Intensified mAb polishing:
linking single-pass tangential flow filtration with anion exchange chromatography

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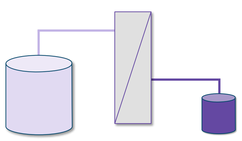
Introduction

Process intensification is an approach to improve operational throughput by running a manufacturing process or unit operation differently. In mAb purification, intensified processing can remove bottlenecks created by high bioreactor titers, increase manufacturing flexibility for multi-product facilities, and reduce cost of goods while increasing the focus on product quality.

This work focuses on intensifying the anion exchange (AEX) mAb polishing step. AEX polishing is commonly used to provide clearance of host cell protein (HCP) and virus impurities. The mAb polishing step can be intensified by pre-concentrating the AEX feed material using Single-Pass Tangential Flow Filtration (SPTFF). This preconcentration enhances AEX operation in two ways:

The benefits of concentration prior to AEX polishing

1 Preconcentrate to reduce pool volume

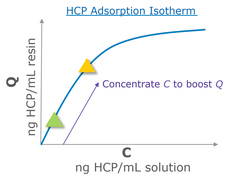


Easier pH/conductivity adjustment
The SPTFF membrane permeates salt while retaining protein, thus reducing adjustment buffer volumes.

Improve facility fit
Reduce tank size by concentrating feed.

Reduce AEX load time
Less volume to load onto AEX column.

2 Preconcentrate to improve HCP clearance

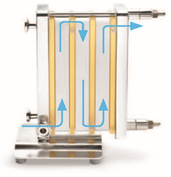


Improve isotherm conditions
HCP concentrations in AEX feed are low, so binding falls within linear portion of isotherm (▲)

By preconcentrating to boost *C*, the binding capacity *Q* is also increased, as dictated by the isotherm (▲)

A new application linking two existing products: SPTFF with Pellicon® cassettes and Eshmuno® Q resin

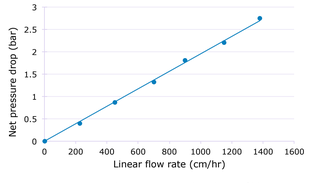
SPTFF allows for product concentration without the need for recirculation. To reach conversion targets in a single pass, residence time in the filter feed channel is extended by increasing the number of filter sections, or by decreasing the feed flux (L/min/m^2, or LMM). Existing Pellicon® cassettes and hardware are used for SPTFF operation.



SPTFF assembly of Pellicon® cassettes at benchscale. Each of the three filter sections contains 0.11 m² membrane area.

Eshmuno® Q anion exchange resin features an 85 µm average particle size, which provides excellent pressure/flow properties. Typical Eshmuno® Q resin conductivity for flowthrough mAb polishing is less than 10 mS/cm.

Eshmuno® Q resin: Pressure/Flow data



Eshmuno® Q resin pressure/flow data. 10 cm ID, 20 cm bed height. 150 mM NaCl solution.

Here, we present a case study with experimental and cost analysis data to support the use of intensified polishing with SPTFF and Eshmuno® Q anion exchange resin.

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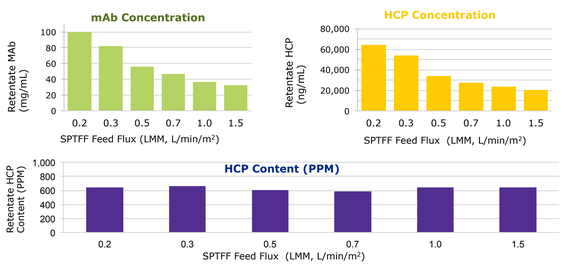
Methods and Results

SPTFF concentration with Pellicon® cassettes

mAb A (pI 8.3) was previously purified by both protein A and cation exchange (CEX) chromatography. The CEX elution pool contained 11 g/L mAb A and approximately 600 ppm HCP. This material was concentrated using a three-section SPTFF setup, where each section contained an 88 cm^2 Pellicon® 3 C-Screen cassette with 30 kD Ultracel® membrane. This SPTFF set up was operated at 6 different feed fluxes, from 0.2 to 1.5 LMM. The retentate mAb and HCP concentrations are shown in data set 1.

Data Set 1. SPTFF concentration of mAb A

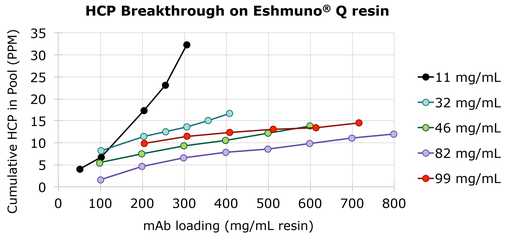


- Higher retentate concentration achieved at lower feed flux
- HCP does not permeate the 30 kD membrane

Effect of feed concentration on Eshmuno® Q resin HCP clearance

In addition to a control sample (unconcentrated CEX eluate), SPTFF material concentrated at several different feed fluxes were evaluated for HCP clearance on Eshmuno® Q resin. Column chromatography experiments were conducted at 200 µL RoboColumn® scale; all conditions evaluated at pH 8.4, 5.5 mS/cm. HCP breakthrough data is shown in data set 2.

Data Set 2. Eshmuno® Q resin HCP clearance



- Unconcentrated control (11 g/L): 10 ppm endpoint at 150 g/L load.
- Increasing concentration results in more shallow breakthrough and increased loading: 82 g/L condition reaches 10 ppm at 600 g/L load (4x improvement).
- Shallow breakthrough curve can be utilized to reduce HCP content in flowthrough pool for improved product quality.
- 99 g/L condition shows 10 ppm breakthrough at 200 g/L load; likely due to mAb-HCP interactions for this molecule.

Effect of feed concentration on Eshmuno® Q resin productivity

Boosting the mass loading on Eshmuno® Q improves resin productivity, since less resin volume is required to polish a given mass of mAb.

$$Productivity\ (g/L/hr) = \frac{M_{Protein}}{V_{Resin}\ x\ t_{cycle}}$$

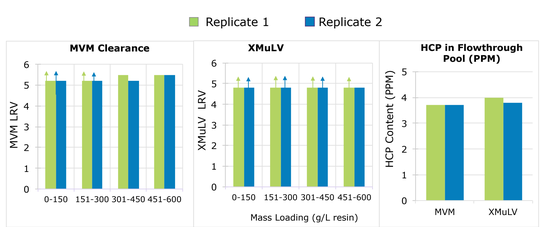
In batch applications, additional productivity gains can be made by taking advantage of reduced load times provided by feed volume reduction. This reduces overall cycle time, further boosting resin productivity. Using data set 2 to calculate productivity, a 5x productivity improvement is achieved for the 82 g/L feed condition.

Eshmuno® Q viral clearance at increased loading

Eshmuno® Q resin is proven to provide excellent viral clearance at mass loadings between 100-300 g/L resin. Given the increased mass loadings that can be achieved by incorporating preconcentration for intensified polishing, it is important to verify that viral clearance is maintained through 600 g/L load.

An 80 g/L solution of mAb A was prepared by SPTFF. MVM and XMuLV viruses were spiked into separate pools of the mAb solution. Virus spiked material was loaded onto Eshmuno® Q resin packed in 1 mL column volume. Experiments conducted at pH 8.4, 5.5 mS/cm. Fractions were collected in 150 g/L load intervals and assayed for virus and HCP content.

Data Set 3. Viral clearance at increased mass loading



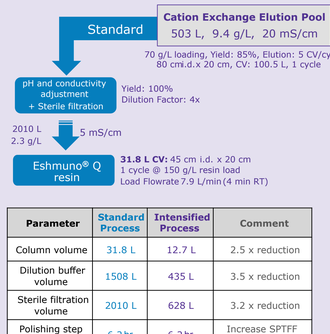
Arrow indicates virus content below limit of detection

- Eshmuno® Q resin provides excellent clearance of MVM and XMuLV viruses while simultaneously removing HCP through 600 g/L load.

Economic Analysis

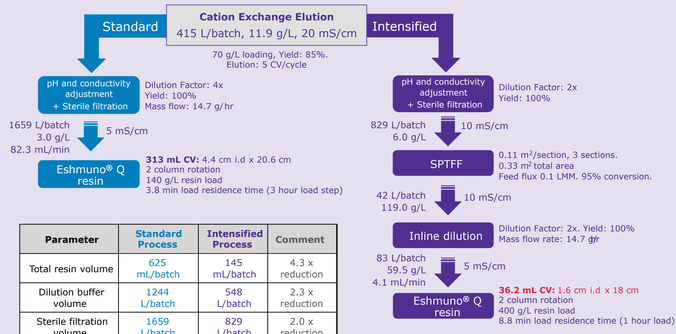
Two economic case studies compare the use of standard polishing and intensified polishing processes. In each case, the intensified polishing approach provides significant savings in resin, buffer, and sterile filtration, leading to an overall reduction in cost. Additionally, the reduced process volumes achieved by SPTFF concentration and minimal dilution improve facility fit by reducing tank volumes and pump requirements.

Case study 1: 2,000 L batch bioreactor, 3 g/L titer, single harvest

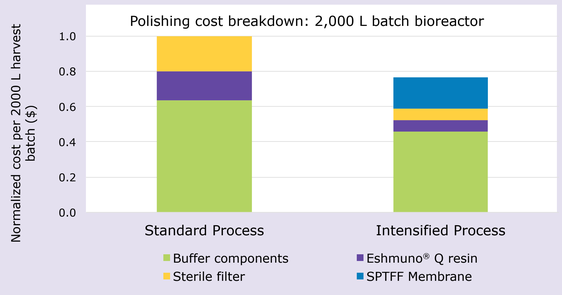


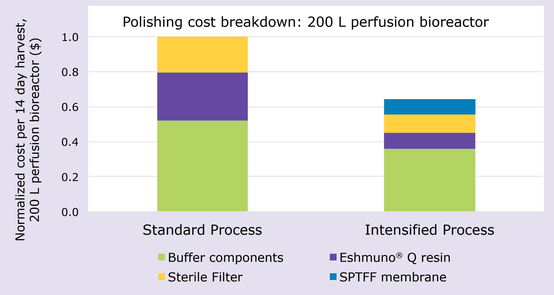
Parameter	Standard Process	Intensified Process	Comment
Column volume	31.8 L	12.7 L	2.5 x reduction
Dilution buffer volume	1508 L	435 L	3.5 x reduction
Sterile filtration volume	2010 L	628 L	3.2 x reduction
Polishing step time	6.2 hr	6.2 hr	Increase SPTFF area to reduce time
Resin productivity	24 g/L/hr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

Case study 2: 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 improvement





Economic calculations assuming 100 re-use cycles per Eshmuno® Q column and 50 re-use cycles per SPTFF device.

Summary

Intensified polishing: A new application which links SPTFF using Pellicon® cassettes and Eshmuno® Q anion exchange resin

- Improve isotherm binding conditions by pre-concentrating feed.
- 4x improvement in Eshmuno® Q resin mass loading while maintaining HCP and viral clearance.

Ideal for next generation continuous process applications

- Concentrate mAb product without recirculation tanks.
- Reduce volume requirements for pH/conductivity adjustment.
- Reduce process tank volumes.

Improve process economics

- Boost Eshmuno® Q resin productivity: increase mass load, decrease cycle time.
- Low SPTFF cost provides significant savings in resin, buffer, and sterile filtration.
- Reductions in tank size and pump requirements improve facility economics.

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