MAb polishing using a new multimodal anion exchanger

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MAb polishing using a new multimodal anion exchanger

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Summary

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 theses challenges, a new multimodal anion exchange chromatography 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 and elute mode.

Here we present a polishing application where a monoclonal antibody (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.

Introduction to Capto adhere ImpRes

Capto adhere ImpRes is a BioProcess™ chromatography medium for high-resolution polishing of MAbs and other biomolecules. The strong, anion exchange, multimodal ligand displays high selectivity compared with traditional ion exchangers, which makes it possible to solve challenging purification problems (Fig 1). Main contaminants in MAb processes, such as aggregates, host cell proteins (HCP), leached protein A, and viruses are efficiently reduced.

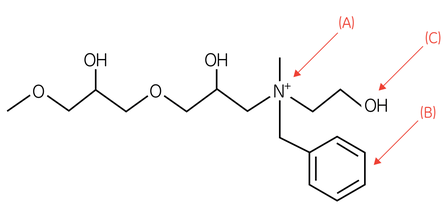

Fig 1. Chemical structure of Capto adhere ImpRes ligand. The adhere ligand binds to the target molecule through multiple types of interactions, of which the most pronounced are ionic (A) and hydrophobic (B) interactions and hydrogen bonding (C).

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).

Robust loading conditions

The dynamic binding capacity (DBC) was determined at three different residence times (RT) on Capto adhere ImpRes and Capto adhere, a related anion exchanger with larger, 75 µm beads.

For Capto adhere ImpRes, the DBC was higher and the residence time had less influence on DBC than shown for Capto adhere between 2 and 8 min RT (Fig 3).

Efficient impurity removal

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).

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 with remained high MAb yield and small pool volume (Table 1, Fig 5).

Table 1. Results from step elution experiment. Start values of aggregates, HCP, and protein A in brackets

Yield (%)	Aggregate conc. (%)	HCP (ppm)	Protein A (ppm)	Pool volume (CV)
90	0.5 (1.2)	30 (500)	< 1 (3)	2.9

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 2. Results from the viral clearance study

VIrus	Log removal (Log_{10})
MuLV	5
MVM	5

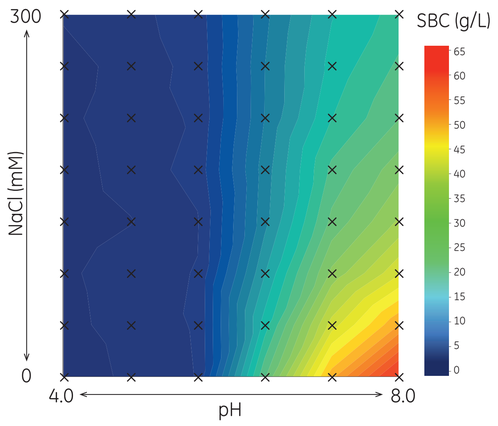

Fig 2. Contour plot from screening in PreDictor Capto adhere ImpRes, 6 µL. Start buffers were sodium acetate and sodium phosphate depending on the pH.

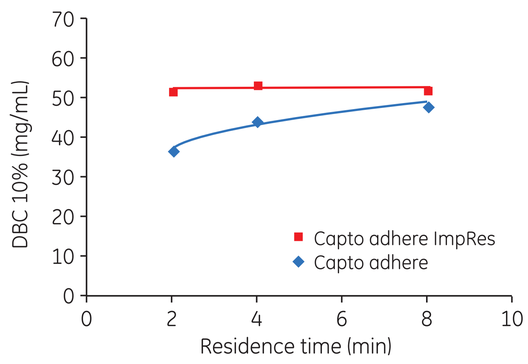

Fig 3. DBC at 2 to 8 min residence time at 90 mM sodium phosphate buffer, pH 7.8 for Capto adhere ImpRes and Capto adhere media. The column used was a Tricorn™ 5/50 (bed height 4.7 cm).

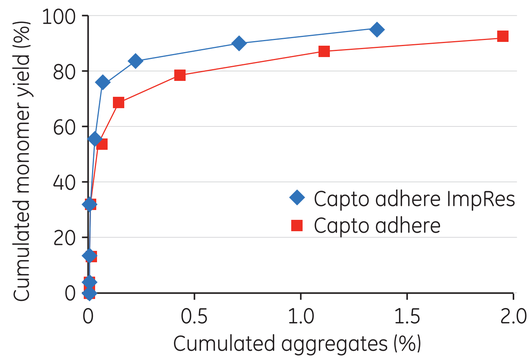

Fig 4. Cumulative monomer yield vs cumulative aggregate content for Capto adhere ImpRes and Capto adhere media.

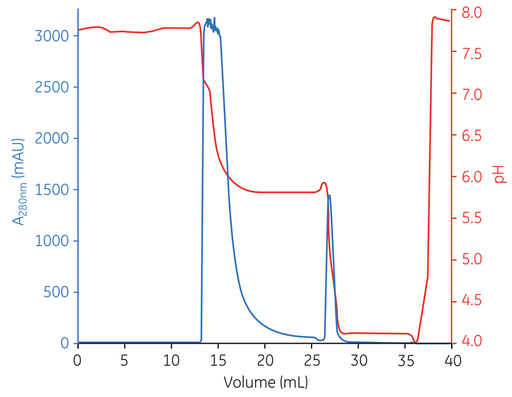

Fig 5. Step elution experiment on Capto adhere ImpRes medium.

Load 30 g MAb/L medium. *Load:* phosphate buffer, pH 7.8; *Elution:* Phosphate/citrate buffer + 100 mM NaCl, pH 5.4; *Strip:* acetate buffer, pH 3.5. *Residence time:* 4 min; *Column:* Tricorn 5/50 (bed height 4.7 cm).

Conclusions

Here we present the 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 MAb was developed in bind and elute mode that showed:

- Effective removal of MAb aggregates, HCP, leached protein A, and viruses with remained high product yields.
- Improved aggregate removal compared to the related anion exchanger, Capto adhere.
- Robustness towards residence time.

References

Application note "Polishing of monoclonal antibodies in bind/elute mode using Capto adhere ImpRes", 29-0273-38 , Edition AA, (2013).

03/2013 29-0493-77 AA