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White Paper

Flow-Through Polishing Process Development: A Holistic Approach

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In the future, biopharmaceutical manufacturing facilities will incorporate fully continuous and automated systems. To achieve this goal, a stepwise approach is being used in which unit operations are intensified and then connected. **Figure 1** shows a typical process for batch mode manufacturing of monoclonal antibodies (mAb) and highlights key areas where intensified processes are being leveraged. It is quite common for upstream processes to make use of an intensified seed train and perfusion bioreactor to improve productivity, speed and efficiency. Downstream processes are being intensified with use of affinity chromatography in continuous operation, in-line virus inactivation, flow-through polishing using a combination of depth filtration, anion exchange, cation exchange and virus clearance, and finally, continuous ultrafiltration and diafiltration.

In this white paper, we describe development and evaluation of a flow-through polishing step, which is critical for the evolution towards a continuous process. We highlight our approach to process development, scouting and integration of different technologies and conclude with an exploration of the effect of different "pre-filters" on extending virus filter capacity.

Flow-Through Polishing Template

Our flow-through polishing template starts with processing the low pH viral inactivation pool through a carbon depth filter followed by flow-through anion exchange (FT-AEX). The pH is then adjusted using an in-line process followed by flow-through cation exchange (FT-CEX) and virus filtration (**Figure 2**).

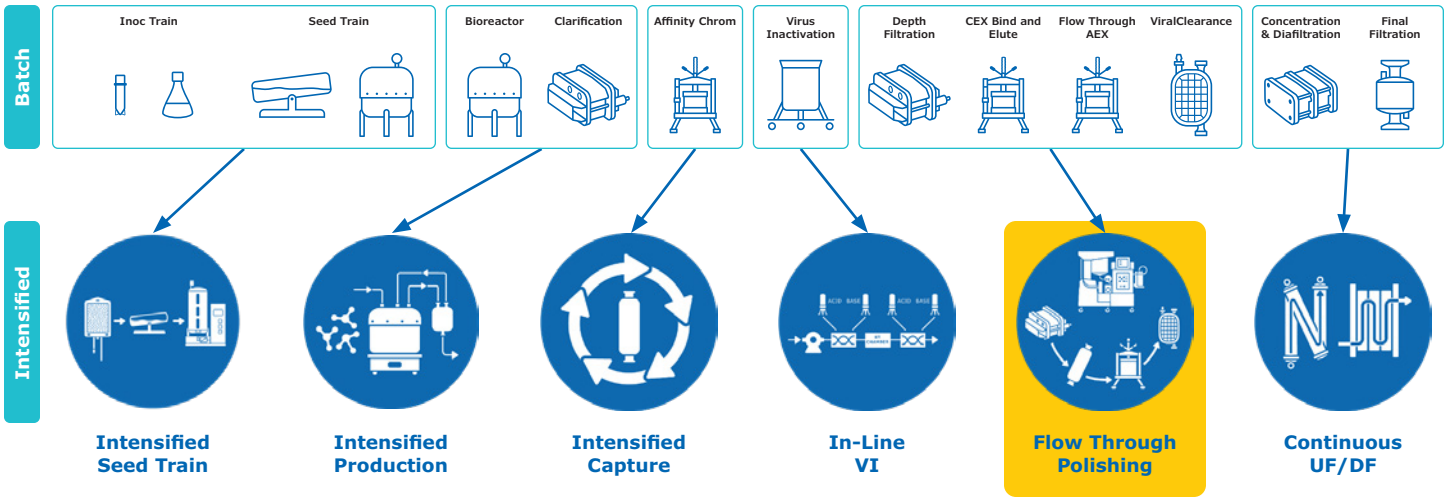


Figure 1. Intensified flow-through polishing is a critical step in the evolution towards a continuous process.

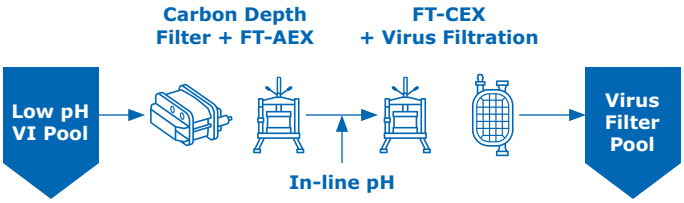


Figure 2. Flow-through polishing template.

To optimize the flow-through polishing template, we recommend a toolbox approach to process development and technology evaluation, as no single solution will be applicable for all molecules. A key consideration during optimization is the type of impurities that must be removed during the polishing step; these will typically include host-cell DNA, viruses, aggregates, host-cell protein (HCP) and leached Protein A. To remove these impurities, different chemistries that work synergistically, in the conditions that are optimum for the overall unit operation, must be selected, as well as the matrices and devices that can accommodate removal of impurities.

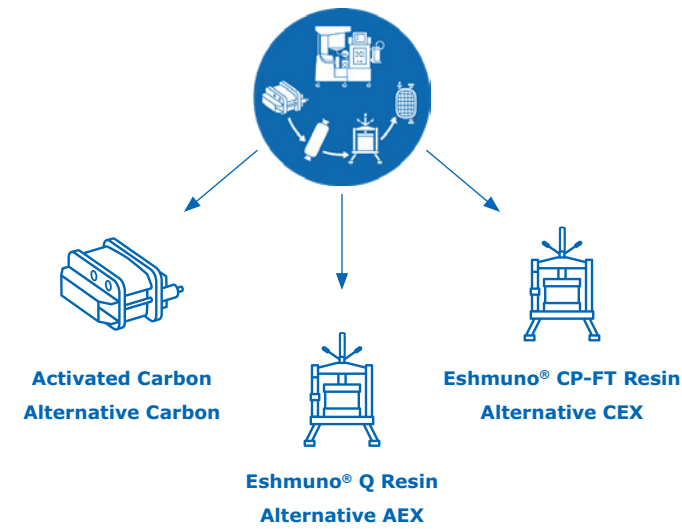
Technology Scouting

In order to optimize the steps in the flow-through polishing template, we evaluated two different technologies for each step – carbon (activated carbon and an alternative), anion exchange (Eshmuno® Q resin and an alternative) and cation exchange (Eshumuno® CP-FT resin and an alternative; **Figure 3A**).

To understand the effect of individual technologies on the success of the polishing step, each is evaluated individually and then their performance is assessed as part of an integrated process (**Figure 3B**). To determine its performance, the cation exchange step was tested individually using a mAb protein A elution pool containing 4-5% aggregates. We then tested anion exchange followed by cation exchange and finally, tested carbon ahead of combined ion exchange steps.

A

Step 1: Scouting



B

Step 2: Integration

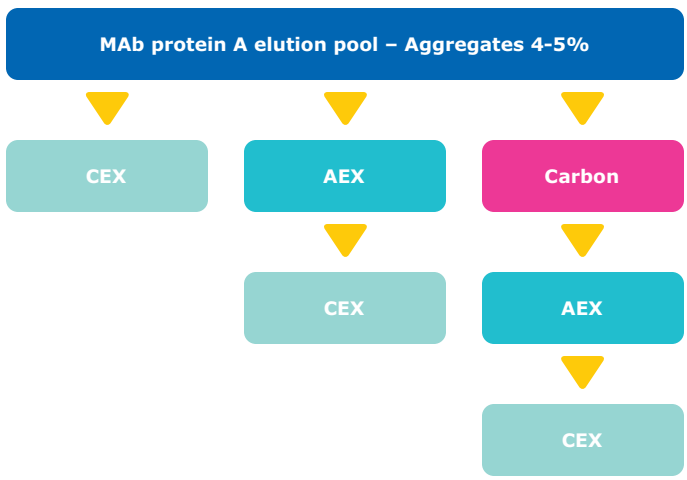


Figure 3. Technology scouting and integration process.

Technology Comparison

The following data sets compare the technologies evaluated for each of the flow-through polishing steps – carbon capture, anion exchange and cation exchange.

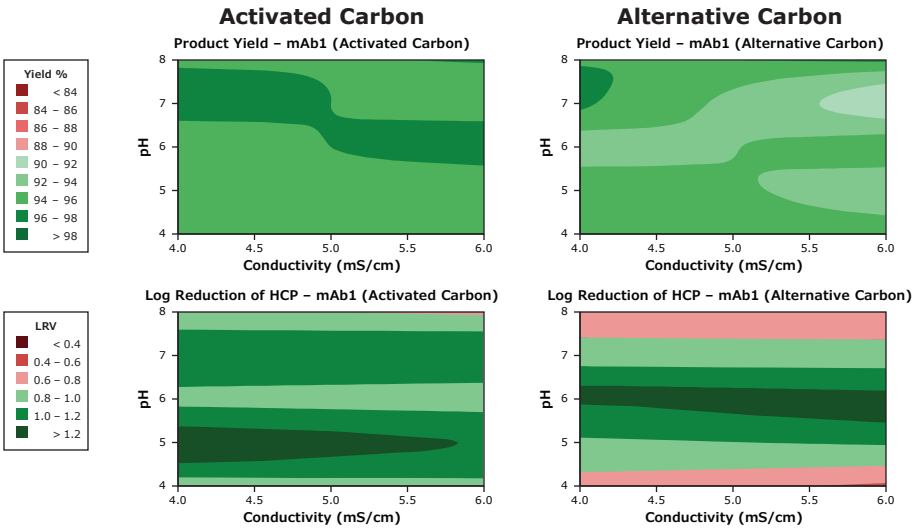
Figure 4 compares the product yield and removal of HCP by activated carbon and an alternative; mAb titer was 23 mg/mL and HCP was 700 ppm. Data are provided in the form of a contour map which defines a process window; this type of presentation facilitates selection of technologies with a broader operating window, which is beneficial when designing continuous processes.

When evaluating yield, both carbon technologies were insensitive towards conductivity, which is desirable for this process. Activated carbon delivered higher yields compared to the alternative. Activated carbon was also more effective in terms of HCP removal performance, which was pH dependent.

A similar comparison was made between Eshmuno® Q resin and an alternative AEX; mAb titer was 17 mg/mL and HCP was 3300 ppm. **Figure 5** shows that the Eshmuno® Q resin outperformed the alternative AEX. Both technologies resulted in a comparable ~0.5 LRV of HCP.

Figure 6 summarizes results of the comparison of Eshmuno® CP-FT resin and the alternative CEX for monomer yield and HCP clearance; mAb titer was 17 mg/mL with 650 ppm of HCP and 3% aggregates. Eshmuno® CP-FT resin delivered better performance in terms of monomer yield and provided robust HCP clearance over the entire pH/conductivity window.

The cation exchange step is also required for aggregate removal. There was greater aggregate removal at a lower pH by the Eshmuno® CP-FT resin compared to the alternative CEX resin. Aggregate clearance was optimized at the higher recommended conductivity for the alternate CEX technology compared to Eshmuno® CP-FT resin (5-15 mS/cm compared to 4-6 mS/cm). This would be an important consideration when formulating process conditions for this technology.



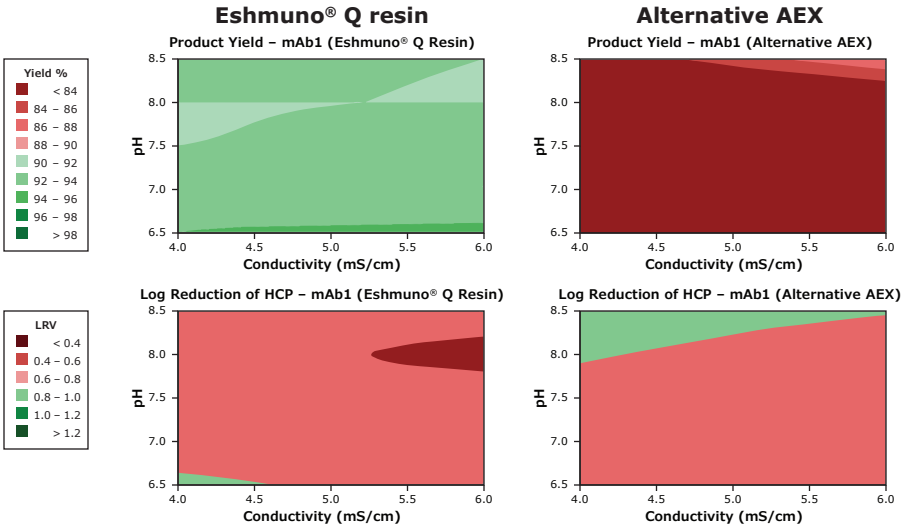
YIELD:

- ✓ Results insensitive to conductivity
- ✓ Yield on Activated Carbon better than on Alternative Carbon

HCP Removal:

- ✓ pH dependent performance
- ✓ Better HCP clearance on Activated Carbon

Figure 4. Activated carbon provided effective removal of HCP with high yields.



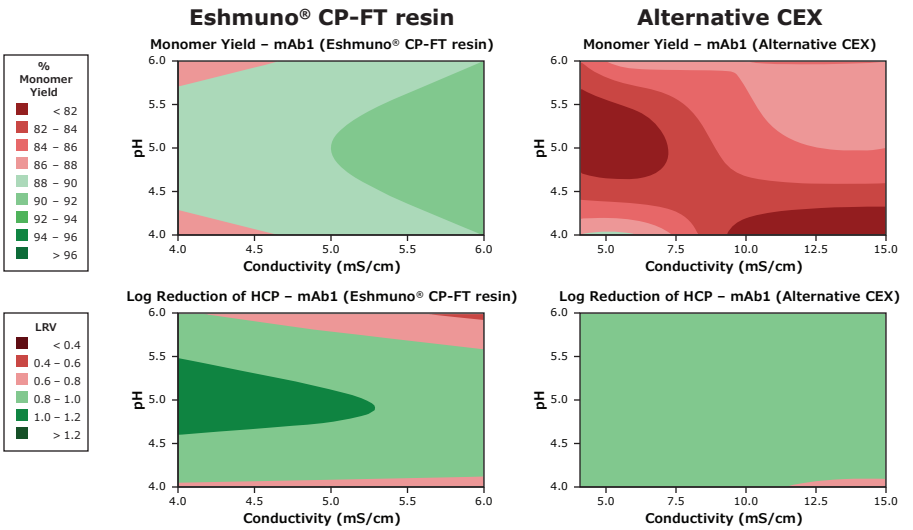
YIELD:

- ✓ Eshmuno® Q resin outperformed alternative AEX
- ✓ Alternative AEX expected to have higher yield at higher loading

HCP Removal:

- ✓ Both technologies resulted in ~0.5 LRV HCP removal

Figure 5. pH dependent HCP clearance with a higher yield on Eshmuno® Q resin



Monomer Yield:

- ✓ Better performance with Eshmuno® CP-FT resin

HCP Removal:

- ✓ Robust HCP clearance over entire pH/conductivity window

Figure 6. Robust HCP clearance using Eshmuno® CP-FT resin.

Technology Integration

Based on results of the comparison studies, the following technologies were used in the following order for flow-through polishing:

- Activated carbon; enabled increased loading on Eshmuno® Q resin
- Eshmuno® Q resin; removed impurities to increase the robustness/loading capacity of Eshmuno® CP-FT resin
- Eshmuno® CP-FT resin; protected Viresolve® Pro virus filter to increase loading capacity

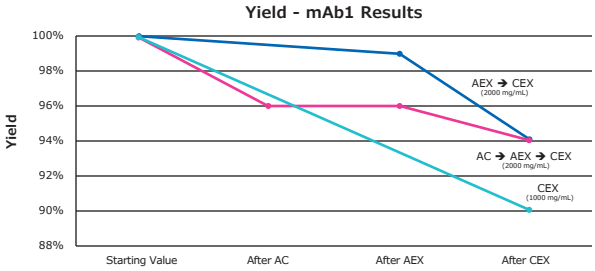
pH and conductivity conditions were as follows:

- Aggregate clearance was optimized at lower conductivity for Eshmuno® CP-FT resin
- Activated carbon: Results were independent of conductivity, and limitedly influenced by pH; conditions selected to match AEX conditions to minimize pH and conductivity adjustment in between
- Anion exchange: pH chosen from scouting for highest HCP removal; higher pH chosen to aid in virus removal
- Cation exchange: pH chosen from scouting with highest HCP removal and lowest aggregate level in the pool

Three experiments were conducted, each with different combinations of technologies (**Figure 7**):

- Eshmuno® CT-FT resin
- Eshmuno® Q resin → Eshmuno® CT-FT resin
- Activated carbon → Eshmuno® Q resin → Eshmuno® CP-FT resin

A



Eshmuno® CP-FT resin:

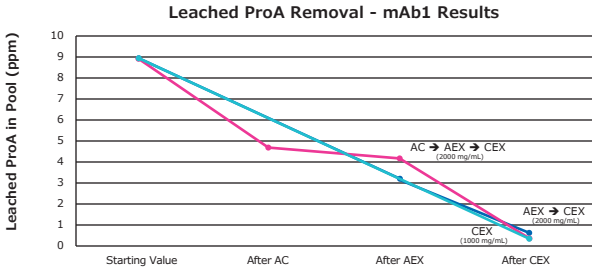
Yield: ≥90%

Leached ProA Removal: ≥96% (0.32 ppm)

HCP Removal: ≥97% (96 ppm)

Aggregate Removal: ≥92% (0.31% in pool)

B



Eshmuno® Q resin → Eshmuno® CP-FT resin:

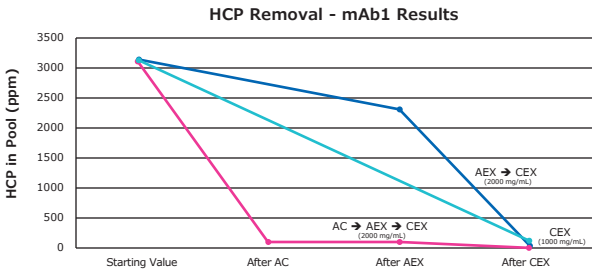
Yield: ≥94%

Leached ProA Removal: ≥92% (0.68 ppm)

HCP Removal: ≥98% (73 ppm)

Aggregate Removal: ≥79% (0.95% in pool)

C



Activated Carbon → Eshmuno® Q resin:

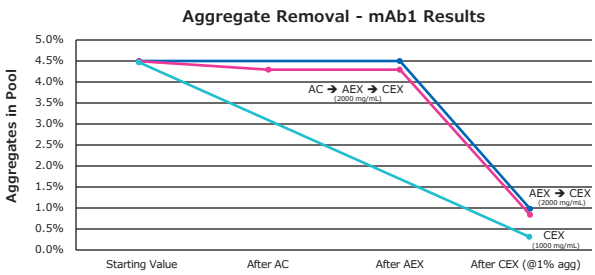
Yield: ≥96%

Leached ProA Removal: ≥50% (4.2 ppm)

HCP Removal: ≥98% (73 ppm)

Aggregate Removal: <5% removal (4.27% in pool)

D



Activated Carbon → Eshmuno® Q resin → Eshmuno® CP-FT resin:

Leached ProA Removal: ≥94% (45 ppm)

Yield: ≥94%

HCP Removal: ≥99% (6 ppm)

Aggregate Removal: ≥82% (0.80% in pool)

Figure 7. Yield (A), removal of leached Protein A (B), removal of HCP (C) and removal of aggregates (D) using the various technology combinations

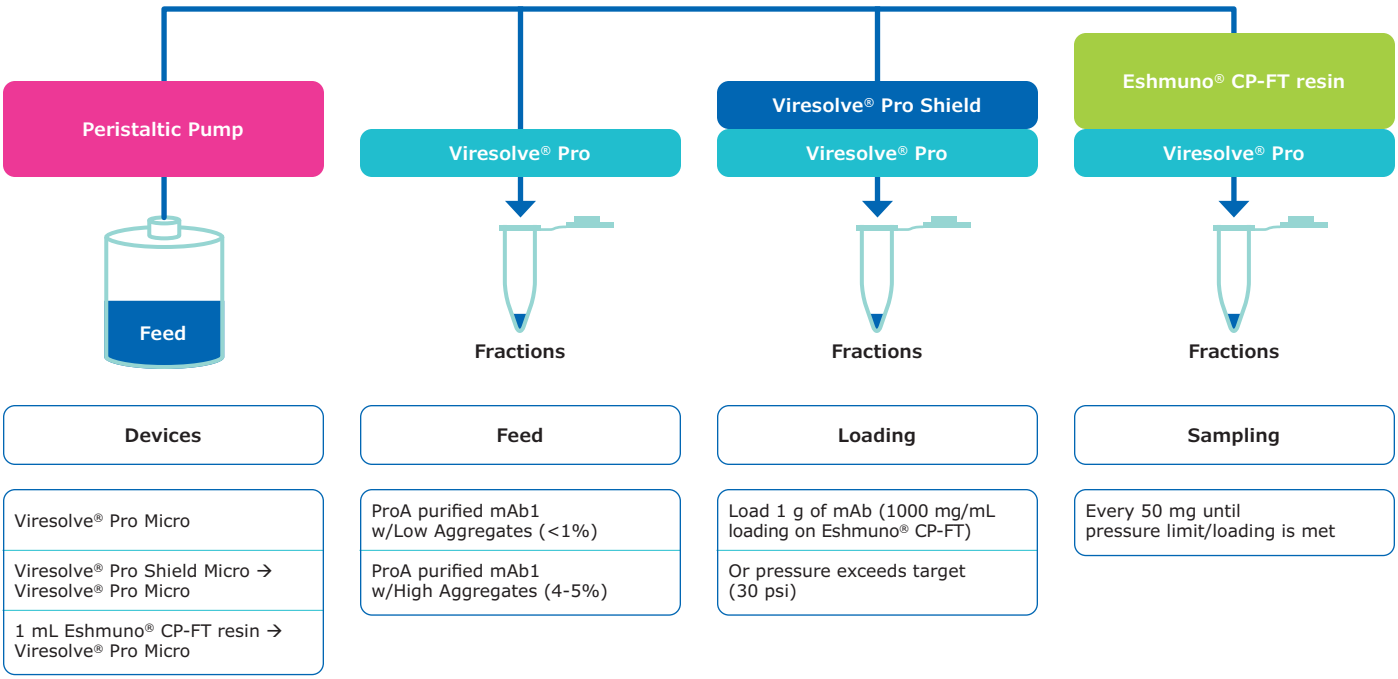


Figure 8. Experimental workflows used to evaluate the effect of feed pretreatment on virus filter capacity.

Loading on the Eshmuno® CP-FT resin was 1,000 g/L at pH 5 and conductivity at 4 mS/cm. For the second set of experiments in which Eshmuno® Q resin was added, loading was 300 g/L. Fractions were collected, analyzed, and then pooled and loaded onto the Eshmuno® CP-FT resin at 2,000 g/L to challenge the cation exchange step. For the third set of experiments, activated carbon was added; fractions were collected, analyzed, and then pooled and passed onto the Eshmuno® Q resin at 2,000 g/L and then pooled again and passed onto the Eshmuno® CP-FT resin at 2,000 g/L

Figure 7A shows the yield for the cation exchange resin alone, and then in combination with the other technologies. The yield was at least 90 percent for all the combinations with cation exchange resin alone being the lowest. This was because, when Eshmuno® CP-FT resin was evaluated alone, it was only loaded to 1,000 mg/mL, while the loading was increased to 2,000 mg/mL for the other two sets of experiments. Similarly, all the technology combinations provided good removal of leached Protein A with Eshmuno® CP-FT resin driving most of the removal (**Figure 7B**).

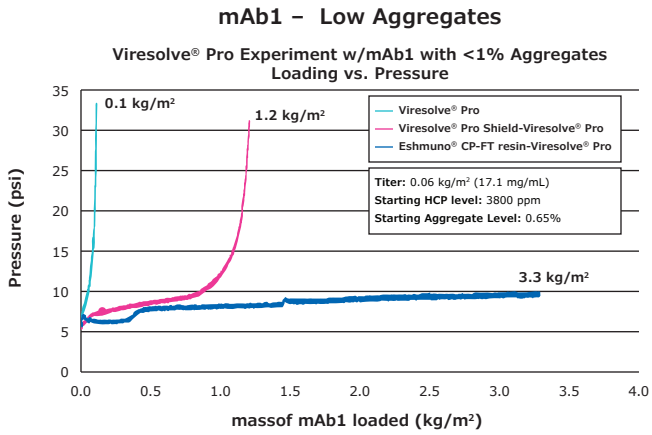
Figures 7C and 7D show results for HCP and aggregate removal, respectively. Activated carbon and cation exchange were the main drivers for HCP removal, and the combination of all three technologies resulted in a significant reduction of more than 99 percent. In terms of aggregate removal, the cation exchange resin was quite successful on its own. When the technologies are combined, it was still the cation exchange that drove most of the aggregate removal, with less than one percent remaining in the pool.

Increasing Virus Filter Capacity

Virus filters are designed to allow passage of most proteins while excluding viruses and can become plugged due to the presence of aggregates in the feed, especially the HMW species. The following experiments demonstrate the ability of the Eshmuno® CP-FT resin to protect Viresolve® Pro virus filter due to its ability to remove aggregates from the feed.

Three experimental workflows were set up to evaluate the effect of different "pre-filters" on virus filter capacity (**Figure 8**).

One feed ran through the virus filter without any prefilters. The second workflow included a Viresolve® Pro Shield filter prior to the virus filter, which can remove aggregates and the third workflow included Eshmuno® CP-FT resin prior to the virus filter. Two mAb Protein A elution pools were used as the feeds; one had aggregates at less than one percent, which is expected after flow-through polishing, while the other feed was Protein A-purified mAb spiked with 4 – 5 percent aggregates. The Eshmuno® CP-FT resin was loaded at 1,000 mg/mL, which is the recommended loading. Pressure for virus filter could not exceed 30 PSI.



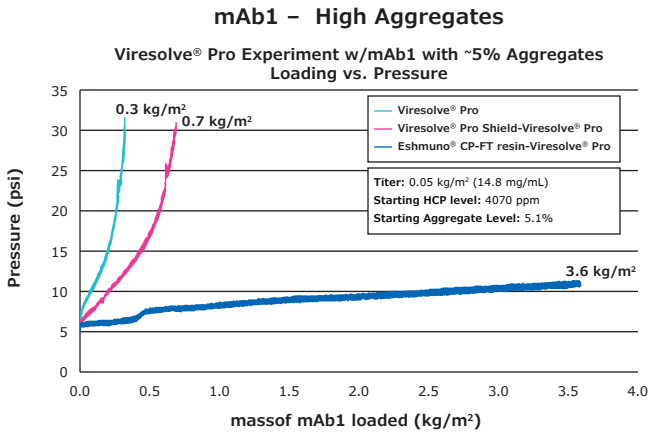
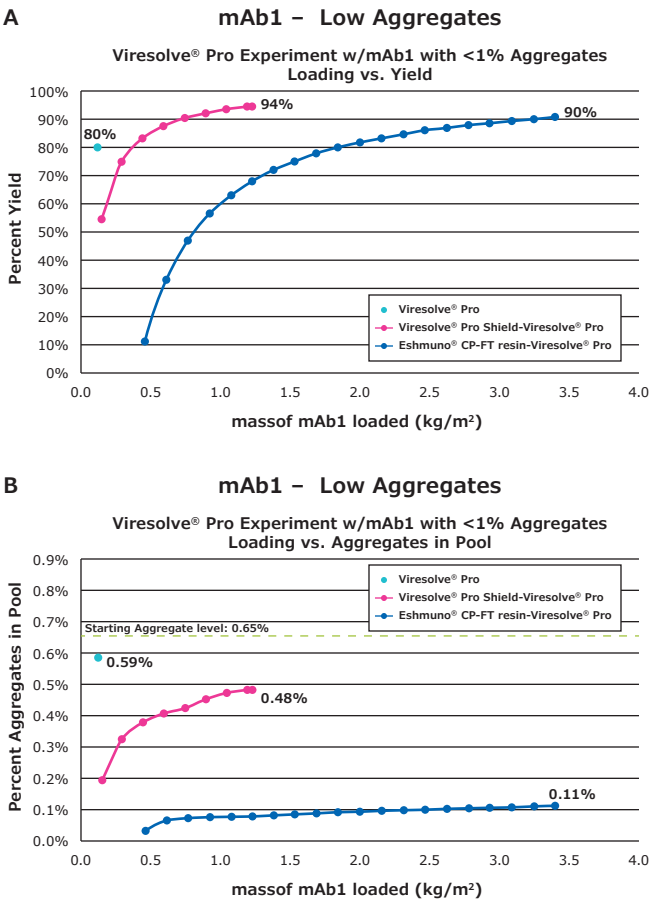
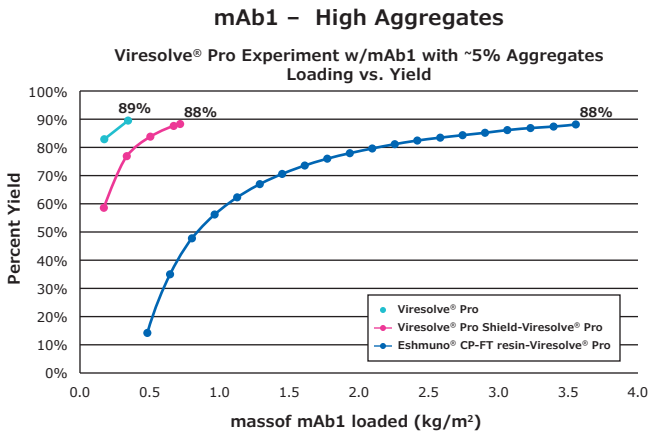


Figure 9. Impact of pretreatment on virus filter loading.





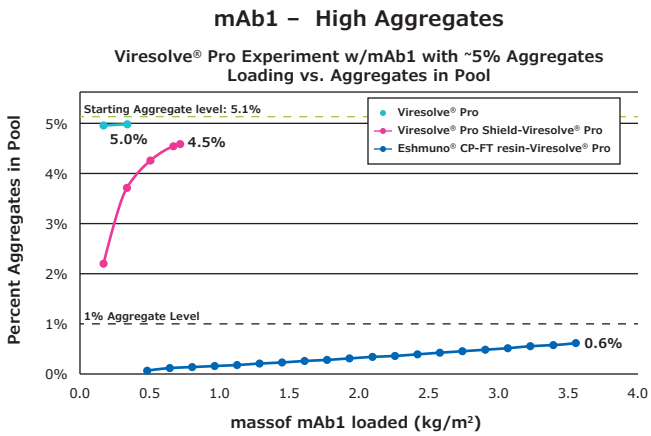


Figure 10. Impact of pretreatment on virus filter loading versus product yield (A) and aggregates (B).

Figure 9 shows a comparison of pressure versus loading for the three different setups. The virus filter by itself would fail as it reached 30 PSI before 0.25 kg/m2 was reached. The Viresolve® Pro Shield removed aggregates enabling loading up to 1.2 kg/m2 with similar results for the high aggregate feed. Incorporation of the Eshmuno® CP-FT resin significantly extended the life of the Viresolve® Pro compared to use of the Viresolve® Pro Shield with an increase of at least 2-4x loading capacity without any pressure spike till end of loading.

Figure 10 shows the product yield (**A**) and aggregates (**B**) versus loading. Higher yields were observed for both low and high aggregate feeds pretreated with Viresolve® Pro Shield and Eshmuno® CP-FT resin (90 and 88 percent respectively). In terms of aggregates, Viresolve ® Pro Shield showed a gradual increase of aggregates which led to filter fouling while Eshmuno® CP-FT resin provided robust aggregate removal throughout loading.

Conclusion

Flow-through polishing is an essential step towards the development of fully intensified, continuous processes. As demonstrated in this white paper, the first step in optimization of a flow-through polishing process requires a holistic perspective. The technology scouting process is molecule dependent and should therefore be performed for each molecule to determine the optimum operating window to maximize yield and impurity removal. A broad process operating window is an important objective as this is extremely important for a continuous process, as is selecting technologies with different mechanisms of impurity removal that can work synergistically in comparable solutions.

The second important takeaway is the advantage provided by designing the flow-through polishing system around a designed-for-purpose cation exchange as the central component of the polishing toolbox. In this study, Eshmuno® CP-FT resin provided robust aggregate removal and contributed to HCP and Protein A removal and was a key enabler for connected and continuous processing. A further advantage of the Eshmuno® CP-FT resin is that it protected subsequent virus filters from clogging.

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MS_WP6732EN Ver. 0.0
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