

# Polishing of monoclonal antibodies using Capto S ImpAct

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# Application note 29-1083-27 AA

Ion exchange chromatography

# Polishing of monoclonal antibodies using Capto<sup>™</sup> S ImpAct

Capto S ImpAct chromatography medium (resin) is a strong cation exchanger (CIEX). The medium is designed for the polishing steps of monoclonal antibodies (MAbs) and a wide range of other biomolecules. In this study, the binding capacity for a MAb and the efficiency in the clearance of impurities using Capto S ImpAct in bind-and-elute mode were evaluated. This study was performed according to the regulatory initiative quality by design (QbD). A wide experimental space was screened with high-throughput process development (HTPD) methodology, followed by an optimization within a narrower region using a design of experiments (DoE) approach. Even at high loading levels of MAb (up to 80 mg/mL medium), the results showed good resolution between monomer and aggregates at a high monomer recovery.

# Introduction

There is a great demand for using MAbs and MAb conjugates as biopharmaceuticals. As a result, more cost-effective, efficient, and flexible process purification schemes are one of the highest priorities for MAb manufacturers. The relative homogeneity of MAbs makes them well-suited for platform processes, which are sets of unit operations, conditions, and methods applied to molecules of a given class. A platform approach is desirable as it saves both time and money in process development. GE Healthcare Life Sciences' MAb toolbox employs protein A-based media, such as MabSelect SuRe™ or MabSelect SuRe LX chromatography media, for capture of the target. After the initial protein A capture step, there is a selection of media available that are well-suited for the polishing steps. One of these options, Capto S ImpAct, is a strong cation exchanger with a polymer-grafted ligand (Fig 1) that displays both high binding capacity and good resolution. The medium allows the use of high flow rates and bed heights, thereby increasing productivity and flexibility in process design.



**Fig 1.** Schematic picture of the polymer-grafted Capto S ImpAct medium. The polymer surface extender is formed by random grafting between the two building blocks pyrrolidone and sulfonate.

# Materials and methods Start material

The MAb (MAb A) used in this study was purified from Chinese hamster ovary (CHO) cell supernatant by protein A affinity chromatography, using MabSelect SuRe medium. Some characteristics of the MAb are shown in Table 1.

Table 1. Characteristics of the antibody used in the study

Antibody	MAb A	
pl*	8.9	
Aggregate content	7%	

\* pl = isoelectric point

# Determination of static binding capacity

Static binding capacity (SBC) was determined in PreDictor<sup>™</sup> Capto S ImpAct, 2 µL 96-well filter plates. All samples and buffers were prepared automatically using a Tecan<sup>™</sup> robot. Equilibration of the medium was performed by addition of 200 µL start buffer per well, followed by agitation at 1100 rpm for 1 min, after which the buffer was removed by centrifugation. The equilibration step was repeated three times. MAb solution (200 µL, 3 mg/mL) was added to each well followed by agitation for 90 min. Unbound material (flow-through fraction) was removed by centrifugation for 1 min, and MAb concentration was determined by measurement of absorbance at 290 nm. SBC was calculated using Assist software.

# Determination of dynamic binding capacity

Dynamic binding capacity (DBC) was determined at 10% breakthrough by frontal analysis using ÄKTA™ pure 25 chromatography system. The UV-absorbance at 305 nm was used for determination of breakthrough. Prior to frontal analysis, the MAb solution was injected by-passing the column (Tricorn™ 5/50) to obtain the absorbance value in the sample.

DBC was calculated according to:

$$DBC_{X\%} = (V_{X\%} - V_0) \times C_0 / V_c$$

where

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

 $V_0$  = system and column void volumes (mL)

 $C_o = MAb$  concentration in the sample (mg/mL)

 $V_c$  = volumetric bed volume (mL)

# Screening of binding conditions

Based on the SBC results, screening of binding conditions was performed in a Tricorn 5/50 column using ÄKTA pure 25. Several conditions, pH and ionic strength, were investigated for optimal aggregate removal.

# Optimization of gradient elution conditions

Conditions (load, start aggregate content, gradient length, residence time during elution, and pH) for gradient elution were investigated using a DoE approach. ÄKTA pure 25 was used with a Tricorn 5/50 column and scouting functionalities included in UNICORN™ 6.3 software.

# Verification of the design

To verify the DoE model, two verification runs were performed in a prepacked HiScreen™ Capto S ImpAct column using ÄKTA pure 25.

# Determination of aggregates and aggregate clearance

Fractions from the chromatography runs were collected and analyzed by gel filtration (size exclusion chromatography) on a Superdex<sup>™</sup> 200 Increase 10/300 GL column. The peaks were integrated and the dimer/aggregate concentrations (in percent) were estimated. Cumulated recovery of monomers was plotted against cumulated aggregates.

# Determination of host cell protein content

The host cell protein (HCP) content was analyzed using ELISA.

# Results and discussion Static and dynamic binding capacity

To find the optimal binding conditions for MAb A, SBC was determined in PreDictor Capto S ImpAct, 2  $\mu$ L 96-well filter plates, where pH and salt concentration were varied. Binding pH was varied between 4.5 and 6.0 and the salt concentration from 0 to 350 mM NaCl. Optimal binding, at the conditions tested, was found over a wide pH range (pH 4.75 to 6.0), at a slightly increased ionic strength (Fig 2). Based on these results, a narrower range of pH and NaCl concentration was used for further investigation of DBC. The DBC results confirmed that an increased ionic strength gives higher dynamic binding capacity (Table 2).



**Fig 2.** SBC measured in 50 mM sodium acetate with protein A-purified MAb. The red area in the contour plot corresponds to the highest SBC, and blue to the lowest. The five circles mark the conditions selected for further DBC studies.

Table 2. DBC of Capto S ImpAct at different ionic strengths and pH values

Buffer	рН	NaCl (mM)	DBC (mg/mL)
50 mM sodium acetate	5.0	-	71
50 mM sodium acetate	5.0	50	109
50 mM sodium acetate	5.5	50	122
100 mM sodium acetate*	6.0	-	124
50 mM sodium acetate	6.0	-	89

\* Buffer capacity was too weak to add 50 mM NaCl at pH 6. Ionic strength was instead increased by increasing acetate concentration.

Table 3. Monomer recovery and aggregate content at different binding conditions

# Screening of binding conditions

An aggregate reduction screening study at different binding and elution conditions was performed in packed columns. The 20 CV gradient elution was either 0 to 450 mM NaCl or 50 to 500 mM NaCl depending on NaCl concentration in the binding buffer. pH was varied from 5.0 to 6.0. The same pH value was used for binding and elution. Collected fractions were analyzed by gel filtration and cumulated recovery of monomer was plotted against cumulated concentration of aggregates (Fig 3). It was found that pH had a significant effect on the resolution between monomers and aggregates (Fig 3 and Table 3). The results show that a better separation between monomer and aggregates can be obtained at lower pH values. The best separation at the tested conditions was found at pH 5. Furthermore, a binding buffer with higher ionic strength resulted in a better separation at the same pH value.



**Fig 3.** Cumulated aggregates vs cumulated MAb monomer recovery after gradient elution at different pH values. The ionic strength (IS) refers to the binding buffer used. See Table 3 for details on binding and elution conditions.

Binding buffer	рН	NaCl (mM)	lonic strength (mM)	Sample load (mg/mL)	Aggregate content at 90% monomer recovery (%)*	Monomer recovery at 1% aggregate content (%)*
50 mM sodium acetate	5.0	50	84	50	0.7	94
50 mM sodium acetate	5.0	-	33	50	1.2	85
50 mM sodium acetate	5.5	50	94	50	1.2	78
100 mM sodium acetate	6.0	-	95	50	1.6	4
50 mM sodium acetate	6.0	-	48	61	1.9	5

\* The elution was a 20 CV gradient from 0 to 450 mM NaCl or 50 to 500 mM NaCl depending on the NaCl content in the binding buffer.

# Optimization of elution conditions using a DoE approach

A DoE approach was used to find the optimal elution strategy for MAb A. Factors in the DoE study were load, start aggregate content, gradient length, residence time during elution, and pH of the elution buffer. Binding conditions were identical for all experiments (50 mM sodium acetate, pH 5.0 + 50 mM NaCl, 4 min residence time). As can be seen in the coefficient plot (Fig 4), the most significant factors for removal of aggregates were gradient length and start aggregate content.

Even at high load (80 mg MAb/mL medium) and high aggregate content (7%) in the sample, the DoE model predicts that the aggregate level is reduced to less than 1% at a monomer recovery of 90% (Fig 5). For HCP removal, no good model was obtained. However, after polishing using Capto S ImpAct the values ranged between 200 and 600 ppm. The average start value was 1500 ppm.



**Fig 4.** Coefficient plot for the response aggregate content at 90% monomer recovery. Positive bars have an increasing effect on the measured response when the parameter setting goes from its low to high value and vice versa for the negative bars. N = number of experiments, DF = degrees of freedom, R<sup>2</sup> describes how well the experiments are fitted to the model, Q<sup>2</sup> describes the predictability of the model, RSD = residual standard deviation, Conf. level = Confidence level



**Fig 5.** 4D contour plot showing the prediction of aggregate content at 90% monomer recovery in the elution pool after gradient elution in 50 mM sodium acetate, pH 5.0 + 500 mM NaCl. The lines represent 50% probability to achieve a specific end aggregate content (numbers in white boxes). CV = column volume.

# Verification of the design

To verify the best conditions for aggregate removal at high start aggregate content (7%) predicted by the model obtained in the DoE study, two experiments were performed. Sample load was 50 and 80 mg/mL medium. The other parameters were kept constant at 8 min residence time, 20 CV gradient length, and pH 5.0 during elution. Column verification of the method was performed in a HiScreen Capto S ImpAct column. The obtained results were in good agreement with the predicted results for monomer recovery and aggregate clearance (Table 4).

Figure 6 shows the chromatogram from the verification of the predicted results from the DoE model at a high sample load (80 mg/mL medium). The chromatogram illustrates that aggregates (green) elute at the tail of the elution peak. The experimental aggregate content (0.9%) at 90% monomer recovery at this load agreed well with the expected value (< 1%).

Table 4. Predicted and experimental results for monomer recovery andaggregate content at 7% start aggregate content

Sample load	Result	Aggregate content at 90% monomer recovery (%)
80 mg/mL	Predicted result	< 1.0
	Experimental result	0.9
50 mg/mL	Predicted result	≤ 0.8
	Experimental result	0.8

Column: HiScreen Capto S ImpAct, CV 4.7 mL Sample: ~10 ma/mL MAb A purified on MabSelect SuRe. buffer exchanged into start buffer Sample load: 80 mg/mL medium Start buffer: 50 mM sodium acetate, pH 5.0 + 50 mM NaCl Elution buffer: 50 mM sodium acetate, pH 5.0 + 500 mM NaCl Flow rate loadina: 1.12 mL/min. residence time 4 min Flow rate elution: 0.59 mL/min, residence time 8 min Gradient: Linear, 20 CV System: ÄKTA pure 25 200





A Monte Carlo simulation was performed using Oracle<sup>™</sup> Crystal ball software. The simulation was used to study how variation in the critical process parameters translates into variation in aggregate content at 90% monomer recovery. Critical process parameters were load, start aggregate content, gradient, residence time, and pH (Table 5). The simulation predicted that 100% of the experiments would result in an aggregate content below 1% when start aggregate was 7% (Fig 7).

Table 5. Critical process parameters used in the Monte Carlo simulation

Factor	Min.	Target	Max.	Distribution
Load	77	80	83	Triangular ± 4%
Start aggregate content	6.65	7	7.35	Triangular ± 5%
Gradient	19.6	20	20.4	Triangular ± 2%
Residence time	7.84	8	8.16	Triangular ± 2%
рН	4.9	5	5.1	Triangular ± 2%
RSD*		0		Normal standard deviation 0.033

\* RSD = residual standard deviation



**Fig 7.** Monte Carlo simulation of aggregate reduction at a load of 80 mg/mL medium and 90% monomer recovery. Number of trials were 10 000. Aggregate levels at 90% monomer recovery were predicted to be maximum 1.0% and minimum 0.71%, with a median value of 0.85%.

# Conclusions

In this work, we present results from a study using Capto S ImpAct, a strong cation exchanger designed for polishing of MAbs. The study was designed according to the QbD regulatory initiative. The results show that Capto S Impact exhibits a high dynamic binding capacity for a wide range of conditions. The medium also demonstrated good resolution between monomer and aggregates at a high monomer recovery.

Furthermore, the study of elution conditions, using a DoE approach, showed that efficient aggregate removal can be achieved in a robust manner even at high loading levels of MAbs. These data show the great potential of Capto S ImpAct for polishing of MAbs in today's bioprocessing industry.

# Ordering information

Products	Quantity	Code number
Capto S ImpAct	25 mL	17-3717-01
Capto S ImpAct	100 mL	17-3717-02
Capto S ImpAct	1 L	17-3717-03
Capto S ImpAct	5 L	17-3717-04
Capto S ImpAct	10 L	17-3717-05
Capto S ImpAct	60 L	17-3717-60
HiTrap™ Capto S ImpAct	5 × 1 mL	17-3717-51
HiTrap Capto S ImpAct	5 × 5 mL	17-3717-55
HiScreen Capto S ImpAct	1 × 4.7 mL	17-3717-47
PreDictor Capto S ImpAct, 2 µL	4 × 96-well filter plates	17-3717-16
PreDictor Capto S ImpAct, 20 µL	4 × 96-well filter plates	17-3717-17
PreDictor RoboColumn™ Capto S ImpAct, 200 µL	8 columns in row	17-3717-71
PreDictor RoboColumn Capto S ImpAct, 600 µL	8 columns in row	17-3717-72
PreDictor Capto CIEX polishing screening, 2 μL/6 μL	4 × 96-well filter plates	29-0955-68
PreDictor Capto CIEX polishing screening, 20 µL	4 × 96-well filter plates	29-0955-67

### **Related literature**

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Capto S ImpAct	29-0670-18
PreDictor 96-well filter plates and Assist software	28-9258-39
PreDictor RoboColumn	28-9886-34
HiScreen prepacked columns	28-9305-81

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