Utilizing Single-Use Technology for Single-Pass TFF Operations

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Introduction

Single-pass tangential flow filtration (SPTFF) is becoming more widely used as a bioprocessing method to reduce volume between operation steps and to increase achievable product concentration. Novel applications of SPTFF include concentration of post-clarified bioreactor harvest, pre- and post-column chromatography, and high concentration at final ultrafiltration. In these instances, SPTFF is used to boost efficiency, overcome plant facility fit constraints, and enable continuous operations between process steps.

In SPTFF, a longer residence time in the feed channel increases conversion of feed-to-permeate and is achieved by decreasing the feed flow rate and/or increasing the number of sections in series to provide the feed a longer path length. For scale up, the feed flow rate, number of sections in series, and pressure are held constant.

To generate a serial flow path, ultrafiltration cassettes are installed in a standard holder with diverter plates inserted in between each cassette to create the SPTFF configuration (Figure 1). However, the cassette assembly is not well suited for single-use process operations, such as in the manufacturing of antibody-drug conjugates. As more bioprocess operations move toward a single-use approach, there is a growing need for more options to streamline single-use unit operations via technologies such as SPTFF.

Figure 1. Single-pass TFF setups. Pellicon® 3 cassettes with diverter plates (left) and single-use Pellicon® Capsules (right).

The Pellicon® Capsule is a new single-use TFF device that is holderless, gamma sterilized, and preservative-free. Pellicon® Capsules can be connected directly from port to port to generate a serial flow path without requiring additional accessories, as shown in Figure 1.

Single-use Pellicon® Capsules were compared to Pellicon® 3 cassettes to provide an option for true, single-use TFF operations in a single-pass mode. Studies were carried out using low and high feed concentrations of a bovine gamma globulin (BgG) solution to compare both filter formats with 30 kDa Ultracel® membrane and C screen. Additionally, scaling work with the capsules was completed using a clarified harvest monoclonal antibody (mAb) feed.

Methods

SPTFF Performance Comparison Using Low and High BgG Concentrations

To challenge the range of SPTFF operations, two feed concentrations of BgG, 1 and 25 g/L, were prepared in PBS buffer. Each feed solution was sterile filtered prior to use.

Three Pellicon® 3 cassettes with 30 kDa Ultracel® membrane and C screen, size 0.11 m^2, were assembled in a Pellicon® mini holder with mini diverter plates (SPTFF mini kit) and torqued to 180 lbs-inch. The three devices were flushed with water, cleaned with 0.1 N NaOH, and integrity tested prior to use.

For the single-use setup, three gamma sterilized Pellicon® Capsules with 30 kDa Ultracel® membrane and C screen, size 0.1 m^2, were assembled in series. An “N” configuration was made such that the capsules were connected port to port, retentate to feed; see Figure 1. The three devices were simply flushed with water prior to use.

Both SPTFF setups were run simultaneously using the same feed material over the course of two days: 1 g/L on day one and 25 g/L on day two. For each feed condition, feed flux excursions (determining conversion and concentration at different feed flow rates), and a stability study at the desired conversion were run to confirm setpoints. Conversion and concentrations were calculated based on volume using Equations 1 and 2, where Y is conversion of feed to permeate and C is concentration.

Equation 1:

$$Y_{Section2} = \frac{Q_{PermSection1} + Q_{PermSection2}}{Q_{PermSection1} + Q_{PermSection2} + Q_{PermSection3} + Q_{Retentate}}$$

Equation 2:

$$C_{SectionX} = \frac{C_{initial}}{(1 - Y_{SectionX})}$$

Equation 1 calculates conversion per section (e.g., for the second section).
Equation 2 calculates the predicted concentration based on conversion.

Initial feed concentrations as well as final concentrations during the stability study were collected and analyzed by UV spectrophotometry to confirm predicted measurements.

Pellicon® Capsule Scaling Study with mAb Clarified Harvest

The mAb used for the scaling study was produced at MilliporeSigma to simulate a typical Chinese hamster ovary (CHO) cell line. The material was harvested, acid precipitated, clarified through a 1.14 m^2 Clarisolve® POD filter, and sterile filtered prior to use. Bench-scale sizing with Pellicon® Capsule size 0.1 m^2 was performed to identify process parameters for scale-up.

A series of feed flux excursions were carried out to determine the optimal feed flow rate for a 10× concentration (90% conversion). For data collection purposes, the feed flow rates were decreased until a 20× concentration was achieved. Initial feed samples and retentate samples were collected to confirm concentration. Samples were also collected and analyzed for turbidity. The titer was measured via analytical Protein A.

Scale up was performed on bulk clarified harvest using Pellicon® Capsule size 0.5 m^2 at the predetermined set of optimal conditions. Once steady-state was achieved, process pressures and weights were recorded at given time points throughout the run to monitor conversion and process stability. Initial feed and final retentate samples were collected and analyzed via analytical Protein A.

Results

Pellicon® 3 Cassette vs Pellicon® Capsule with 30 kDa Ultracel® Membrane and C Screen

Feed flux excursions were carried out at 1 g/L BgG concentration and compared. The results in Figure 2 show comparable performance between C screen cassette and capsule for all three SPTFF sections.

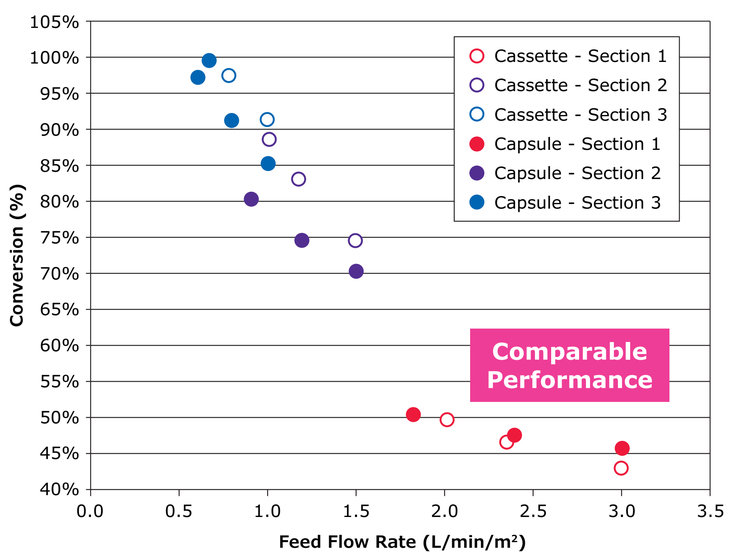

Figure 2. Conversion vs feed flow rate. Feed flux excursions for 1 g/L BgG.

Figure 3 shows the results of the third SPTFF section for 25 g/L BgG solution with a logarithmic fitted line plot to compare the data for capsule versus cassette. The results show the performance is comparable for the C screen cassette and the capsule. Similar curves were captured for sections 1 and 2 (data not shown).

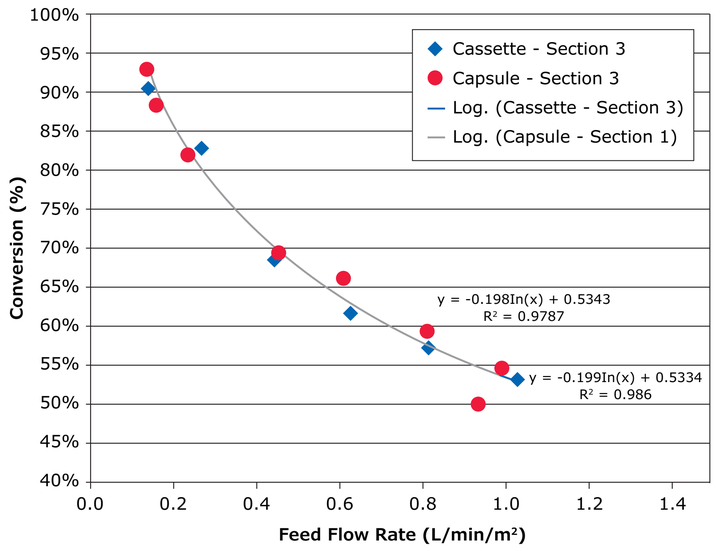

Figure 3. Conversion vs feed flow rate for section 3. Feed flux excursions for 25 g/L BgG.

The average pressure drop of the C screen cassette and capsule was assessed for stability comparison purposes using both BgG feeds, 1 g/L and 25 g/L. Figure 4 shows that the pressure drop profile for both capsule and cassette formats is comparable.

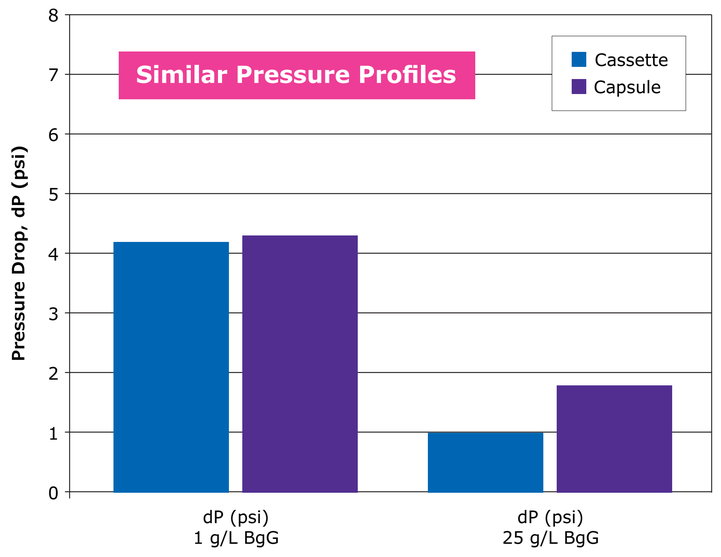

Figure 4. Average pressure drop cassette vs capsule.

Pellicon® Capsule Scaling: 0.1 m^2 to 0.5 m^2 with mAb Feed

Figure 5 shows the performance of the 0.1 m^2 capsule during the scale-down trial. Turbidity data show the stability of the molecule as the concentration is increased. The predicted concentration curves were calculated from the data and compared to the measured concentrations. As shown, the predicted and actual concentrations are well aligned. Our target concentration for the scale-up study was 12.8 g/L. To determine the feed flow rate required for a 3-section assembly, we simply identified the resultant x axis value at this concentration to obtain 0.84 L/min/m^2.

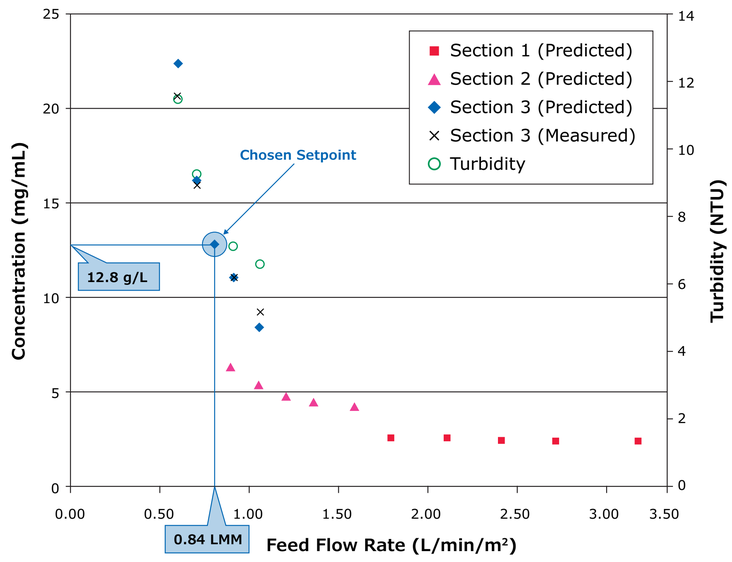

Figure 5. mAb concentration and turbidity vs feed flow rate for capsule scale-down study. LMM = L/min/m^2

Figure 6 shows the performance of the scale-up study using 0.5 m^2 capsules at feed flow rate of 0.84 L/min/m^2, as determined by the scale-down study. The graph shows the overall stability of concentration and conversion over the course of the run. The final pooled concentration is shown by the "x“ symbol. The concentration matched nicely with the predicted concentration of 12.8 g/L.

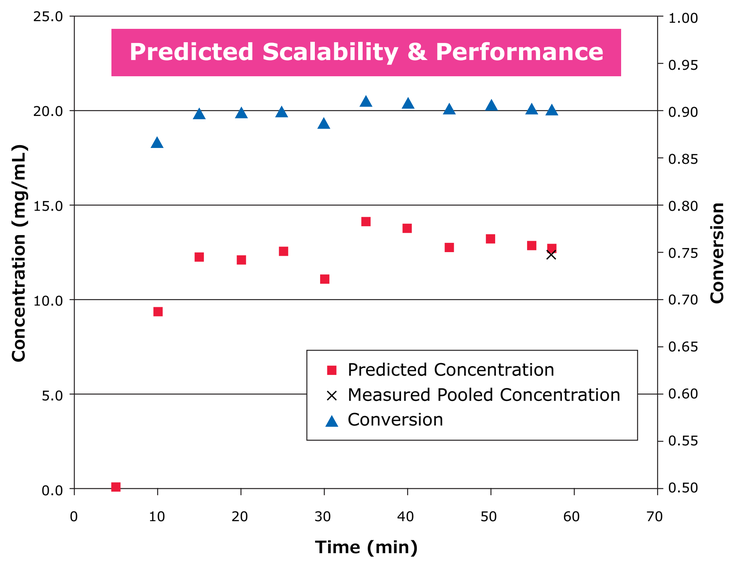

Figure 6. mAb conversion and concentration stability over time during capsule scale-up study.

Summary

- The single-use Pellicon® Capsule is very easy to install and run in SPTFF operation, with less overall installation time and hardware compared to cassettes.
- Pellicon® Capsules show comparable performance to Pellicon® 3 cassettes at both low and high feed concentrations, with both SPTFF assemblies achieving high conversions as well as high yield and recoveries (data not shown).
- Pellicon® Capsules scale between sizes (0.1 m^2 to 0.5 m^2). The scale-down trial provided reliable predicted versus measured concentrations. Stability of conversion and concentration was maintained throughout the run at predetermined setpoints.

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