Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place

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Lifetime performance study of MabSelect SuRe™ LX during repeated cleaning-in-place

The performance of MabSelect SuRe LX, an alkali-stabilized, protein A-derived affinity medium (resin) for capturing monoclonal antibodies, was evaluated over 100 purification cycles that included cleaning-in-place with 0.1 M sodium hydroxide. Results showed that dynamic binding capacity was maintained and that yield was constant throughout the study. Ligand density also remained unaltered. In addition, levels of leached protein A and host cell protein were almost constant. Extended studies with higher sodium hydroxide concentrations confirmed good stability. These data suggest that MabSelect SuRe LX can be used repeatedly during production-scale purification and deliver products that meet predetermined quality and safety standards over a long working life.

Introduction

Increasing demand for monoclonal antibodies (MAbs) as biopharmaceuticals has promoted the development of cell cultures with very high expression levels. Over the last 20 years, the antibody titers of mammalian cell culture have risen dramatically. Today, we commonly see titers of 5 to 10 g/l, and expression levels as high as 15 g/l or greater have been reported. The demand for purification processes that can handle this increase has risen accordingly.

The large-scale purification of MAbs usually consists of two or three chromatographic steps, and protein A affinity media are often the first choice for initial capture because they deliver high purity (> 99%) and yield in a single step. Protein A affinity chromatography also requires minimum conditioning prior to loading and allows rapid transfer to a stable process intermediate.

The MabSelect™ family of affinity chromatography media, which is based on the protein A ligand, has found wide acceptance among large-scale commercial manufacturers of biopharmaceutical MAbs. This media family has expanded to meet the developing needs of large-scale manufacture. A recent addition is MabSelect SuRe LX, which is based on the same rigid high-flow agarose matrix and alkali-stabilized ligand as MabSelect SuRe. This ligand withstands rigorous CIP and sanitization procedures with 0.1 or 0.5 M sodium hydroxide (NaOH). Compared with MabSelect SuRe, however, MabSelect SuRe LX offers 20% to 50% higher dynamic binding capacity at slightly longer residence times (e.g., 6 to 10 min).

This Application note describes a study to quantitate the long-term chromatographic performance of MabSelect SuRe LX during repeated purification cycles that use 0.1 or 0.5 M NaOH for cleaning-in-place (CIP). Parameters measured included dynamic binding capacity, yield of antibody, and levels of leached protein A and host cell proteins (HCP).

Materials and methods

General lifetime study with MAb-containing feedstock and 0.1 M NaOH for CIP

MabSelect SuRe LX was packed in a Tricorn™ 5/100 column (bed height 10 cm, column volume 1.96 ml). The column was then subjected to repeated cycles of a clarified, harvested cell culture fluid (HCCF) with a MAb concentration of 0.8 g/l. Human polyclonal IgG (hIgG) was used to determine the dynamic binding capacity at 10% breakthrough (DBC, 10% breakthrough). Residence times were 4 or 6 min. Table 1 outlines this scheme.

Adsorbed antibodies were eluted by five column volumes of 0.1 M citrate, pH 3.0. Cleaning-in-place was performed with 0.1 M NaOH (contact time 15 min) after each elution cycle.

Table 1. Scheme for the initial MabSelect SuRe LX performance lifetime study with 0.1 M NaOH

Cycle no.	Load*†	Residence time (min)	Cycle no.	Load*†	Residence time (min)
1	hIgG	4 and 6‡	52	hIgG	4
2	hIgG	4	53	Blank	"
3	HCCF	"	54	hIgG	6
5	Blank	"	60	hIgG	4
10	hIgG	"	61	HCCF	"
11	HCCF	"	62	Blank	"
12	Blank	"	72	hIgG	"
20	hIgG	"	83	hIgG	"
21	HCCF	"	92	hIgG	"
22	Blank	"	97	HCCF	"
32	hIgG	"	98	Blank	"
41	hIgG	"	99	hIgG	"
42	HCCF	"	100	hIgG	6

*All remaining cycles: HCCF (harvested cell culture fluid) applied to 80% of DBC, 10% at a residence time of 4 min, without fraction collection or analysis, hIgG = human IgG.

† New batch of HCCF after cycle 87.

‡ Cycle 1: 6 min residence time measured on a separate column.

Determination of dynamic binding capacity by frontal analysis

UV absorbance at 280 nm was used to determine breakthrough. Prior to frontal analysis, the IgG solution was injected to by-pass the column and thereby obtain a maximum absorbance value. Human IgG (Gammanorm™, Octapharma) in PBS was applied to the column until approximately 10% or 80% breakthrough was attained. Unbound material was washed out with PBS buffer and CIP was performed with 0.1 M NaOH and a contact time of 15 min. DBC at 10% and/or 80% breakthrough was then calculated according to:

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

where $V_{X\%}$ = applied volume of sample at X% breakthrough, C_0 = sample concentration (mg/ml), V_c = geometric total volume (ml), and V_0 = void volume (ml).

Determination of MAb concentration and yield

Concentration determinations were made by analytical chromatography on a 1 ml HiTrap™ column packed with MabSelect SuRe LX. Yield was calculated according to:

$$\text{Yield (\%)} = 100 \times (V_{pool} \times C_{pool})/(V_{in} \times C_0)$$

where V_{pool} = volume of pooled fractions (ml), C_{pool} = MAb concentration in pool (mg/ml), V_{in} = volume of sample loaded onto the column (ml), and C_0 = sample concentration (mg/ml).

HCP clearance and protein A leakage

Sample was applied to a final load of 80% of the DBC, 10% breakthrough. After washing out unbound material, elution was performed with 0.1 M citrate pH 3.0 and 1 ml fractions were collected in tubes containing 0.1 ml 1 M Tris, pH 9. This was followed by CIP with 0.1 M NaOH and finally re-equilibration with PBS.

HCP levels were measured using commercial anti-CHO HCP antibodies (Cygnus Technologies Inc. USA). Essentially, an ELISA methodology was adapted to Gyrolab™ Workstation LIF (Gyros AB, Uppsala, Sweden) using Gyrolab Bioaffy™ 20HC microlaboratory discs. Leakage of the MabSelect SuRe LX protein A ligand was measured using a commercial ELISA kit (Repligen Corp., Waltham, MA, USA).

Alkaline stability study with repeated cycling of 0.1 or 0.5 M NaOH

MabSelect SuRe LX was packed in Tricorn 5/100 columns that were subjected to repeated cycles with 5 column volumes (CV) PBS buffer, 5 CV of 0.1 M acetic acid pH 3.0, 2.2 CV of 0.1 or 0.5 M NaOH (contact time 15 min/cycle; room temperature, i.e. 21°C to 23°C) and 5 CV of PBS buffer. Frontal analysis at 6 min residence time was regularly performed with human IgG.

Results

General lifetime study with MAb-containing feedstock

One hundred cycles with 0.1 M NaOH CIP were performed. Table 2 and Figure 1 summarize the results. The dynamic capacity of MabSelect SuRe LX decreased slightly after 10 cycles but then remained constant. Yield was high (> 97%) and stable throughout the study. There were no significant changes in leached protein A or HCP levels over the 100 cycles.

Table 2. Results from MabSelect SuRe LX lifetime study with 0.1 M NaOH as CIP agent

Cycle no.	DBC, 10% Res. time 6 min	HCP (ng/ml)	HCP (ppm)	Protein A (ppm)	Remark
1	60.6				
3		2700	222	13	
11		2750	197	11	
12		31			Blank
21		2650	187	16	
22		16			Blank
42		2700	192	11	
43		< 4.6			Blank
54	58.8				
61		2700	196	15	
62		< 4.6			Blank
97		1250	86	13	New feed
100	58.3				

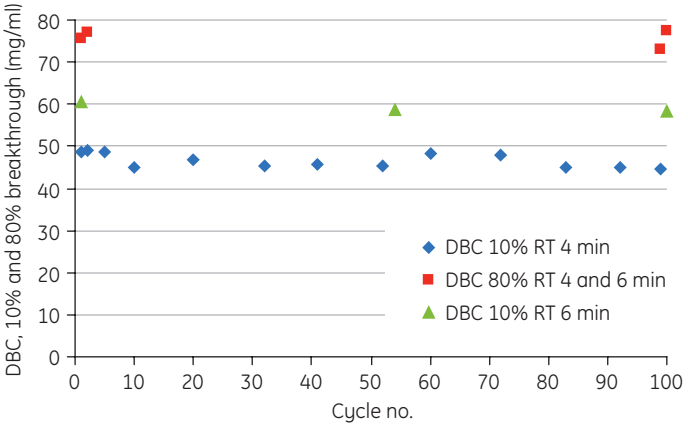

Fig 1. Dynamic binding capacity measurements for MabSelect SuRe LX over 100 purification cycles with 0.1 M NaOH as CIP agent.

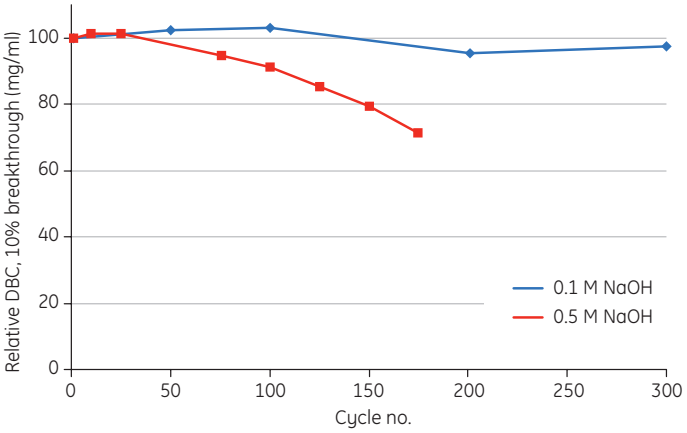

Fig 3. DBC of MabSelect SuRe LX is stable at 0.1 M NaOH for up to 300 purification cycles. At 0.5 M NaOH, DBC begins to decline earlier.

Overlays of chromatograms from five runs with HCCF (spanning purification cycle 7 to cycle 80) showed almost identical results (Fig 2). Carry-over levels were consistently low and no trend could be seen.

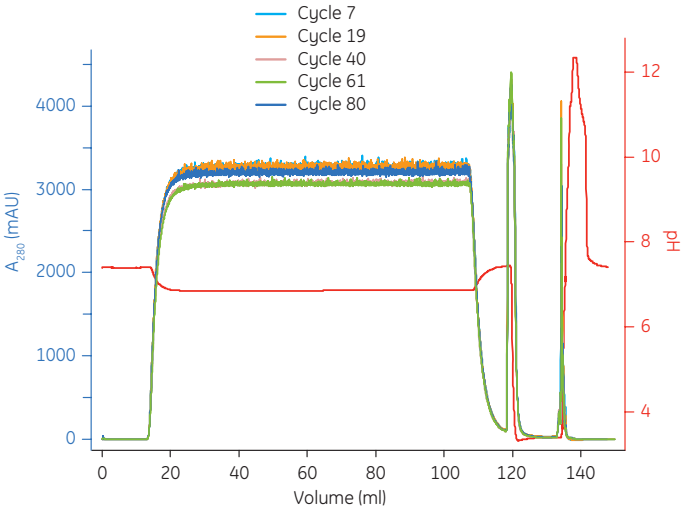

Fig 2. Overlay of chromatograms from runs with HCCF (0.8 g MAb/l from cycle 3). A new batch of HCCF with a MAb concentration of 1.1 g/l was applied from cycle 87.

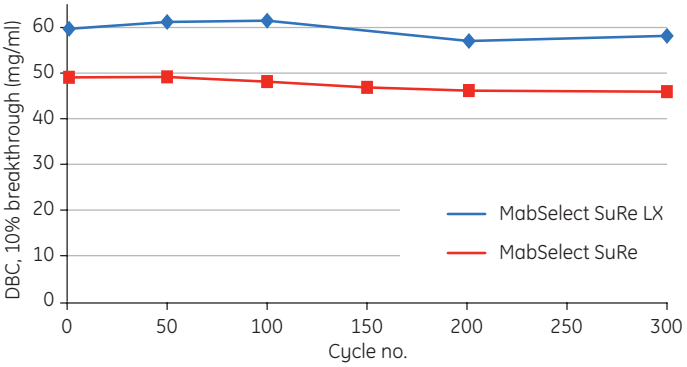

Fig 4. DBC results obtained with MabSelect SuRe LX and MabSelect SuRe for 300 cycles of CIP with 0.1 M NaOH.

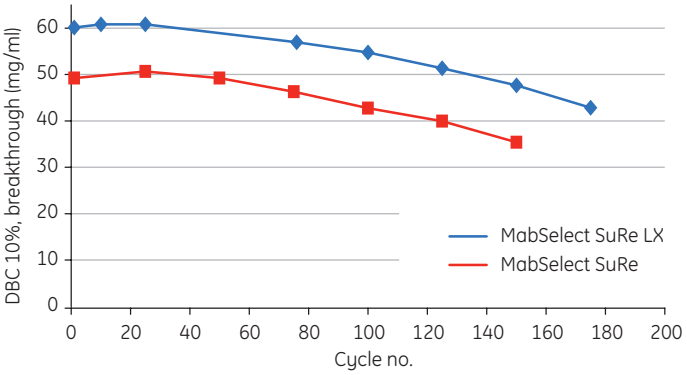

Fig 5. DBC results obtained with MabSelect SuRe LX and MabSelect SuRe for 175 and 150 cycles, respectively of CIP with 0.5 M NaOH.

Alkaline stability study

Figure 3 summarizes the results of the stability study on MabSelect SuRe LX. For 0.1 M NaOH, DBC is relatively stable (> 95% of DBC) for as many as 300 cycles. For 0.5 M NaOH, DBC gradually decreases after cycle 25. Nevertheless, it still remains above 90% for 100 cycles at this higher concentration.

To compare MabSelect SuRe LX with the other alkali-stable, protein A-based medium in the MabSelect family (MabSelect SuRe), lifetime studies were performed using CIP with 0.1 and 0.5 M NaOH. Results are shown in Figures 4 and 5. The greater DBC of MabSelect SuRe LX is evident from both. Following CIP with 0.5 M NaOH, 80% of DBC remained after 150 cycles for MabSelect SuRe LX and after 125 cycles for MabSelect SuRe, indicating 20% longer lifetime for MabSelect SuRe LX.

In addition, DBC was measured at two different residence times (2.4 and 6 min) with 0.5 M NaOH. Figure 6 shows the results. DBC decreased significantly faster at the shorter residence time. For MabSelect SuRe, 80% of DBC remained after approximately 80 cycles with a 2.4 min residence time, compared with 125 cycles at 6 min residence time. At 6 min residence time, MabSelect SuRe LX retained 80% DBC after 150 cycles, confirming the exceptional binding capacity seen in Figures 4 and 5.

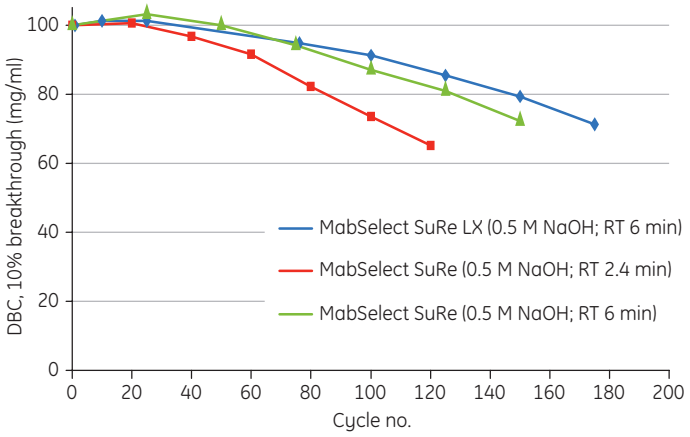

Fig 6. DBC results obtained with 0.5 M NaOH at two residence times (RT 6 min or 2.4 min) for MabSelect SuRe compared with MabSelect SuRe LX at RT 6 min.

Conclusions

The lifetime study with MAb-containing feedstock demonstrates that the product quality, dynamic binding capacity, and yield of MabSelect SuRe LX were stable over 100 purification cycles, and that the levels of leached protein A and HCP were consistently low.

These data demonstrate that the protein A ligand of MabSelect SuRe LX is very stable and that it withstands repeated and effective CIP procedures with NaOH as cleaning agent. Repeated cycling with buffers shows that longer residence time not only results in increased dynamic binding capacity, but also in prolonged lifetime.

Ordering information

Product	Code no.
MabSelect SuRe LX, 25 ml	17-5474-01
MabSelect SuRe LX, 200 ml	17-5474-02
MabSelect SuRe LX, 1 l	17-5474-03
MabSelect SuRe LX, 5 l	17-5474-04
MabSelect SuRe LX, 10 l	17-5474-05
Tricorn 5/100 column	28-4064-10

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