Lifetime performance study of MabSelect PrismA during repeated cleaning-in-place cycles

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Lifetime performance study of MabSelect™ PrismA during repeated cleaning-in-place cycles

This work demonstrates a lifetime performance study on MabSelect PrismA protein A chromatography resin. The study was conducted for 100 purification cycles using mAb-containing samples, including cleaning in place (CIP) with 0.5 M NaOH at a contact time of 15 min in each cycle. Results show that dynamic binding capacity (DBC), antibody recovery, and clearance of impurities, such as antibody aggregates, host cell protein (HCP), host cell DNA (hcDNA), and leached protein A, were maintained at a similar level throughout the study. Furthermore, an alkaline-stability study was performed for 300 cycles, using 0.5 M NaOH as cleaning agent, and for 150 cycles using 1 M NaOH as cleaning agent. Generated data indicates that the protein A ligand of MabSelect PrismA is stable and that it withstands repeated and effective CIP procedures with high concentrations of NaOH as cleaning agent over a long resin working life.

Introduction

Since the first commercially approved mAb in 1980s, this class of therapeutic molecules has become the largest and fastest growing segment of biopharmaceuticals. With advances in upstream procedures, the mass of mAb that is being sent for further downstream processing is increasing, adding pressure to the initial capture step.

Protein A capture resins provide an efficient antibody purification platform, and have followed a highly synergistic evolutionary path with the biotherapeutic mAbs over the past 30 years. Annual productivity gains of above 4.5% and increases in binding capacity of more than 5.5% have been observed for the protein A capture step (1).

With increasing mAb titers, however, the impurity level of the cell culture feed also increases. If not removed, these impurities can build up on the resin and start to leach into the process material in subsequent cycles. An efficient cleaning of the column in each cycle will prevent a decrease in resin capacity due to column fouling and minimize the risk of carryover between cycles and batches. Harsh cleaning conditions will result in efficient cleaning, but can at the same time destroy sensitive resins.

MabSelect PrismA affinity chromatography resin is based on an optimized high-flow agarose base matrix and a genetically engineered protein A-derived ligand. The resin features enhanced alkaline stability and binding capacity over its predecessor MabSelect products. With the enhanced properties of both the base matrix and ligand, MabSelect PrismA offers a significantly increased capacity to help resolve bottlenecks in manufacturing-scale operations. The significantly improved alkaline stability of the resin ensures more efficient cleaning and sanitization to prevent carryover and enhance bioburden control in the downstream antibody capture step.

This work aims to demonstrate the long-term chromatographic performance of MabSelect PrismA during repeated purification cycles using 0.5 M NaOH for CIP. Parameters monitored include remaining DBC as well as antibody recovery and purity in terms of leached protein A and clearance of aggregates, HCP, and hcDNA. The enhanced alkaline stability of MabSelect PrismA was also demonstrated in a study with buffers only (no sample) and with cleaning using 0.5 or 1.0 M NaOH included in each cycle. The remaining capacity was monitored during 300 cycles for 0.5 M NaOH and during 150 cycles for 1.0 M NaOH.

Materials and methods

For these studies, MabSelect PrismA was packed in Tricorn™ 5/50 columns (bed height 5 cm, column volume 0.88–0.94 mL) for the lifetime study ,and in Tricorn 5/100 columns (bed height 10 cm, column volume 1.96 mL) for the alkaline-stability study. Sample load was performed at 4 min residence time for study with antibody-containing samples. The predecessor products MabSelect SuRe™, MabSelect SuRe LX, and MabSelect SuRe pcc were included in the study as reference resins.

Lifetime study with mAb-containing sample and 0.5 M NaOH for CIP

Packed columns were subjected to repeated cycles with load of mAb-containing cell culture supernatant. Adsorbed mAb was eluted with three column volumes (CV) of 50 mM acetate, pH 3.5 followed by a strip with 100 mM acetic acid, pH 2.9. CIP was performed with 0.5 M NaOH (15 min contact time) after each elution cycle. The purification protocol is outlined in Table 1.

DBC at 10% breakthrough (Q_{B10}) was determined by frontal analysis using ÄKTAexplorer 10 system. Breakthrough capacity was also determined by periodic counter-current chromatography (PCC) during cycling using the ÄKTA™ pcc chromatography system in a four-column (4C) setup.

Table 1. mAb purification protocol

Sample	Human IgG (~ 5 mg/mL)	mAb1/mAb2 (4.3/2.2 mg/mL)
Equilibration	20 mM phosphate, 150 mM NaCl, pH 7.4	4 min
Load	mAb sample was applied to the column until 10% breakthrough (mAb concentration 4.28 mg/mL)	4 min
Wash 1	5 CV of 20 mM phosphate, 500 mM NaCl, pH 7	4 min
Wash 2	1 CV of 50 mM acetate, pH 6	4 min
Elution	3 CV of 50 mM acetate pH 3.5	4 min
Strip	100 mM acetic acid, pH 2.9	4 min
CIP	3 CV of 0.5 M NaOH	15 min (contact time)
Re-equilibration	3 CV of 20 mM phosphate, 150 mM NaCl, pH 7.4	4 min

CV = column volumes

Alkaline-stability study with repeated cycling using 0.5 or 1.0 M NaOH for CIP

The alkaline stability study was performed on the ÄKTA pure chromatography system. Packed columns were subjected to repeated cycles with 5 CV of PBS buffer; 5 CV of 0.1 M acetic acid, pH 3.0; and 3 CV of 1.0 or 0.5 M NaOH (contact time 15 min/cycle). Frontal analysis at 6 min residence time was regularly performed with human IgG. The alkaline stability using 0.5 M NaOH was determined for 300 cycles. For 1.0 M NaOH, alkaline stability was determined for 150 cycles.

Determination of DBC by frontal analysis

Determination of DBC was performed by frontal analysis, using the ÄKTAexplorer 10 system for the lifetime study and the ÄKTA pure for the alkaline-stability study. Eluted protein was monitored by UV absorbance at 280 nm. Prior to frontal analysis, human IgG (gammanorm™, Octapharma) solution was injected to by-pass the column to obtain a maximum absorbance value. Thereafter, human IgG in PBS was applied to the column until approximately 10% breakthrough was attained. Unbound material was washed out with PBS buffer and CIP was performed with 0.5 M NaOH at a contact time of 15 min. Q_{B10} was calculated according to:

$$Q_{B10} = (V_{10\%} - V_0) \times C_0/V_c$$

where

$V_{10\%}$ = applied volume of sample at 10% breakthrough

C_0 = mAb concentration (mg/mL)

V_c = geometric total volume (mL)

V_0 = void volume (mL)

Determination of breakthrough capacity using ÄKTA pcc

Determination of breakthrough capacity was performed using the ÄKTA pcc system in a 4C PCC setup. With the built-in dynamic control functionality of the system, the mAb breakthrough was monitored in real time and Q_{B10} was determined during cycling.

Analyses

Concentration of mAb in the cell culture supernatant was measured by surface plasmon resonance (SPR) using the Biacore™ T100 system.

Aggregate clearance was determined by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column. Peaks were integrated and percentage of aggregates were determined.

HCP content in the elution fractions and product pools was 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.). The MabSelect SuRe ligand was used as reference for MabSelect SuRe, MabSelect SuRe LX, and MabSelect SuRe pcc. The MabSelect PrismA ligand was used as a reference for MabSelect PrismA.

Levels of hcDNA were determined by an in-house qPCR method, using primers and probes as described previously (2). Samples were automatically prepared using a MagMax™ Express 96-deepwell magnetic particle processor and PrepSEQ™ Residual DNA Sample Prep kit. Real-time PCR was performed using the StepOnePlus™ system (Thermo Fisher Scientific), and by using the StepOne™ software for evaluation.

Results

Lifetime study with mAb-containing sample

Lifetime study with mAb-containing cell culture supernatant was performed for 100 cycles, including CIP using 0.5 M NaOH at a contact time of 15 min in each cycle. DBC results obtained by conventional chromatography, using the ÄKTAexplorer 10 system are summarized in Table 2. As determined by PCC using ÄKTA pcc, remaining Q_{B10} after 100 cycles was 91% for MabSelect PrismA, 83% for MabSelect SuRe LX, 62% for MabSelect SuRe pcc, and 61% for the MabSelect SuRe resin (Fig 1). Q_{B10} determined by conventional chromatography agrees well with Q_{B10} determined by PCC. For

MabSelect SuRe, MabSelect SuRe LX, and MabSelect SuRe pcc, the decrease in Q_{B10} resulted in a decreased amount of mAb in the elution pool, while elution pool concentration remained stable for MabSelect PrismA. Aggregate concentrations in elution pools were relatively stable, that is, no significant trend could be observed. Concentrations of HCP and leached protein A were also relatively stable throughout the study. Table 3 summarizes the results for the MabSelect PrismA, MabSelect SuRe, MabSelect SuRe LX resins, and MabSelect SuRe pcc.

Table 2. Q_{B10} determined by conventional chromatography for 100 cycles including CIP with 0.5 M NaOH, 15 min/cycle

	Cycle no.	Q_{B10}	Remaing capacity (%)
MabSelect SuRe	1	46.2	
	51	40	87
	100	26.9	58
MabSelect SuRe LX	1	50.3	
	51	46.1	92
	100	40.8	81
MabSelect SuRe pcc	1	74.8	
	51	62.3	83
	100	44.9	60
MabSelect PrismA	1	68.2	
	51	65	95
	100	62.3	91

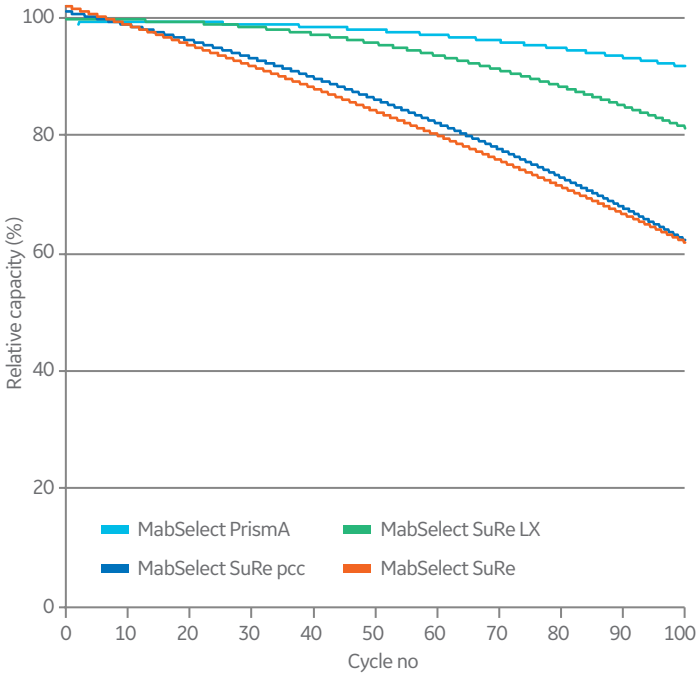

Fig 1. Relative remaining Q_{B10} determined using the ÄKTA pcc system for 100 cycles including CIP with 0.5 M NaOH, 15 min/cycle.

Table 3. Results from performance lifetime study for 100 cycles including CIP with 0.5 M NaOH, 15 min/cycle using ÄKTA pcc

Resin	Cyle no.	Pool volume (CV)	Pool concentration (mg/mL)	Aggregate (%)	HCP* (ppm)	hcDNA† (ppm)	Protein A (ppm)
MabSelect SuRe	1	1.8					
	20	1.8	19.6	2	372	1	3
	58	1.6	14.4	1.4	507	2	1
	74	1.4	13.5	1.3	452	2	1
	90	1.3	12.1	1.5	407	2	11
MabSelect SuRe LX	1	1.9					
	20	1.9	21.1	2	308	0	12
	58	1.8	18.4	1.5	460	1	1
	74	1.9	17.8	1.5	646	1	1
	90	1.8	16.9	1.7	369	2	1
MabSelect SuRe pcc	1	2					
	20	1.8	28.8	2.3	327	1	3
	58	1.9	21.2	1.6	340	1	2
	74	1.8	19	1.5	530	1	2
	90	1.8	19.4	1.8	342	1	2
MabSelect PrismA	1	1.9					
	20	1.9	27.9	2.3	304	0	5
	58	1.9	23.9	1.7	471	1	4
	74	2	24.4	1.8	337	1	5
	90	2	25.3	2.1	460	1	3

* HCP at start 1.4 × 105 ppm
† hcDNA at start 8037 ppm

Alkaline-stability study

Using 0.5 M NaOH for CIP at a contact time of 15 min/cycle, the remaining Q_{B10} of MabSelect PrismA after 300 cycles was more than 95% (Fig 2). MabSelect SuRe LX exhibits a remaining Q_{B10} of approximately 90% after 100 cycles, approximately 80% after 200 cycles, and about 50% after 300 cycles. For MabSelect SuRe resin, the remaining Q_{B10} was 30%, 40%, and 60% lower than the original Q_{B10} after 100, 200, and 300 cycles, respectively.

Using 1.0 M NaOH for CIP at a contact time of 15 min/cycle, the remaining Q_{B10} of MabSelect PrismA after 100 cycles was almost 100% (Fig 3). After 100 cycles, Q_{B10} started to decrease slowly but was still around 90% of initial capacity after 150 cycles. For the reference MabSelect SuRe LX resin, an 8% loss of the origin capacity was observed already after 50 cycles. After 150 cycles, only 50% of original capacity remained. For the MabSelect SuRe and MabSelect SuRe LX resins, remaining Q_{B10} can be increased by using 0.1 M NaOH for CIP as per original recommendation.

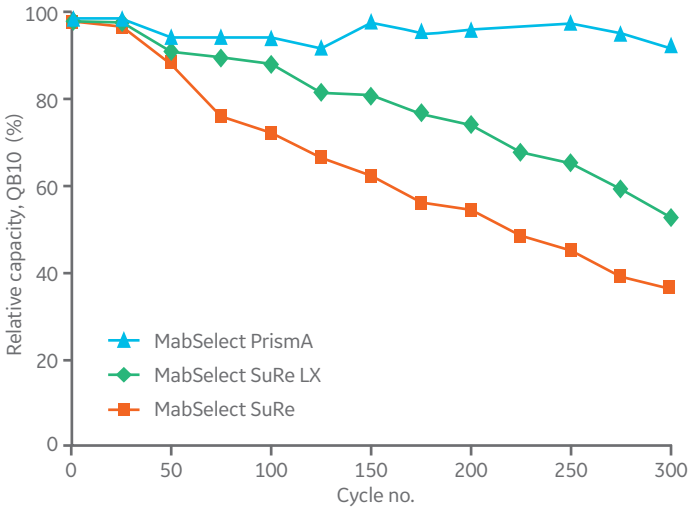

Fig 2. Relative remaining capacity (Q_{B10}) for 300 cycles, including CIP with 0.5 M NaOH for 15 min/cycle.

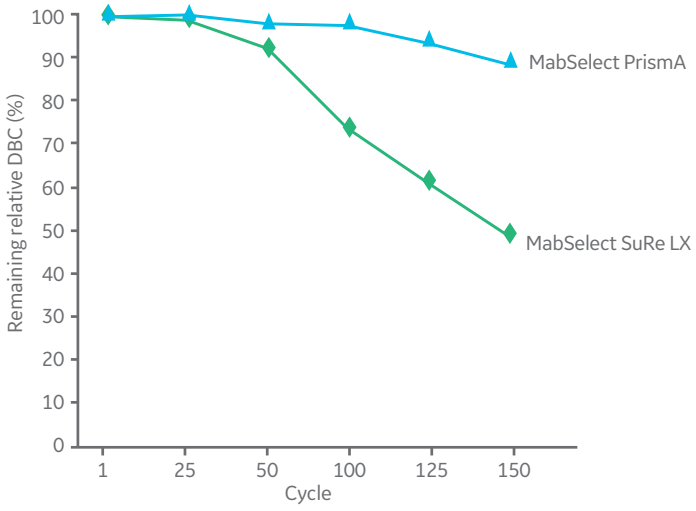

Fig 3. Relative remaining capacity (Q_{B10}) for 150 cycles, including CIP with 1.0 M NaOH for 15 min/cycle.

Conclusion

The lifetime study with antibody-containing sample demonstrates that the dynamic binding capacity and performance of MabSelect PrismA in terms of mAb elution pool volume and purity are stable over the 100 purification cycles that were conducted with CIP using 0.5 M NaOH at a contact time of 15 min included in each cycle. The alkaline-stability study shows that MabSelect PrismA can withstand a NaOH concentration of 0.5 M for 300 cycles, and a NaOH concentration as high as 1.0 M for 150 cycles. The results show that the protein A ligand of MabSelect PrismA is stable and that it withstands repeated and effective CIP procedures with high concentrations of NaOH as cleaning agent over a long resin working life.

References

1. Bolton *et al*. The role of more than 40 years of improvement in Protein A chromatography in the growth of the therapeutic antibody industry. *Biotechnol Prog* **32**, 1193–1202 (2016).
2. Hu *et al*. Optimization and validation of DNA extraction and real-time PCR assay for the quantitative measurement of residual host cell DNA in biopharmaceutical products. *J Pharm Biomed Anal* **88**, 92–95 (2014).

Ordering information

Product	Description	Product code
MabSelect PrismA	200 mL	17549802
MabSelect SuRe	200 mL	17543802
MabSelect SuRe LX	200 mL	17547402
MabSelect SuRe pcc	200 mL	7549102
Tricorn 5/50 column	5 mm i.d., 50 mm tube height	28406409
Tricorn 5/100 column	5 mm i.d., 100 mm tube height	28406410

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