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Multimodal chromatography media to resolve purification platform challenges and strategies to handle 'tricky' MAbs

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Abstract

Monoclonal antibodies (Mabs) are commonly purified using platform approaches, where the initial protein A capture step is followed by two polishing steps. Protein A capture is a generic step that does not need extensive optimization. Designing a robust polishing step, however, requires process optimization, in which it is crucial to also consider MAb stability. Here, we describe the screening and optimization of conditions for the final polishing step of a challenging MAb prone to aggregation at pH values above 6. Both the traditional Capto™ Q anion exchange (AIEX) chromatography medium (resin) and the multimodal Capto adhere ImpRes AIEX medium were evaluated. The advantages of using a multimodal anion exchange (AIEX) medium over a traditional AIEX medium for the MAb used here will be presented. Capto adhere ImpRes allowed a wider operational window and was shown to be a more suitable choice for the final MAb polishing step.

Introduction

Even if MAbs share many properties, all antibodies will not behave exactly the same. Certain MAbs will be more challenging to purify, for example, due to a complex impurity profile or aggregate formation under certain conditions. In such cases, an expanded MAb purification toolbox, with different options primarily in the polishing steps, can be beneficial. Here, MabSelect SuRe™ LX protein A medium was used for MAb capture. In the first polishing step, Capto S ImpAct cation exchange (CIEX) medium was used to reduce aggregate content to acceptable levels (~ 1%). The main purpose of the second polishing step is to reduce host cell protein (HCP). For this step, both traditional AIEX media as well as multimodal AIEX media, are available (Fig 1).

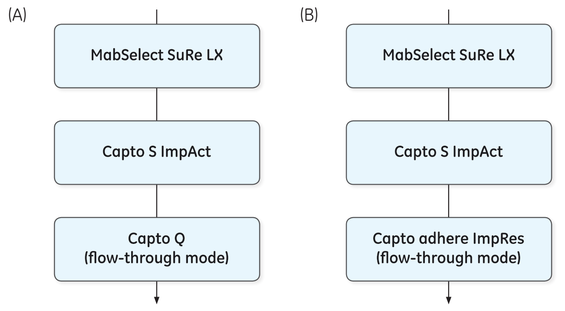

Fig 1. (A) Standard three-step MAb purification process using Capto Q AIEX medium in the last polishing step. (B) Alternative tree-step MAb purification process with Capto adhere ImpRes as multimodal AIEX medium in the last polishing step.

Materials and methods

MAb stability

A central composite face-centered (CCF) design was used to investigate pH and salt dependence of MAb stability. MAb (2 g/L) was incubated for 48 h in 25 mM sodium acetate or 25 mM sodium phosphate buffers of pH 4.5, 6.0, or 7.5 with NaCl concentrations between 0 and 250 mM.

Screening of conditions for the Capto Q step

For screening of conditions for the Capto Q polishing step (flow-through [FT] mode) using Tricorn™ 5/50 columns (1 mL column volume [CV]), a full factorial design at two levels with three center points (11 experiments in total) was used. The investigated factors and their ranges are given in Table 1. A MAb, purified on MabSelect SuRe LX and conditioned to the investigated run conditions, was used as sample.

Table 1. Factors and their ranges used for screening of conditions for the Capto Q polishing step

Factor	Low	High
pH	5.5	7.5
NaCl (mM)	0	50
Load (g MAb/L medium)	50	250

Screening of conditions for the Capto adhere ImpRes step

For the Capto adhere ImpRes screening study, PreDictor™ 96-well filter plates with 6 µL chromatography medium in each well were used. The medium was equilibrated with loading buffers of various pH and conductivity before 200 µL of MAb-containing samples, conditioned to the different loading conditions, were added. A 90 min incubation was performed under vigorous shaking of the plate on an orbital shaker. Unbound material (flowthrough) was analyzed for monomer and aggregate content using size exclusion chromatography (SEC) Superdex 200 Increase 10/300 GL.

Optimization of the Capto adhere ImpRes step

The most promising conditions for the Capto adhere ImpRes step, identified in the 96-well plate study, where used to set the ranges for the column optimization experiments in Tricorn 5/50 columns (1 mL CV) (FT mode). The optimization study was performed using the built-in design of experiments (DoE) module of ÄKTA™ avant chromatography system. A quadratic CCF design with three center points (17 experiments in total) was used. The investigated factors and their ranges are given in Table 2. A MAb, purified on MabSelect SuRe LX and buffer exchanged to the investigated conditions, was used as sample.

Table 2. Factors and their ranges used for optimization of the Capto adhere ImpRes polishing step

Factor	Low	High
pH	5.5	6.5
NaCl (mM)	0	300
Load (g MAb/L medium)	100	200

Verification of process conditions

Verification of conditions for the polishing steps was performed in Tricorn 5/50 columns (1 mL CV). A MAb, purified using MabSelect SuRe LX and Capto S ImpAct, was used as sample. Sample load for Capto Q polishing was 120 g MAb/L medium, at a residence time of 2 min. Sample load for Capto adhere ImpRes polishing was 150 g MAb/L medium, at a residence time of 5.4 min. The flowthrough was analyzed for MAb monomer and aggregate content, HCP, and leached protein A.

Results

MAb stability

During characterization of the MAb used in this study, aggregation was found to be pH dependent. The influence on aggregation was investigated by varying pH and salt concentration, and as can be seen in Figure 2, only pH was found to be a significant factor for aggregation. For highest stability, pH should be as low as possible.

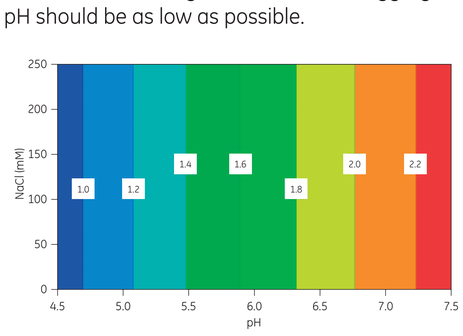

Fig 2. Aggregate levels (white squares) after MAb incubation under various pH and salt conditions for 48 h.

Capto Q condition screening

During the screening of conditions for Capto Q, the aggregate clearance was non-significant. As the MAb is prone to aggregation at the conditions used, statistical models could not be achieved for this response. For the HCP removal, a good model (R^2 = 0.998, Q^2 = 0.941) was achieved. Load had only a small impact on HCP levels, with higher loads corresponding to slightly higher HCP levels. The response surface plot at a sample load of 150 g/L medium is shown in Figure 3. As can be seen, a pH above 7 and a low salt concentration were required for sufficient HCP removal using Capto Q in FT mode.

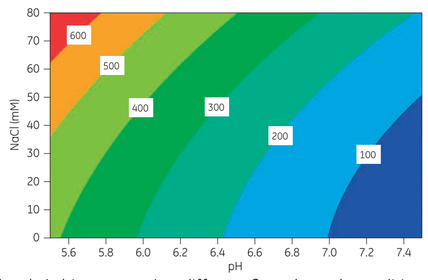

Fig 3. HCP levels (white squares) at different flow-through conditions using Capto Q.

Capto adhere ImpRes selectivity screening

For a flow-through step, the binding of monomer should be low. As shown in Figure 4, Capto adhere ImpRes exhibits higher binding capacity for MAb monomer and aggregates at pH above 6.5. The selected conditions were further explored in a column study.

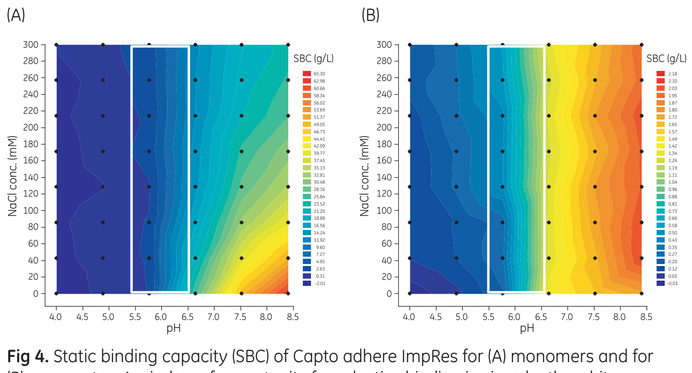

Fig 4. Static binding capacity (SBC) of Capto adhere ImpRes for (A) monomers and for (B) aggregates. A window of opportunity for selective binding is given by the white square.

Optimization of the Capto adhere ImpRes step

Results from optimization of the Capto adhere ImpRes step is shown in Figure 5. Good models for HCP removal were obtained with R^2 and Q^2 values above 0.9. Depending on pH, sufficient HCP removal can be obtained at salt concentrations below 150 mM. At the same time, aggregate removal is favored at a high pH, although with curvatures in the plots. Our investigations have shown that a low pH and intermediate salt concentration is advantageous for high yields (data not shown). Thus, the conditions selected for final column experiments were pH 6.3 and 125 mM NaCl. This salt concentration is half that from the previous Capto S ImpAct step, which facilitates coupling of the two steps with an in-line dilution process.

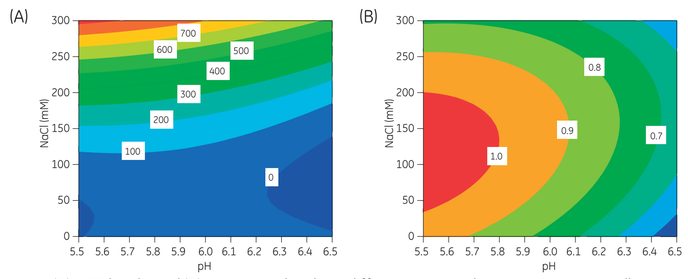

Fig 5. (A) HCP levels and (B) aggregate levels at different run conditions using Capto adhere ImpRes.

Process verification

The sample used for verification had been subjected to a prior polishing step using Capto S ImpAct and had an aggregate content of approximately 1%, 150 ppm HCP, and less than 1 ppm leached protein A ligand. The chromatograms from the verification runs on Capto adhere ImpRes and Capto Q are shown in Figure 6, where also the final level of impurities in the flowthrough pool is given. Both media enabled efficient HCP removal at high yield, while, as expected, the aggregate levels were lower using Capto adhere ImpRes.

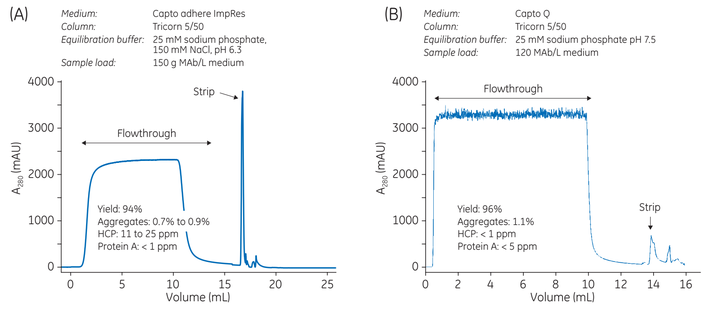

Fig 6. Verification of process conditions for (A) Capto adhere ImpRes and (B) Capto Q. The chromatogram in A was obtained using a 2 mm UV cell, while for the chromatogram in B, the UV cell was 10 mm.

Conclusion

Although the traditional Capto Q AIEX medium has a high ability to remove HCP, the required run conditions caused aggregation of the MAb used in this study. Capto adhere ImpRes allowed the use of a lower pH to enhance MAb stability. As salt was included in the elution buffer from the previous polishing step, the ability to operate at elevated salt concentrations is an additional benefit of Capto adhere ImpRes. To solve the purification task of the challenging MAb used in this study, the traditional Capto Q medium can be replaced by the multimodal Capto adhere ImpRes medium.

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