Optimization of dynamic binding capacity and aggregate clearance in a monoclonal antibody polishing step

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Optimization of dynamic binding capacity and aggregate clearance in a monoclonal antibody polishing step

Capto™ S ImpAct is a strong cation exchange (CIEX) chromatography medium (resin) designed for monoclonal antibody (MAb) polishing steps. In this study, optimization of binding conditions targeting high dynamic binding capacity (DBC) and aggregate clearance was performed. High-throughput process development (HTPD) methodology was applied, using different HTPD tools at various stages of the development process. PreDictor™ 96-well filter plates were used for initial screening of static binding capacity (SBC) and determination of the experimental space, whereas determination of DBC was performed in PreDictor RoboColumn™ units using a design of experiments (DoE) approach. Lab-scale columns were used for selectivity studies and verification of optimal binding condition. The DBC at 10% breakthrough (Q_{B10}), determined to 109 mg MAb/mL medium in lab-scale columns, correlated well with the Q_{B10} obtained using PreDictor RoboColumn units. With a sample load of 76 mg MAb/mL medium (70% of Q_{B10}), the obtained aggregate content was less than 1% at a monomer yield of 93%.

Introduction

With a growing pipeline of new targets and an increased competition, cost-effective purification process schemes are one of the highest priorities for biopharmaceutical manufacturers. Downstream processing of MAbs are commonly performed using purification platforms, comprising a protein A medium in the initial capture step, an intermediate polishing step using a CIEX medium, and the use of a traditional anion exchange (AIEX) or multimodal medium in the final polishing step. Even with an existing purification platform, there is still a need to refine process conditions for new MAb targets coming through the pipeline. In most cases, the initial capture step and the final AIEX step are being operated under fairly similar process conditions for the different MAbs and require limited or no process development. In contrast, the operating conditions for the CIEX step usually require somewhat more optimization to efficiently reduce MAb aggregates and fragments, host cell protein (HCP), and leached protein A ligand, at a high monomer yield. This work describes optimization of a MAb polishing step including Capto S ImpAct medium. Acceptance criteria for this process step were an aggregate content of less than 1% at a monomer yield of above 90% in the product pool.

Materials and methods

Starting material

The MAb used in this study was produced using Chinese hamster ovary (CHO) cells. MAb capture from cell supernatant was performed using MabSelect SuRe™ LX protein A affinity chromatography medium. The isoelectric point (pI) of this MAb is 8.4. MAb aggregate concentration in the eluate from the MabSelect SuRe LX step varied between 2% and 3%.

Screening of SBC

SBC was determined using a PreDictor Capto S ImpAct, 2 µL 96-well filter plate. Loading buffers of pH 4.5 to 8 with 0 to 250 mM NaCl were prepared automatically using a robotic liquid handling system (Tecan, Männedorf, CH). Equilibration of the medium was performed by addition of 200 µL loading buffer per well, followed by agitation at 1100 rpm for 1 min, after which the buffer was removed using a vacuum manifold. Equilibration was repeated three times. After the last equilibration, buffers were removed by centrifugation at 500 × g for 1 min. MAb-containing samples (3.9 mg/mL) of 200 µL, conditioned to the different loading conditions,

were added to each well followed by agitation for 1 h 45 min. The unbound material (flowthrough) was collected into UV transparent plates by centrifugation and protein concentration was determined spectrophotometrically at 280 nm. Spectrophotometric data was entered into Assist software and SBC was calculated.

Determination of DBC

MAb elution buffer from the protein A capture step was exchanged to various loading conditions using PD-10 Desalting columns and MAb concentrations were adjusted to 16 mg/mL. Eight PreDictor Capto S ImpAct RoboColumn 600 µL units were operated in parallel on a robotic liquid handling system (Tecan, Männedorf, CH). The RoboColumn units were equilibrated with 10 column volumes (CV) of the different buffers at a flow rate of 2.5 µL/s. Samples of 12 × 600 µL (total sample load of 7.2 mL) were loaded on the RoboColumn units at a flow rate of 1.85 µL/s, corresponding to a column residence time of 5.4 min. The flowthrough was collected in 200 µL fractions into UV transparent 96-well collection plates. Protein concentration was monitored spectrophotometrically at 300 nm.

The sample load (mg MAb/mL medium) was calculated according to the following equation:

$$\text{Sample load} = \frac{C_0(V_L - V_0)}{V_C}$$

Where

V_L = accumulated volume per fraction (mL)

V_0 = system and column void volume (mL)

C_0 = MAb concentration in the sample (mg/mL)

V_C = volumetric bed volume (mL)

In this study, V_0 was approximated to equal V_C. The breakthrough (breakthrough concentration [C]/start concentration [C_0]) was plotted against the sample load. The sample load at 10% breakthrough (i.e., where C/C_0 is equal to 0.1) was determined for the different loading conditions.

Verification of DBC

Buffer of the MAb eluate from the protein A capture step was exchanged for 50 mM sodium acetate, 50 mM NaCl, pH 5.0 using PD-10 Desalting columns and MAb concentration was adjusted to 16.7 mg/mL. Q_{B10} was determined by frontal analysis using Capto S ImpAct packed in a Tricorn™ 5/100 column connected to an ÄKTA™ chromatography system. Protein concentration was monitored spectrophotometrically at 305 nm.

Q_{B10} was calculated according to the following equation:

$$Q_{B10} = \frac{C_0(V_{10\%} - V_0)}{V_C}$$

where

$V_{10\%}$ = load volume (mL) at 10% breakthrough

V_0 = system and column void volumes (mL)

C_0 = MAb concentration in the sample (mg/mL)

V_C = volumetric bed volume (mL)

Selectivity study

Buffer of the MAb eluate from the protein A capture step was exchanged for various chromatographic conditions (equilibration buffers) using PD-10 Desalting columns. Sample was loaded at 50 mg MAb/mL medium on Capto S ImpAct packed in a Tricorn™ 5/100 column connected to an ÄKTA system. A flow rate of 0.38 mL/min, corresponding to a column residence time of 5.4 min, was used. A linear gradient with a NaCl increase of 350 mM over 20 CV was applied for elution. Fractions of 0.75 mL were collected during elution and 200 µL of each fraction was transferred to a 96-well plate. To stabilize the MAb, 10 µL of 1.25 M sodium acetate, pH 4.5 was added to each well.

Verification of optimal binding condition at high sample load

MAb eluate in 50 mM sodium acetate from the protein A capture step was adjusted to pH 5.0 and NaCl was added to a concentration of 50 mM. Sample was loaded at 76 mg MAb/mL medium on Capto S ImpAct in a Tricorn 5/100 column connected to an ÄKTA system. A flow rate of 0.38 mL/min, corresponding to a column residence time of 5.4 min, was used. A linear gradient from 50 to 400 mM NaCl in 50 mM sodium acetate, pH 5.0 over 20 CV was applied for elution. Fractions of 0.75 mL were collected during elution and 150 µL of each fraction were transferred to a 96-well plate. To stabilize the MAb, 7.5 µL of 1.25 M sodium acetate, pH 4.5 was added to each well.

Determination of aggregate concentration

Fractions from the chromatography runs were collected and analyzed by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column. The peaks were integrated and the dimer/aggregate concentrations (in percent) were calculated. For the gradient elution runs, cumulated recovery of monomers was plotted against cumulated aggregate concentration.

Determination of HCP and protein A content

HCP content in the elution fractions and product pools were analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies Inc.) and Gyrolab™ workstation (Gyros AB). Protein A content was determined using a commercially available ELISA kit (Repligen Corp.).

Results and discussion

Screening of SBC

SBC was determined using the Assist software (Fig 1). SBC was shown to increase with increasing pH from 5 to 6 and with increasing NaCl concentrations up to 40 mM. However, highest SBC was obtained at pH 6.6 without salt. As previous studies have shown that the used MAb is not stable at a pH above 6.0 (data not shown), the experimental space for further determination of DBC and aggregate removal was set to a pH from 5.0 to 6.0 at a NaCl concentration from 0 to 50 mM.

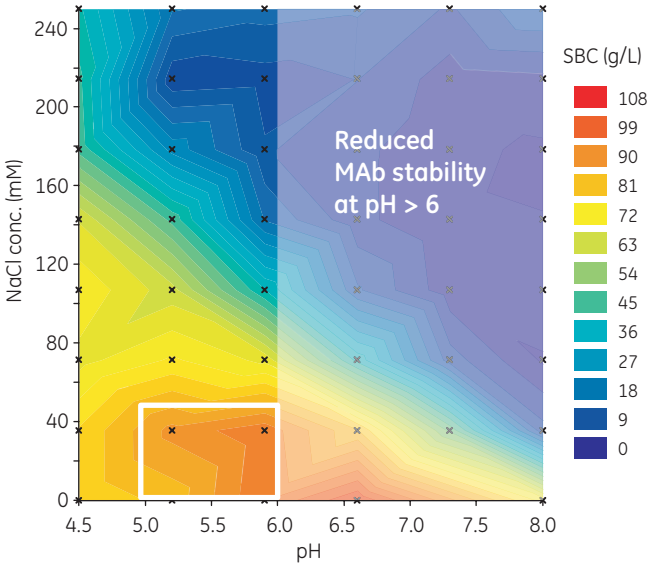

Fig 1. Contour plot from SBC screening in a PreDictor Capto S ImpAct, 2 µL plate. As this MAb was not stable at pH > 6.0, the experimental space for further determination of DBC and aggregate removal was set to a pH from 5.0 to 6.0 at a NaCl concentration from 0 to 50 mM (circumscribed).

Determination of DBC

PreDictor RoboColumn Capto S ImpAct, 600 µL units were used for fast determination of DBC using a DoE approach. As factors, pH in the range from 5.0 to 6.0 and NaCl concentration from 0 to 50 mM were selected. One additional run at pH 4.5 was added to this study. Breakthrough curves are displayed in Figure 2.

DBC increased with increasing pH and NaCl concentration in the explored range, with the lowest DBC of 74 mg MAb/mL medium at pH 4.5 and no salt and the highest DBC of 116 mg MAb/mL medium at pH 6 and 50 mM NaCl. DBC at different binding conditions are listed in Table 1.

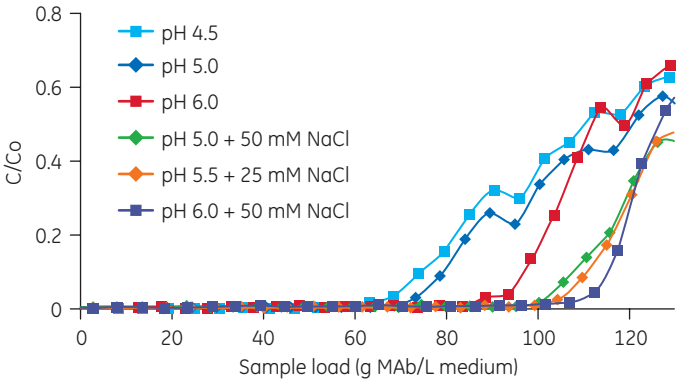

Fig 2. Overlay of breakthrough curves for the different binding conditions. Fractions of 200 µL from the column effluent during sample load were collected in UV transparent plates. The breakthrough curve for pH 5.5, 25 mM NaCl (center point) is presented as an average of triplicate determinations.

Table 1. DBC at different binding conditions

Binding buffer	pH	NaCl (mM)	DBC (mg MAb/mL medium)
50 mM sodium acetate	5.0	-	80
50 mM sodium acetate	6.0	-	97
50 mM sodium acetate	5.0	50	108*
50 mM sodium acetate	6.0	50	116
50 mM sodium acetate (center point)	5.5	25	112, 112, 108
50 mM sodium acetate	4.5	-	74

* Condition resulting in a DBC above 100 mg MAb/mL medium and aggregate concentration below 1%.

Evaluation of aggregate removal

The selectivity between MAb monomer and aggregates using Capto S ImpAct was determined using a DoE approach. As factors, loading pH in the range from 5.0 to 6.0 and NaCl concentration from 0 to 50 mM were selected. MAb aggregate concentration and HCP content in the elution pool at 90% monomer yield were monitored as responses. Elution was performed using linear gradients with NaCl increase from 0 to 350 mM, from 25 to 375 mM, or from 50 to 400 mM over 20 CV. Thus, the gradient slope was equal (+ 350 mM NaCl) for all loading conditions.

Loading pH was shown to be a significant factor for aggregate concentration in the elution pool. Lower pH resulted in a lower aggregate concentration. A loading pH of 5.0, with or without 50 mM NaCl, resulted in less than 1% aggregates at a MAb monomer yield of 90%. Loading at higher pH (5.5 and 6.0) resulted in aggregate concentrations above 1% in the elution pool at 90% monomer yield. Fig 3 shows cumulated monomer yield versus cumulated aggregate concentration for the different binding and elution conditions tested.

The HCP content in the eluate from the MabSelect SuRe LX step ranged from 350 to 600 ppm. The HCP content in the elution pools at 90% MAb yield ranged from 144 to 169 ppm after the Capto S ImpAct step, which is equivalent to a two- to four-fold HCP reduction. No correlation between the HCP reduction and loading conditions was observed. Results are listed in Table 2.

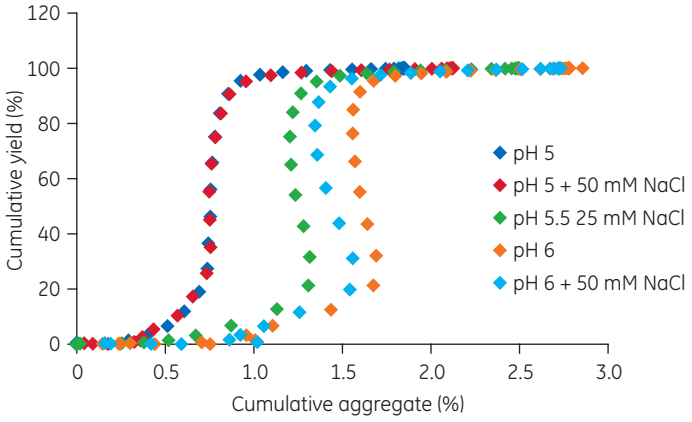

Fig 3. Cumulated monomer yield vs cumulated aggregate concentration for the different binding and elution conditions. Under the conditions tested, the most efficient aggregate removal was obtained at loading pH 5.0, irrespective of salt concentration.

Table 2. MAb aggregate and HCP content at 90% monomer yield under different binding and elution conditions

Binding buffer	Binding pH	Binding NaCl (mM)	Elution buffer (gradient in 20 CV)	Aggregate conc. at 90% yield (%)	HCP conc. at 90% yield (ppm)
50 mM sodium acetate	5.0	0	50 mM sodium acetate, 350 mM NaCl, pH 5.0	0.83	148
50 mM sodium acetate	6.0	0	65* mM sodium acetate, 350 mM NaCl, pH 6.0	1.32	162
50 mM sodium acetate	5.0	50	50 mM sodium acetate, 400 mM NaCl, pH 5.0	0.86	155
50 mM sodium acetate	6.0	50	66* mM sodium acetate, 400 mM NaCl, pH 6.0	1.4	169
50 mM sodium acetate (center point)	5.5	25	50 mM sodium acetate, 375 mM NaCl, pH 5.5	1.20, 1.26, 1.26	147

*The buffer capacity was too low at 50 mM sodium acetate when NaCl was added at pH 6.0.

Verification of binding conditions and selectivity at high sample load

Process optimization resulted in a DBC of more than 100 mg MAb/mL medium at pH 5.0 with 50 mM NaCl in the loading buffer. The use of these conditions and a load of 50 mg/mL resulted in a less than 1% aggregate content at a monomer yield of above 90% in the product pool. The DBC was verified by determination of Q_{B10} to 109 mg MAb/mL medium in a Tricorn 5/100 column, which correlates well with the results from the RoboColumn run (108 mg MAb/mL medium).

The selectivity of Capto S ImpAct between MAb monomers and aggregates was also verified at a higher sample load of 76 g MAb/L medium, corresponding to 70% of Q_{B10} (109 mg MAb/mL medium). At a monomer yield of 93%, the obtained aggregate concentration was 0.9%, the HCP concentration was 170 ppm, and the concentration of leached protein A from the capture step was less than 1 ppm (Fig 4).

Sample:	MAb in 50 mM sodium acetate, 50 mM NaCl, pH 5.0
Medium:	Capto S ImpAct (B/E mode)
Column:	Tricorn 5/100
Load:	76 mg MAb/mL medium (70% of Q_{B10})
Residence time:	5.4 min
Binding buffer:	50 mM sodium acetate, 50 mM NaCl, pH 5.0
Wash:	5 CV of binding buffer
Elution buffer:	50 mM sodium acetate, 50 to 400 mM NaCl in 20 CV
System:	ÄKTA system

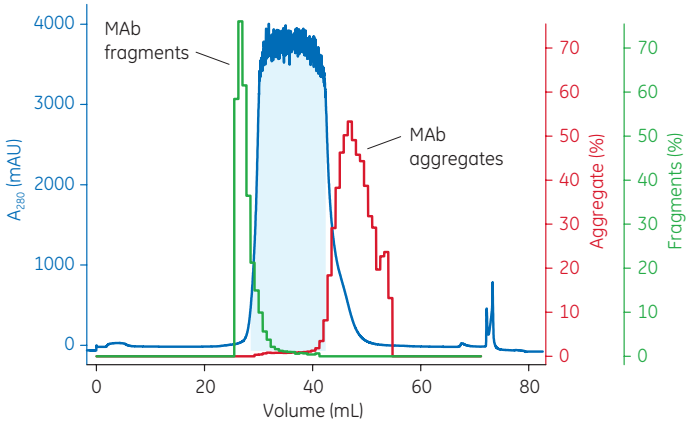

Fig 4. Selectivity of Capto S ImpAct between MAb monomers and aggregates at high sample load. The green histogram shows the amount of fragments in the fractions and the red histogram shows the amount of aggregates. The light blue area under the curve corresponds to pooled product fractions.

Conclusion

This application note describes optimization of DBC and aggregate clearance in a MAb polishing step with Capto S ImpAct CIEX medium. Using a DoE approach, a DBC of more than 100 mg MAb/mL medium was achieved. By optimizing this polishing step, the set acceptance criteria of less than 1% aggregate content at a monomer yield of above 90% in the final product pool could be fullfilled even at the high sample load of 76 mg MAb/mL medium. HCP concentration was 170 ppm, and the concentration of leached protein A ligand from the MAb capture step was less than 1 ppm.

The results show that Capto S ImpAct efficiently removes impurities such as MAb aggregates, HCP, and protein A, with high monomer recovery even at high sample loads.

Ordering information

Product	Size	Product code
Capto S ImpAct	25 mL	17371701
Capto S ImpAct	100 mL	17371702
Capto S ImpAct	1 L	17371703
HiTrap™ Capto S ImpAct	5 × 1 mL	17371751
HiTrap Capto S ImpAct	5 × 5 mL	17371755
HiScreen™ Capto S ImpAct	1 × 4.7 mL	17371747
PreDictor Capto S ImpAct, 2 µL	4 × 96-well filter plate	17371716
PreDictor Capto S ImpAct RoboColumn 600 µL	8 columns in row	17371772
Superdex 200 Increase 10/300 GL	1 × 24 mL	28990944

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Bio-Sciences Corp., 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan
For local office contact information, visit www.gelifesciences.com/contact

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