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Application Note

Intensified polishing using single-pass tangential flow filtration (SPTFF) with anion exchange chromatography

Introduction

The bioprocessing industry is interested in Next Generation Processes with higher flexibility, lower costs, and higher product quality. Single-pass tangential flow filtration (SPTFF) can be used to intensify manufacturing processes to meet these goals. Here, SPTFF preconcentration is used to intensify the anion exchange (AEX) polishing step in monoclonal antibody (mAb) processing for improved impurity removal and column productivity. This intensified polishing approach can be linked with upstream steps for a more continuous process which eliminates tankage and hold time, and enables the use of smaller polishing columns to improve product quality at higher throughputs.

Conventional polishing

It is common in mAb downstream purification processes to use a flow through AEX resin as a final impurity adsorption step. This step uses positively charged quaternary ammonium groups to adsorb negatively charged host cell protein (HCP) and virus impurities while allowing the positively charged therapeutic mAb product to flow through unbound. In many cases, the feed material is pre-diluted to reduce the solution conductivity below 10 mS/cm for optimal impurity adsorption, and typical mass loadings for AEX resin polishing are 100 – 250 g mAb/L resin. As a result, the conventional polishing approach requires relatively large columns with long process times.

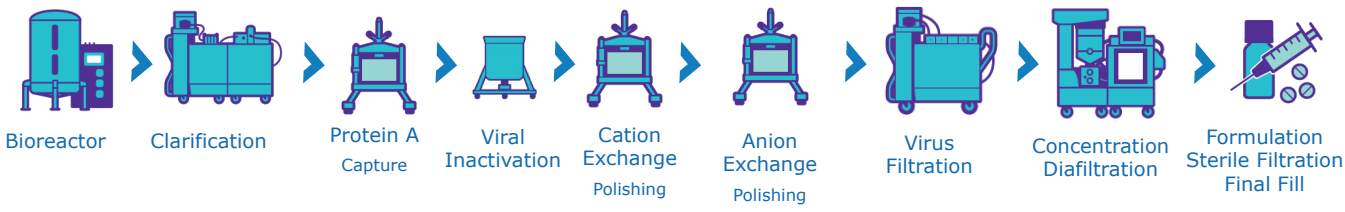


Figure 1. Conventional Polishing
Conventional anion exchange polishing in a mAb process

Intensified polishing

The new application described here uses inline SPTFF to continuously pre-concentrate the mAb feed prior to the AEX column. Our SPTFF assembly uses conventional ultrafiltration cassettes and holders to achieve high concentrations in just one pass through the cassettes. Unlike conventional batch TFF, the single pass approach does not require product recycling, simplifying implementation [1].

When SPTFF is applied to the AEX feed, it concentrates both the mAb product and the HCP impurities, since both species are retained by the membrane. Increasing the concentration of HCP in solution improves its adsorption to the AEX resin, as dictated by the thermodynamic isotherm relating a protein's solution concentration to its adsorbed concentration [2]. In general, HCP levels in the AEX feed solution are dilute, in the range of a few hundred PPM, and so HCP binding falls within the linear portion of the thermodynamic isotherm. Concentrating the HCP in solution by SPTFF improves isotherm binding conditions, allowing for increased impurity adsorption. As a result, mass loading on the AEX resin can be significantly improved by incorporating SPTFF pre-concentration.

In addition to improving the AEX resin's capacity for HCP impurities, SPTFF can also be used to de-bottleneck the polishing step. If conductivity adjustment occurs prior to SPTFF, the membrane will concentrate proteins retained by the membrane while maintaining the same low conductivity as excess buffer ions permeate the membrane. The mass of impurities to be removed by AEX remains constant, however the reduced pool volume correlates to shorter AEX load times, allowing more cycles to be run with a smaller column for a higher productivity process. Alternatively, conductivity adjustment can occur after SPTFF concentration in order to reduce dilution buffer requirements. The volume reductions facilitated by SPTFF result in smaller tank size and pump flow rate requirements for improved facility fit.

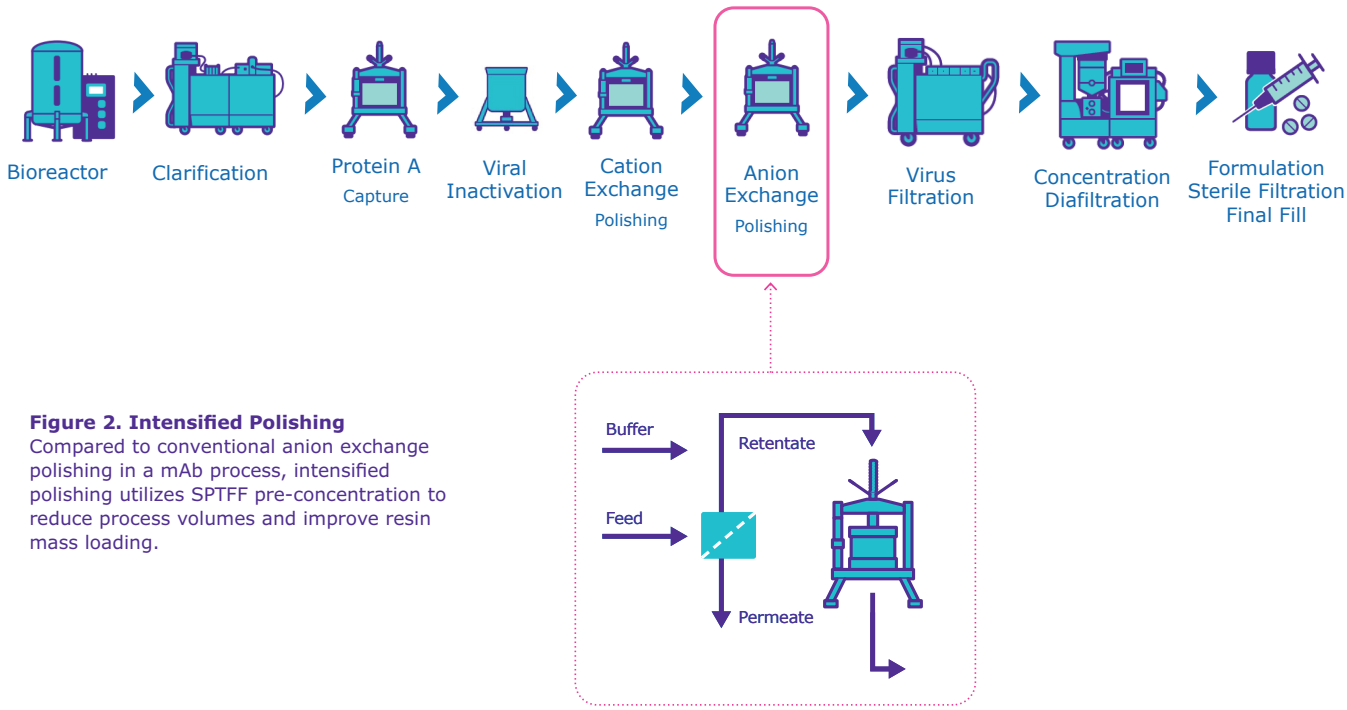


Figure 2. Intensified Polishing
Compared to conventional anion exchange polishing in a mAb process, intensified polishing utilizes SPTFF pre-concentration to reduce process volumes and improve resin mass loading.

Case Study

An IgG1 mAb (pI 8.3) was expressed in CHO culture and purified by Clarisolve® Pod clarification, ProSep® Ultra Plus capture, and Eshmuno® S cation exchange (CEX) chromatography. The CEX eluate was adjusted to pH 8.4, 5.5 mS/cm, and contained a mAb concentration of 11 mg/mL with 662 PPM HCP. This feed material was used to evaluate SPTFF and AEX steps as separate unit operations in order to assess the individual performance of each step.

For SPTFF concentration, 3 sections of 88cm^2 Pellicon® 3 C-screen cassettes with 30 kD Ultracel® membrane were assembled into a mini cassette holder, and arranged in series with separator plates [1]. The pH and conductivity adjusted CEX pool was fed into the SPTFF assembly at several different feed cross-flow rates ranging from 1.5 – 0.2 L/min/m^2 (LMM). For all feed cross-flow rates evaluated, a constant pressure of 10 psig was maintained on the retentate line. Permeate and retentate samples were collected at each feed cross-flow rate set point and monitored for mAb and HCP content. As seen in Figure 3A, decreasing feed cross-flow rate corresponded to increased retentate mAb concentration. A similar trend was observed for HCP concentration (Figure 3B). HCP co-concentrated with the mAb, and no protein was detected in permeate samples.

Along with an un-concentrated control, SPTFF retentate samples collected at the experimental feed cross-flow rate conditions were used to evaluate the effect of feed concentration on AEX resin performance. Polishing was conducted with Eshmuno® Q resin, a chromatography resin featuring 85 µm polyvinyl ether beads functionalized with a positively charged TMAE ligand. For a range of feed concentrations, HCP uptake was evaluated in batch binding experiments using 200 µL of loose resin challenged to 1.1 g mAb/mL resin in a stirred vessel. After a 4 hour incubation, the amount of HCP bound to the resin was determined by mass balance calculations. The results (Figure 4) showed that equilibrium HCP binding capacity increases with HCP feed concentration. This is consistent with operation in the linear region of an equilibrium binding isotherm.

Packed column Eshmuno® Q resin experiments were subsequently conducted with 200 µL resin volume RoboColumn® devices operated at a 3.8 minute residence time. During loading, flowthrough fractions were collected and assayed for HCP and mAb content. Figure 5 plots the cumulative flowthrough HCP content as a function of mAb loading for each of the feed concentrations evaluated. It was observed that preconcentration from 11 mg/mL through 82 mg/mL results in a shallower breakthrough curve, allowing for increased loading at a target HCP clearance level. This behavior was consistent with the increased equilibrium binding capacity observed in loose resin experiments. At the 99 mg/mL concentration, an initial breakthrough of 10 PPM HCP was

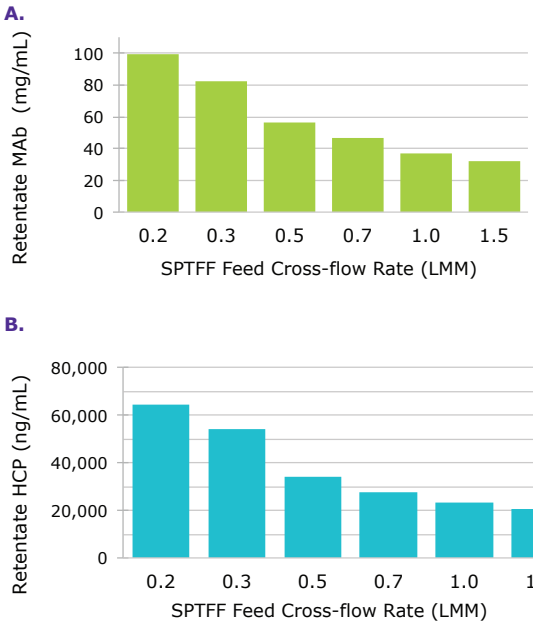


Figure 3A & 3B.
Retentate mAb (A) and HCP (B) concentration as a function of SPTFF feed cross-flow rate. At low feed cross-flow rate, residence time in the feed channel increases, corresponding to increased retentate concentration.

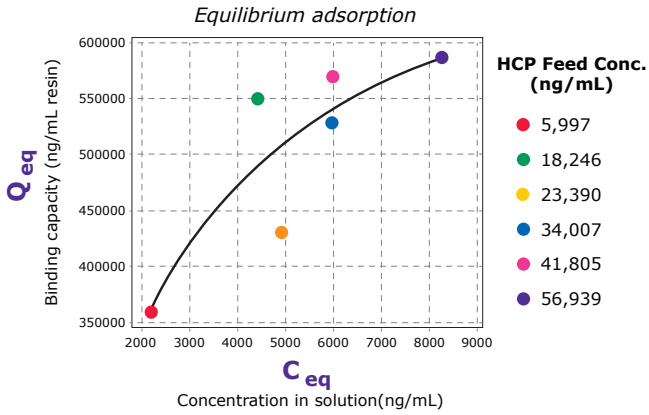


Figure 4.
Experimental equilibrium adsorption isotherm data relating Eshmuno® Q HCP binding capacity and solution concentration.

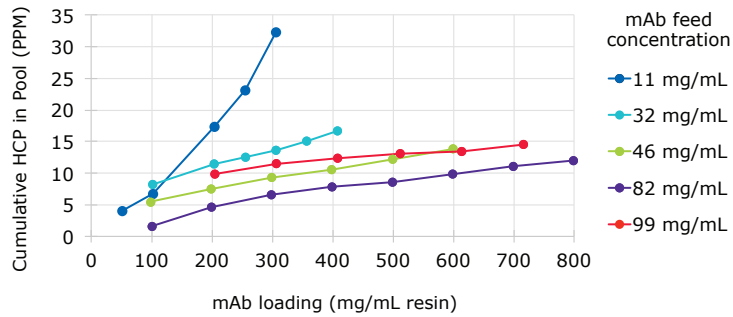


Figure 5.
Eshmuno® Q resin HCP breakthrough as a function of mAb loading for several different feed concentrations: an unconcentrated control (11 mg/mL), and four different concentrations prepared by SPTFF (32 – 99 mg/mL).

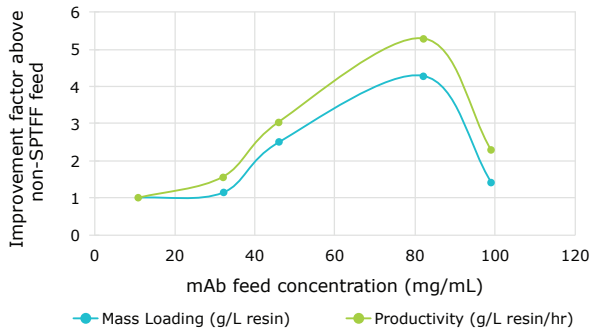


Figure 6.
Mass loading and productivity improvement factor as compared to the non-SPTFF concentrated control condition (11 mg/mL mAb concentration). Concentrating to 80 mg/mL by SPTFF provides an approximate 4x improvement in loading, and a 5x improvement in Eshmuno® Q productivity.

observed at 200 mg/mL resin load. This effect was attributed to HCP-mAb interactions, which for this particular molecule became more prevalent at higher concentrations.

From Figure 5, preconcentrating the Eshmuno® Q feed material delayed breakthrough of HCP in the flowthrough pool, resulting in better product quality. Using a representative HCP endpoint of 10 PPM, pre-concentration was shown to boost loading from about 150 g mAb/L resin at 11 mg/mL feed to 600 g mAb/L resin at 82 mg/mL feed, a 4x improvement in resin mass loading [3]. Additionally, the reduced feed volume shortens the Eshmuno® Q load time, thus reducing overall cycle time for a more productive process. Figure 6 shows that a 5x improvement in resin productivity (g mAb/L resin-hr) was obtained, as calculated from the feed concentrations and mass loadings shown in Figure 5.

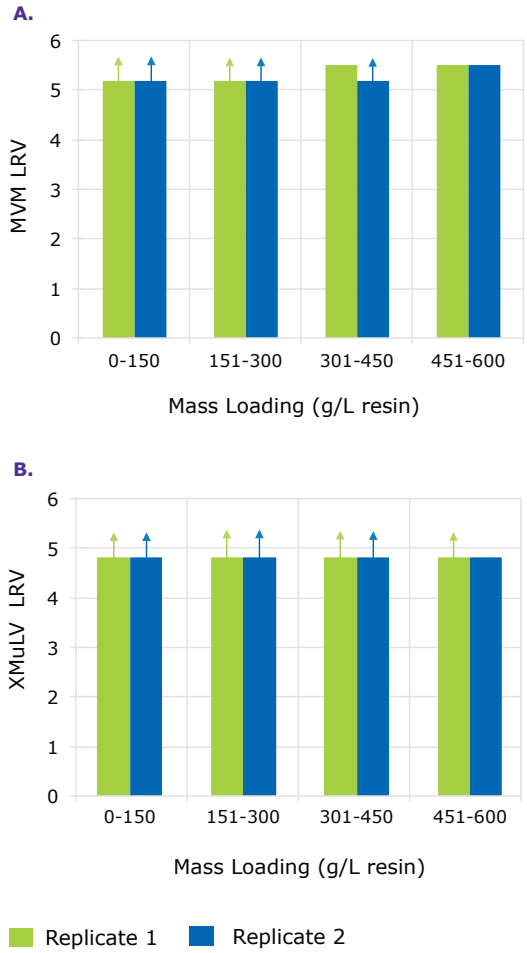


Figure 7A & 7B.
MVM (A) and XMuLV (B) viral clearance data on Eshmuno® Q through 600 g mAb/L resin load. Arrows indicate virus content below limit of detection.

In addition to HCP clearance, Eshmuno® Q resin is also used as a viral clearance step in mAb processing. Eshmuno® Q is proven to provide excellent viral clearance at conventional load levels (100 – 250 g mAb/L resin)[4]. However given the increased mass loadings that can be achieved by incorporating SPTFF pre-concentration, viral clearance experiments were conducted using concentrated mAb at higher resin load levels. The same mAb molecule evaluated in HCP clearance experiments was used for viral clearance testing, again at pH 8.4, 5.5 mS/cm. After concentrating the mAb to 80 g/L by SPTFF, MVM and XMuLV were spiked into separate pools of the mAb solution. The virus spiked material was used to challenge Eshmuno® Q resin packed in 1 mL column volume format through 600 g mAb/L resin load. Flowthrough fractions were assayed for viral clearance, as shown in Figure 7. The results show that excellent clearance of both MVM (> 5 LRV) and XMuLV (> 4 LRV) was maintained through 600 g mAb/L resin load. Flowthrough fractions were also assayed for HCP content, which was less than 10 PPM for all conditions and replicates (data not shown).

Batch Implementation

The image in Figure 8 shows a simplified example that demonstrates the implementation of linked intensified polishing batch operation. A 3-section SPTFF setup (0.1 m^2 area per section) is connected to a 10 mL Eshmuno® Q column. A 0.22 µm Sterivex™ filter is employed upstream of the column as an air trap and to provide bioburden protection. Additionally, inline adjustment of pH and/or conductivity can take place using a secondary pump (Figure 9A). Depending on the amount of dilution required and the SPTFF concentration factor, dilution can occur either before or after the SPTFF assembly.

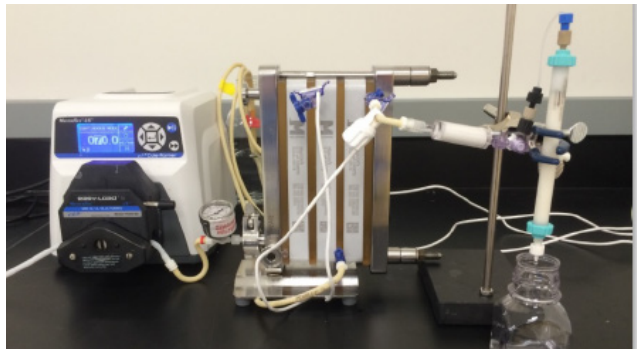
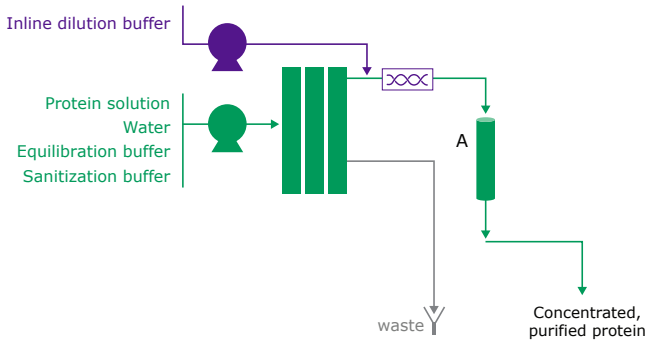


Figure 8.
Laboratory set-up showing an Eshmuno® Q resin packed column linked directly to the SPTFF retentate for intensified polishing.

Linked Implementation

To allow for continuous operation linked directly to the upstream step, a two column rotation can be employed. Column wash, regeneration and equilibration require some time, so cycling between columns approximately once per hour is convenient. The low flow rates used for SPTFF keep the pressures low and permit inline operation of SPTFF with the column. A 0.3 m^2 SPTFF setup connected to a 10 mL Eshmuno® Q column can process 50-110 g mAb/day, a rate which aligns with a 20 – 50 L perfusion bioreactor process. As shown in Figure 9B, a single SPTFF set up can be used to continuously concentrate mAb product prior to the cycled columns. Daily sanitization or cleaning between lots may be required.

A.



B.

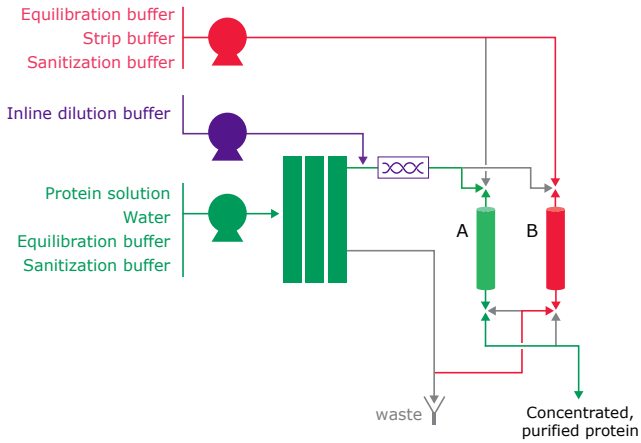


Figure 9.
Implementation of linked intensified polishing. A single Eshmuno® Q column can be utilized for batch processing (A). For continuous operation, a single SPTFF assembly can be connected to a rotation of multiple Eshmuno® Q resin packed columns (B).

Economic Benefits

An economic comparison of conventional and intensified polishing was conducted for both batch and perfusion bioreactor cases. In the first case, a single 2,000 L batch bioreactor harvested at 3 g/L titer is considered (Figure 10A). Adjusting for yield loss and dilution of upstream purification steps, the polishing feed volume is 503 L, with 9.3 g/L mAb concentration and 20 mS/cm conductivity. To process this on Eshmuno® Q resin using conventional polishing techniques, a large volume of dilution buffer is needed to reduce the conductivity to 5 mS/cm, and a relatively large Eshmuno® Q column is required to accommodate a loading of 150 g mAb/L resin. In the intensified polishing case, conductivity adjustment takes place after SPTFF concentration, significantly reducing the dilution buffer volume as compared to the conventional case. The mAb is fed into the Eshmuno® Q resin packed column at 47 g/L concentration, resulting in increased mass loading (nearly 400 g mAb/L resin), and thus a reduction in required resin volume.

In the perfusion bioreactor case, a 200 L bioreactor with 1.5 g/L titer is continuously harvested at a rate of 1.5 vessel volumes per day over the course of a 14 day batch (Figure 10B). Adjusting for yield loss and dilution of upstream purification steps, the polishing feed volume is 415 L/batch with 11.9 g/L mAb concentration and 20 mS/cm conductivity. Again, the incorporation of SPTFF significantly lessens the volume of dilution buffer required to reduce the conductivity prior to Eshmuno® Q polishing. Resin mass loading is improved by boosting the feed concentration, and as a result the intensified case requires less resin for a more productive manufacturing process.

Despite the additional cost of SPTFF membrane, the intensified polishing approach leads to significant savings in resin, buffer, and sterile filtration costs, resulting in improved process economics.

Benefits Summary

Case study A

2,000 L fed-batch bioreactor, 3 g/L titer, single harvest

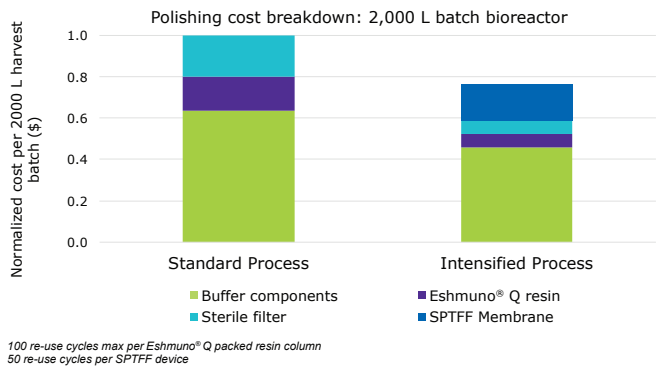
Parameter	Standard Process	Intensified Process	Comment
Column volume	31.8 L	12.7 L	2.5 x reduction
Dilution buffer volume	1508 L	435 L	3.5 x reduction
Sterile filtration volume	2010 L	628 L	3.2 x reduction
Polishing step time	6.2 hr	6.2 hr	Increase SPTFF area to reduce time
Eshmuno® Q resin productivity	24 g/L/hr	59 g/L/hr	2.5 x improvement

Case study B

200 L perfusion bioreactor, 1.5 g/L titer, continuous 14 day harvest at 1.5 vessel volumes/day

Parameter	Standard Process	Intensified Process	Comment
Total resin volume	625 mL/batch	145 mL/batch	4.3 x reduction
Dilution buffer volume	1244 L/batch	548 L/batch	2.3 x reduction
Sterile filtration volume	1659 L/batch	829 L/batch	2.0 x reduction
Resin productivity	24 g/L/hr	101 g/L/hr	4.3 x improve

A.



100 re-use cycles max per Eshmuno® Q packed resin column
50 re-use cycles per SPTFF device

B.

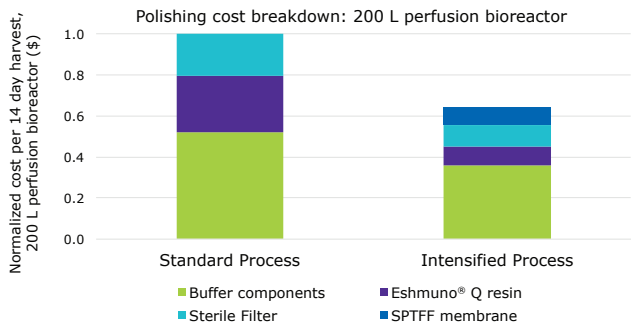


Figure 10.
Economic and facility fit calculations for a 2,000 L batch bioreactor case (A) and a 200 L perfusion bioreactor case (B). In each case, intensified polishing leads to significant savings in resin, buffer, and sterile filtration membrane, reducing process costs.

Summary

Intensified polishing is a new application which combines Pellicon® SPTFF cassettes and Eshmuno® Q anion exchange resin. SPTFF pre-concentration of Eshmuno® Q feed material allows for improved isotherm binding conditions, significantly boosting resin mass loading and productivity. Experimental data shows that a 4x improvement in mass loading and a 5x improvement in productivity can be achieved while maintaining excellent clearance of HCP (10 PPM) and virus (> 5 LRV MVM, > 4 LRV XMuLV).

Intensified polishing is ideal for implementation in continuous processing applications. SPTFF eliminates the need for product recirculation tanks, and the concentration step helps to improve facility fit by reducing tank volumes, pump flow rate requirements, and buffer volumes for pH/conductivity adjustment.

The improved resin mass loading and facility fit provided by intensified polishing directly contributes to improved process economics. Significant savings in process buffers, sterile filtration requirements, and resin volume justify implementation of SPTFF for intensification of the mAb polishing step.

References

1. Merck Application Note. *Single-pass tangential flow filtration*. Lit. No. AN5572EN00, 2014.
2. Veladyudhan A. and Horvath C. 1994. *Adsorption and ion-exchange isotherms in preparative chromatography*. J Chromatog A. 663: 1-10.
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