GE Healthcare
Life Sciences

Polishing of monoclonal antibodies in bind/elute mode using Capto™ MMC ImpRes

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Summary

A rapid procedure to establish a robust second step for the purification of a MAb using Capto MMC ImpRes in bind/elute mode is shown. The results from optimization of the loading conditions show high yield of monomeric MAb, as well as good clearance of aggregate, host cell protein (HCP), and leached protein A

Introduction to Capto MMC ImpRes

Capto MMC ImpRes is a chromatographic medium (resin) based on a multimodal cation exchange ligand (Fig 1). The ligand constitutes a hydrophobic part, a weak cation exchange group, and groups that can promote hydrogen bonds. The multimodal ligand in combination with optimized ligand density and bead size allow for improved high-resolution polishing compared with traditional ion exchange media as well as existing multimodal chromatography media.

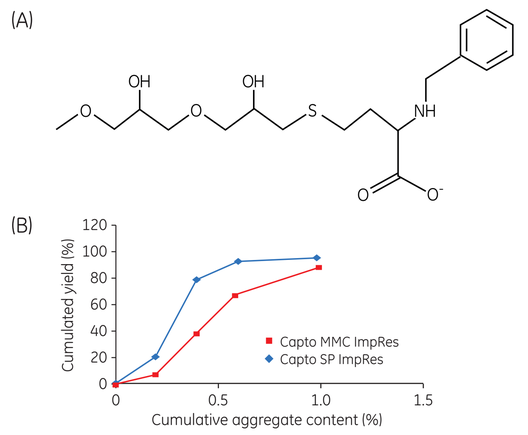

Fig 1. (A) Chemical structure of Capto MMC ImpRes ligand. (B) Capto MMC ImpRes shows improved aggregate removal compared with the traditional ion exchange medium, Capto SP ImpRes.

Experimental & Results

Screening SBC

To find optimal binding capacity for the MAb, static binding capacity (SBC) was determined in 6 µL PreDictor™ Capto MMC ImpRes 96-well plates. Binding pH was varied between pH 4.0 and 8.0 and the salt concentration from 0 to 500 mM NaCl. The results show that the highest SBC was obtained at approx. pH 6.0 and NaCl concentration of 0 to 150 mM (Fig 2).

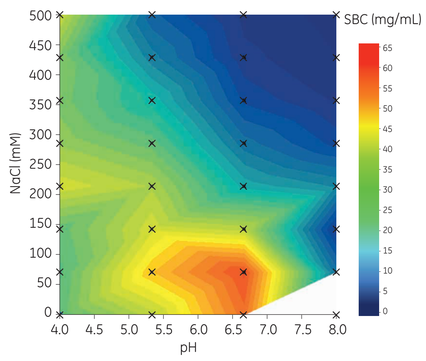

Fig 2. Contour plot from screening in PreDictor Capto MMC ImpRes, 6 µL. Start buffers were sodium acetate, pH 4.0 and 5.3; sodium phosphate, pH 6.3; Tris, pH 8.0.

Verification DBC

The conditions for optimal dynamic binding capacity (DBC) were verified in column format. Two different pH values were used and NaCl concentration was varied. The results of the column DBC measurements were in line with observations in the previous PreDictor plate experiment; the conditions giving the highest DBC were pH 6.0 with a NaCl concentration of 100 mM (Fig 3). Minor effects on DBC from residence time were observed under these conditions (data not shown).

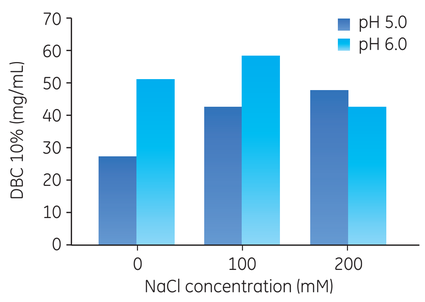

Fig 3. DBC at 4 min residence time and pH 5.0 and 6.0. Column was Tricorn™ 5/50, bed height 4.7 cm. Binding buffer A) pH 5: 50 mM sodium acetate B) pH=6: 25 mM sodium phosphate, 25 mM sodium citrate.

Screening elution conditions

In order to optimize the yield and determine if pool volume and aggregate removal was affected by loading conditions, a salt gradient was applied at two different loading pH (Fig 4). Eluted pools containing 90% of the MAb had low levels of aggregates, HCP, and protein A (Table 1).

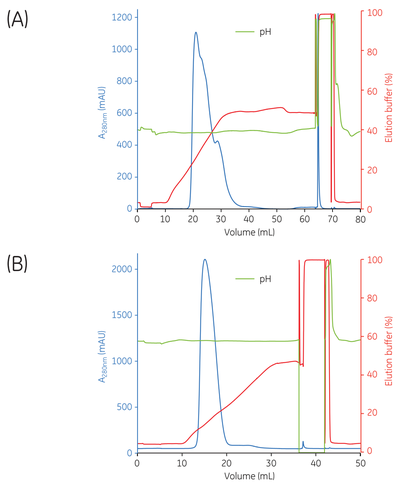

Fig 4. Elution of the MAb in a gradient from 0.1 to 1 M NaCl at two different pH: 5.0 (A) and 6.0 (B). Tricorn 5/50 column, bed height 4.7 cm was used. Residence time was 4 min.

Three-factor screening and validation

After establishing the elution conditions in terms of pH and salt, the effect of gradient length, sample load, and residence time on aggregate content and pool volume was investigated by a full factorial design. Good models were obtained for both aggregate content (Fig 5) and pool volume (data not shown). The model validation in HiScreen™ Capto MMC ImpRes column confirmed the low aggregate content in the eluted material.

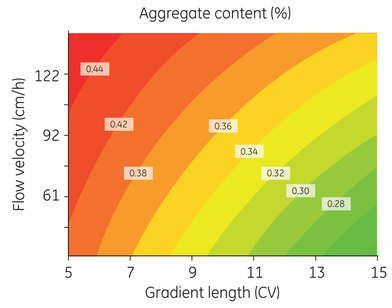

Fig 5. Contour plot for aggregate content. Sample load was varied between 30 and 42 g/L, gradient length 5–15 CV and flow velocity 37–140 cm/h, corresponding to 2–8 min residence time. Loading conditions were 0.1 M NaCl, pH 6.0 and elution was done with gradient up to 1 M NaCl.

Table 1. Levels of impurities at different load pH

Sample	Aggregate at 90% yield (%)	Pool volume (CV)	HCP (ng/mL)	Protein A (ng/mL)
Post PrA	2	-	250	15
pH 5.0	0.04	14.1	16	< 3*
pH 6.0	0.22	5.4	56	< 3*

* Limit of quantitation

Table 2. Comparison of predicted and actual result when verifying model in HiScreen format

	Accumulated yield (%)	Aggregate at 90% yield (%)	Pool volume (CV)
Predicted	NA	0.34	4.1
Actual	99	0.39	4.0

Discussion

Binding conditions were swiftly established in high-throughput process development (HTPD) format and verified in column format. The screening in the first step also supports the results from investigation of elution conditions in column format. In HTPD format, a maintained SBC was observed at high NaCl concentration combined with low pH . To elute the protein from the column, pH had to be increased. A significantly larger pool volume was observed at pH 5.0 compared to pH 6.0, indicating stronger binding at lower pH. Optimization of additional factors, such as gradient length, residence time, and sample load further decreased the pool volume while a low aggregate content was achieved.

Conclusion

This work describes a rapid procedure to establish a robust second step in bind/elute mode for the purification of a MAb using Capto MMC ImpRes. Good agreement between batch mode screening and column experiments was observed. By optimizing running conditions, a process step giving good aggregate removal was achieved. The obtained model was validated in HiScreen column format confirming the performance. Excellent agreement between predicted and actual performance was observed.

References

Application note "Polishing of monoclonal antibodies using Capto MMC ImpRes in bind/elute mode", 29-0373-49 Data file Capto MMC ImpRes, 29-0356-74

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