MAb polishing using a new multimodal anion exchanger

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Introduction

• Multimodal chromatography has proven to be a powerful tool for solving difficult separation challenges, one of them being aggregate removal while maintaining high yields.

• To address these challenges, a new multimodal anion exchange medium (resin) with a particle size designed for polishing (40 µm) was developed. The new medium, Capto™ adhere ImpRes, displays high resolution, making it suitable for use in bind elute mode.

• In this work we present a polishing application where a MAb was purified in bind and elute mode using Capto adhere ImpRes. This medium gives improved resolution, yield, and higher dynamic binding capacity as well as efficient removal of main contaminants in Mab processes such as aggregates, host cell proteins (HCP), leached protein A, and viruses.
Introduction to Capto™ adhere ImpRes

• Capto adhere ImpRes is a BioProcess™ chromatography medium for high-resolution polishing of monoclonal antibodies (MAbs) and other biomolecules.

• The strong anion exchange multimodal ligand displays high selectivity compared with traditional ion exchangers, which allows the possibility to solve challenging purification problems.

• Main contaminants in MAb processes such as aggregates, host cell proteins (HCP), leached protein A and viruses are efficiently reduced.
Introduction to Capto™ adhere ImpRes

**Fig 1.** Chemical structure of Capto adhere ImpRes ligand. The adhere ligand binds to the target molecule through multiple types of interactions, of which the most pronounced are ionic (A) and hydrophobic (B) interactions and hydrogen bonding (C).
Condition screening in PreDictor™ plates

- To find optimal binding conditions for the MAb, static binding capacity (SBC) was determined in 6 μL PreDictor Capto™ adhere ImpRes 96-well plates.
- Binding pH was varied between pH 4.0 and 8.0 and the salt concentration from 0 to 300 mM NaCl.
- The results show that the highest SBC (approximately 65 g/l was obtained at high pH and low conductivity (Fig 2).

Fig 2. Contour plot from screening in PreDictor Capto adhere ImpRes, 6 μL. Start buffers were sodium acetate and sodium phosphate depending on the pH.
Robust loading conditions

- The dynamic binding capacity (DBC) was determined at three different residence times (RT) on Capto™ adhere ImpRes and Capto adhere (75 μm).

- The DBC for Capto adhere ImpRes is higher and less sensitive to RT than Capto adhere between 2 and 8 min residence time.

*Fig 3.* DBC at 2 to 8 min residence time at 90 mM sodium phosphate buffer, pH 7.8 for Capto adhere ImpRes and Capto adhere media. The column used was a Tricorn™ 5/50 (bed height 4.7 cm).
The capability to separate monomers from aggregates was evaluated by running linear gradient elution experiments on Capto™ adhere ImpRes and Capto adhere.

Fractions from the elution peak were analyzed by Size Exclusion Chromatography.

Results showed improved aggregate removal for Capto adhere ImpRes compared to Capto adhere (Fig 4).

Fig 4. Cumulative monomer yield vs cumulative aggregate content for Capto adhere ImpRes and Capto adhere.
Efficient impurity removal

- Based on the gradient elution experiment, a step elution protocol was developed for Capto™ adhere ImpRes.
- The results showed that aggregates HCP and residual protein A were efficiently removed at a high yield and with a small pool volume (Table 1, Fig 5).

**Table 1.** Results from step elution experiment. Start values of aggregates, HCP and Protein A within brackets.

<table>
<thead>
<tr>
<th>Yield (%)</th>
<th>Aggregate conc. (%)</th>
<th>HCP (ppm)</th>
<th>Protein A (ppm)</th>
<th>Pool volume (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>0.5 (1.2)</td>
<td>30 (500)</td>
<td>&lt; 1 (3)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Fig 5. Step elution experiment on Capto adhere ImpRes. Load 30 g Mab/l medium. Load: phosphate pH 7.8; Elution: Phosphate/citrate + 100 mM NaCl pH 5.4; Strip: acetate pH 3.5. Residence time, 4 min; Column: Tricorn™ 5/50 (bed height 4.7 cm).
Viral clearance

- The final step elution protocol was tested for removal of two model viruses, murine leukemia virus (MuLV) and minute virus of mice (MVM).
- The load was spiked with the viruses and the elution pool was analyzed using an infectivity assay.
- Capto™ adhere ImpRes showed efficient viral clearance for both viruses (Table 2).

<table>
<thead>
<tr>
<th>Virus</th>
<th>Log removal (Log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuLV</td>
<td>5</td>
</tr>
<tr>
<td>MVM</td>
<td>5</td>
</tr>
</tbody>
</table>
Conclusions

• In this work, we present results from a case study using Capto™ adhere ImpRes, a multimodal anion exchanger with a particle size designed for polishing.

• A polishing step for a monoclonal antibody was developed in bind and elute mode that showed:
  • Effective removal of MAb aggregates, HCP, leached protein A and viruses at high yields.
  • Improved aggregate removal compared to Capto adhere.
  • Robustness towards residence time.
References

Acknowledgments

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