Single-Pass TFF (SPTFF) Evaluation in a mAb Process to Debottleneck Tank Size Limitations

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Introduction

Tangential Flow Filtration (TFF) is widely used in the biopharmaceutical industry for monoclonal antibodies (mAbs) or biosimilars purification at different steps.

Typical TFF steps concentrate product through volume reduction to achieve high yields. For mAbs, TFF usually utilizes cellulosic membranes with a Nominal Molecular Weight Limit (NMWL) of 30kD, and a target yield >95%.

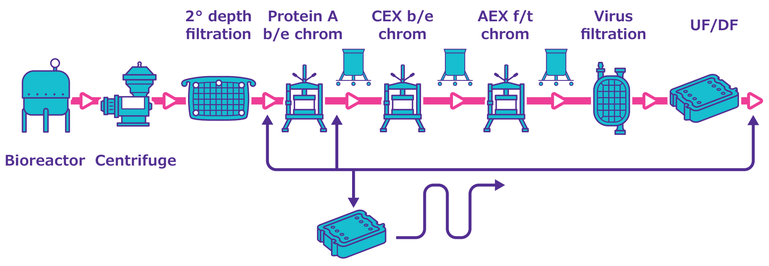
Today, biopharmaceutical companies and CMOs can face facility fit issues during the tech transfer of new molecules or next generation processes of marketed products. In particular, they may face size limitations of their existing tanks compared to the required volume of liquid. The main objective of this evaluation is to assess how easily single-pass TFF (SPTFF) could be implemented before and/or after a purification step in order to reduce volumes, footprint, costs of capital investment, and time.

Single-pass TFF is a new way to use an existing technology. The product feed is constantly concentrated during a single pass through serialized TFF device up to the targeted final concentration. A recirculation loop is not required, simplifying hardware settings and reducing hold up volume and footprint. This allows higher product recovery while reducing the risk of product damage associated with traditional recirculation. Single-pass TFF is also a convenient way to reduce volumes, helping to eliminate tank bottlenecks.

In this poster we will highlight a mAb case study, where SPTFF is applied to overcome these challenges.

Methods

MilliporeSigma proposes a continuous processing template that includes SPTFF steps:



EMD Serono tested SPTFF at three possible locations in the process template, including at the final concentration step.

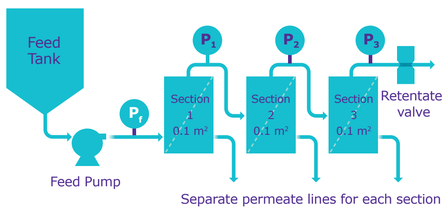
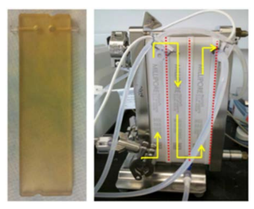


Figure 1: SPTFF Schematic



Picture 1: SPTFF Diverter Plate and set up

Protocol

Three sections (1 88 cm² cassette per section with diverter plates) were installed in a Pellicon® Mini holder (**Figure 1**; Picture 1) to allow a serial flow through the devices. Flush, sanitization, NWP measurement, and buffer equilibration steps were then completed.

Subsequently, Feed Flux excursion experiments were performed. The cumulative conversion and concentration was evaluated against the feed flux after each section of the single-pass process for the three process feed streams.

In SPTFF, the residence time given by the feed flow rate plays an important role as it defines how long the liquid stays under pressure in the filtration device and allows feed to be converted into permeate.

The different feed fluxes (range from 8 to 0.2 liters per minute per meter squared (LMM) depending on the feed and concentration tested) were tested from the highest to the lowest in order to progressively increase the residence time and product concentration in the retentate.

For each feed flux tested, we measured retentate flow, three permeate flows, feed and retentate pressures, as well as the concentrations in the feed, retentate and permeates.

After the optimization trials, a process simulation (concentration) was done for the 6 different feed solutions.

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Objectives

The objectives of the trials were to test the performance of SPTFF with Ultracel® 30 kDa membranes with C screens on three different feedstocks for two different mAbs (mAb A+mAb B):

- Clarified harvest feed (starting concentration around 2.5-3 g/L)
- Post-Protein A feed (starting concentration around 11 g/L)
- Drug substance feed (starting concentration around 5 g/L)

The predefined success criteria were the following:

- Protein recovery greater than 95%, less than 5% aggregates
- At least 5x concentration on Clarified Harvest and Intermediate Bulk
- Final concentration of at least 100 g/L on drug substance feed

Figure 2. Clarified Harvest solution

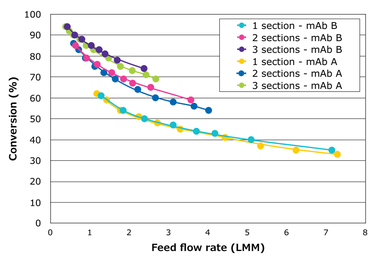


Figure 3. Post Protein A solution

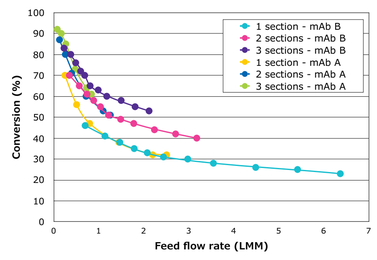


Figure 4. Drug substance solution

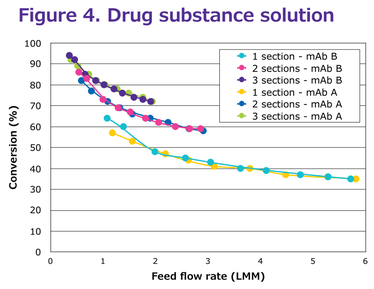
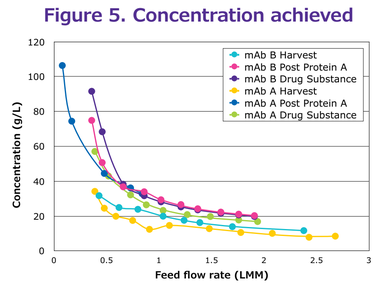


Figure 5. Concentration achieved



Results

Conversion ratio

The measured conversion (ratio of feed flow compared to the filtrate flow) is plotted for each section in the following graphs, using the calculated area normalized by feed flux (**Figures 2-4**). By lowering the pump speed, the feed flux is reduced. There is a higher conversion ratio as the liquid being processed is given a longer residence time in the TFF devices. Therefore, at a lower feed flux, the conversion for each section is increased compared to a higher feed flux.

The following conclusions were drawn from the data:

- The first section contributes the most to the conversion rate
- For clarified harvest feed, up to 95% conversion rate was achieved at 1 LMM feed flow rate which corresponds to a 20 times concentration factor (**Figure 2**)
- For post-Protein A feed (**Figure 3**):
  - For mAb A, up to 90% conversion rate was achieved (10 times concentration factor)
  - For mAb B, up to 80% conversion rate was achieved (5 times concentration factor)
- For Drug Substance feed (**Figure 4**), up to 90% conversion for both mAbs was achieved (10 times concentration factor)

Concentration achieved

By taking the data used to plot conversion and calculating the achieved concentration after the third section of the SPTFF setup, the graphs in **Figure 5** could be generated for the two tested mAb solutions.

Here we can conclude that a concentration factor above 5 could be easily reached for all tested feed streams. Despite a 10-times concentration, the 100 g/L final concentration was not reached for the Drug Substance feed. Due to the lower starting material concentration of 5 g/L, lower feed fluxes than used in this trial would have been required.

Discussion

Process Design for mAb A

In **Figure 6**, an example for mAb A is plotted, where three sections in series have been chosen for experiments. By simple interpolation of the curve using the targeted conversion of 90%, it is possible to identify the:

- corresponding Feed Flux (~ 0.55 LMM)
- number of sections (3 in this example)
- required total filtration area and the area needed per section to achieve the target conversion

At 10 times concentration (90% conversion rate), the feed flux using three sections is determined to be 0.55 LMM. For 2300 L Drug Substance of mAb A to be concentrated in 90 minutes (~25.55 L/min), the total area needed in the three sections in series will be approximately 46 m². This total area is equally distributed among the three sections and leads to ~15.3 m² per section. If a longer processing time is acceptable, the TFF membrane area could be proportionally reduced, thus also increasing yield by decreasing dilution during recovery.

Aggregate Levels

Looking at the aggregate level after the three-section SPTFF process, the success criteria was met: the level remained below 5% for each trial performed (**Figure 7**).

mAb Recovery

For product recovery (**Figure 8**), the success criteria (95% yield) was achieved for all of the scenarios. The data showed the recovery without any buffer displacement or flush of the system at the end of the process. Adding a flush step would allow reaching even higher recovery percentage (but would add dilution of the final product).

Figure 6: mAb A - SPTFF Conversion Chart - 3rd section

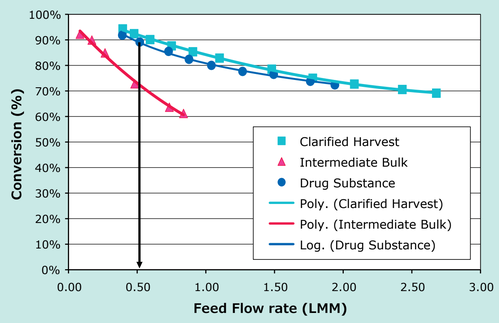


Figure 7: SPTFF Aggregation mAb A

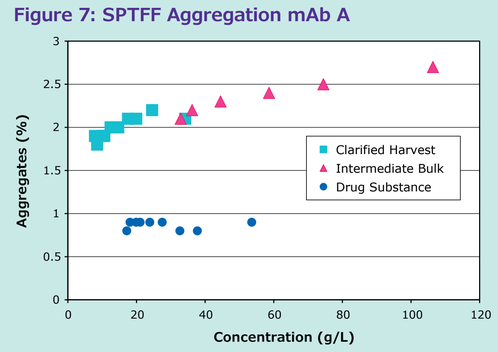
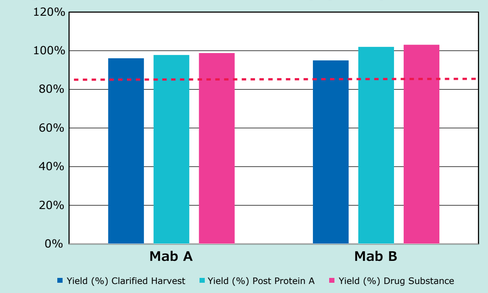


Figure 8: SPTFF - mAb Recovery (Yield)



Summary

Single-pass TFF runs at constant operating conditions throughout the process, simplifies the required hardware, and allows high concentration factors and high product recovery without significant dilution by reducing hold-up volume. It also reduces the risk of product damage associated with multiple pump passes during recirculating TFF operations.

SPTFF is a continuous operation and can be linked with other process steps, which can help decrease volumes to eliminate tank bottlenecks or reduce chromatographic column sizes. The SPTFF application is especially valuable in existing facilities where space may be limited or where a process has to fit into an existing facility layout.