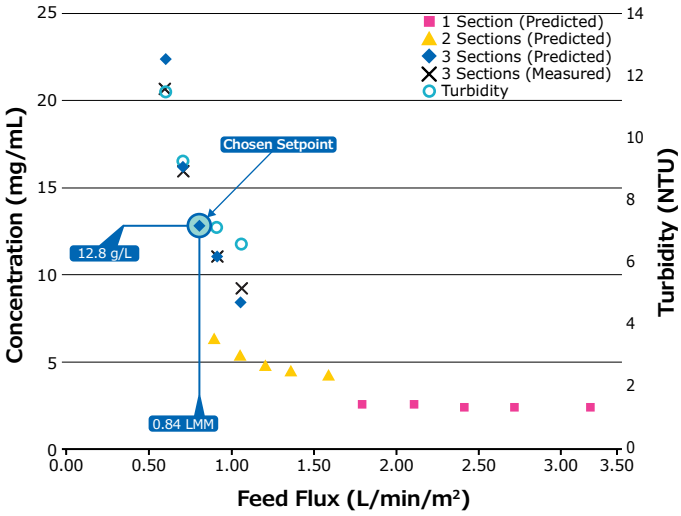

Figure 7. Results of 0.1 m² capsule, 3-section series SPTFF scale-down study.

Scale-Up Study

For the pilot run, three 0.5 m² Pellicon® Capsules were arranged in series, flushed, and equilibrated with buffer prior to processing. The feed pump was set to 0.84 LMM to match the optimal feed flux determined from the scale-down system. For this experiment, the retentate was not returned to the feed vessel for a stabilization period: retentate and permeate were both collected in vessels on scales and measured at regular intervals throughout the run to monitor process stability.

Retentate pressure was initially set at 4 psi and manually increased to 13 psi to match the feed channel pressure drop from the scale-down study and hit the desired conversion. Much like the pressure differences seen between the Pellicon® Capsule 0.1 m² and Pellicon® 3 cassette 0.11 m², pressure differences are seen between the 0.1 m² and 0.5 m² capsules—Figure 8 shows this comparison. For the 0.1 m² capsule only, less pressure is needed to achieve the desired conversion. However, when comparing pressure drop between the different capsule sizes, the values are the same.

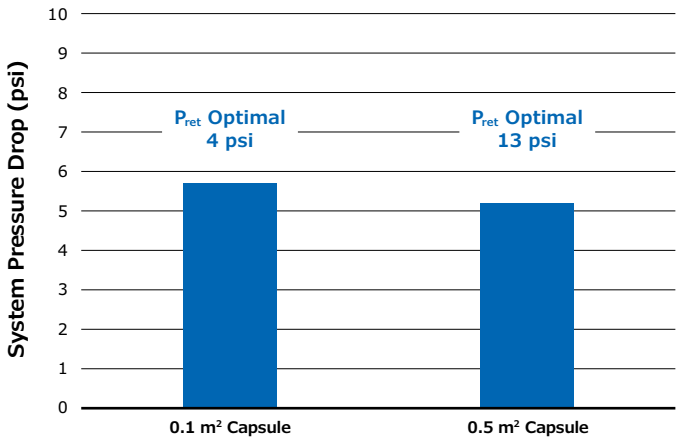

Figure 8. Pressure drop across feed channel for 0.1 and 0.5 m² capsules at 0.84 LMM.

Once stability was achieved, the process ran uninterrupted for approximately 1 hour at consistent concentration and conversion. The initial low readings indicate the period it took to displace buffer and establish a gel layer on the membrane surface. Measured concentration of a pooled sample, shown in Figure 9 as ○, confirmed the predicted output of the batch. Both conversion and predicted concentration were monitored and tracked throughout the run and are plotted in Figure 9 with the measured pool sample.

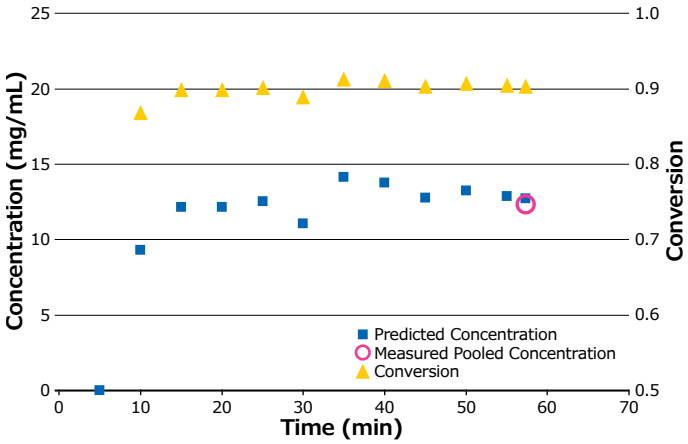

Figure 9. Process stability for pilot scale run.

Summary

The single-use Pellicon® Capsule is ideally suited for single-pass processing of biopharmaceuticals.

This application note has shown how to:

- Establish the optimal operating conditions and configuration for single-pass TFF
- Properly scale when using Pellicon® Capsules and Pellicon® 3 cassettes

References

1. Application Note, Single-pass tangential flow filtration. Lit. No. AN5572EN00.
2. User Guide, Pellicon® Capsule and Pellicon® Capsule Stand. Lit. No. UG1549EN.
3. Lutz, H. Ultrafiltration for Bioprocessing, Chapter 5. Woodhead Publishing Div. of Springer, Cambridge, UK; 2015.

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