GE Healthcare
Life Sciences

Application note 28-9875-25 AA

Affinity chromatography

Dynamic binding capacity study on MabSelect SuRe™ LX for capturing high-titer monoclonal antibodies

The dynamic binding capacity (DBC) of MabSelect SuRe LX, an alkali-stabilized, protein A-derived affinity medium (resin) for capturing monoclonal antibodies (MAbs), was measured at different residence times and feed concentrations. DBC increased with increased residence time, while MAb concentration did not have any significant influence. MabSelect SuRe LX and MabSelect SuRe DBC were also compared for seven different MAb-containing feedstocks. At longer residence times, MabSelect SuRe LX displayed 20% to 46% higher DBC. The productivity of both was also analyzed. Results suggest that over its lifetime, MabSelect SuRe LX could purify up to 50% more antibody than MabSelect SuRe.

Introduction

Due to greater economic pressure on MAb production, the antibody titers in mammalian cell culture have increased dramatically over the last 20 years. Today, it is possible to see titers of 1 to 8 g/l, and feedstock expression levels as high as 15 g/l and greater have been reported. As a consequence, the demand for efficient chromatographic purification during manufacture has also increased. Unless this demand is met, purification operations risk limiting the throughput they can deliver, which will result in costly downstream processing bottlenecks.

MAb purification strategy

The large-scale purification of MAbs usually consists of two or three chromatographic steps. Protein A is the affinity chromatography ligand of choice for first-step capture as its high selectivity gives excellent purity (typically > 99%) plus high yields. Furthermore, protein A-based media are easy to use at both small and large scale.

Protein A media therefore form the basis of a platform approach to MAb purification. Subsequent downstream processing can be performed using a variety of chromatography techniques and combinations, especially ion exchange, multimodal, and hydrophobic interaction chromatography.

Key role for MabSelect™ affinity media

Based on the protein A ligand, the MabSelect family of affinity chromatography media (MabSelect, MabSelect Xtra™ and MabSelect SuRe) has found wide acceptance among large-scale commercial manufacturers of biopharmaceutical MAbs. A recent addition is MabSelect SuRe LX, which comprises the same rigid high-flow agarose matrix and alkali-stabilized, protein A-derived ligand as MabSelect SuRe. MabSelect SuRe LX thus enjoys the same performance attributes such as alkali and protease stability as well as generic elution. Compared with MabSelect SuRe, however, MabSelect SuRe LX offers more than a 20% increase in DBC at a slightly longer residence time. At 6 min residence time, for example, the medium's capacity for human IgG is approximately 60 g/l.

This study investigated the performance of MabSelect SuRe LX during MAb capture by measuring its DBC at different residence times and feed concentrations. MabSelect SuRe LX data were also compared with MabSelect SuRe. In addition, process economy parameters, specifically medium productivity and utilization during manufacturing, were also evaluated for both media.

Materials and methods

Samples

Frontal analysis was performed with both human IgG and clarified CHO cell culture supernatants containing various monoclonal antibodies.

Sample preparation

Clarified CHO cell culture supernatant was used either in its native state or after spiking with purified MAb to final concentrations of approximately 5 and 10 mg MAb/ml. The resulting MAb concentrations were analyzed by analytical chromatography using a 1 ml HiTrap™ MabSelect SuRe column.

Frontal analysis for determining dynamic binding capacity

MabSelect SuRe LX and MabSelect SuRe were packed in Tricorn™ 5/100 columns (bed height 10 cm, column volume 1.96 ml). Frontal analysis was performed with native and spiked feeds. Clarified CHO cell culture supernatant was applied to the column until approximately 10% breakthrough was achieved. This was followed by washing out unbound material with PBS buffer, elution with 0.1 M citrate, pH 3.0, and cleaning-in-place (CIP) with 0.1 M NaOH. Selected fractions during sample loading were analyzed on a HiTrap MabSelect SuRe column using the buffers named above. Elution peaks were integrated and their areas in mAU × ml were compared to the peak area for the start material. The volume applied at 10% breakthrough ($V_{10\%}$) was defined from the fraction with 10% of the peak area in the start material.

For human IgG, $V_{10\%}$ was determined from the UV curve at 280 nm. Dynamic binding capacity at 10% breakthrough was calculated according to Equation 1.

$$DBC_{10\%} = (V_{10\%} - V_0)\, C_0/V_c \qquad [1]$$

where C_0 = antibody concentration (mg/ml), V_c = geometric total volume (ml), and V_0 = void volume (ml).

Process economy analyses

Productivity was measured (as g/l/h) while processing progressively higher titer feedstocks.

Purification potential ($P_{potential}$), which is used to show the manufacturing advantage of a medium, is defined as the mass of antibody that can be processed through the lifetime of a given quantity of media. It can be calculated from the product of the mass of antibody purified per batch through a certain volume of medium and the number of batches that can be processed through the same volume. This is outlined in Equation 2.

$$\text{Purification potential, } P_{potential} = \underbrace{\frac{\text{Mass to be purified per batch (kg)}}{\text{Column volume (l)}}}_{\substack{\text{Mass of antibody to be purified per volume of medium used}}} \cdot \underbrace{\frac{N_{life}{}^{\dagger}}{N_{cycles/batch}}}_{\substack{\text{Number of batches that can be processed through a given volume of medium}}} \qquad [2]$$

$^{\dagger}N_{life}$ = life of medium

Media utilization in manufacturing as well as related parameters were evaluated via purification potential analysis.

Results and discussion

Dynamic binding capacity vs residence time

Table 1 shows the DBC results for MabSelect SuRe LX for monoclonal IgG1 and human IgG at 10% breakthrough (DBC, 10% breakthrough) determined at residence times between 1 and 10 min (Fig 1).

Table 1. DBC for human IgG and monoclonal IgG1 vs residence time on MabSelect SuRe LX

Residence time (min)	DBC, 10% breakthough (mg/ml)	
	Human IgG	Monoclonal IgG1
1	18.3	
2.4	37.1	39
6	60.5	63
8	67.7	
10	69.2	68

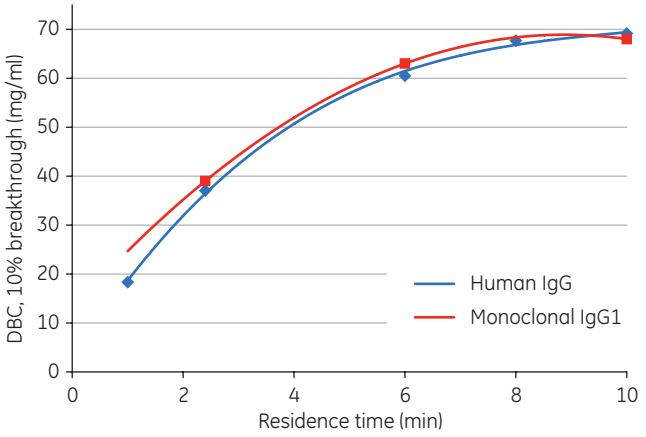

Fig 1. DBC increases as a function of residence time.

Comparing corresponding data for both media (Fig 2) shows that their DBC is almost equivalent at short residence times (≤ 3 min), while MabSelect SuRe LX shows significantly higher DBC than MabSelect SuRe at longer residence times.

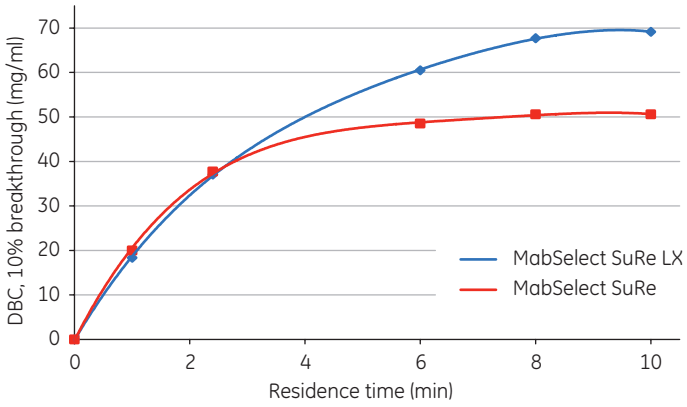

Fig 2. DBC for human IgG versus residence time. MabSelect SuRe LX has higher DBC at longer residence times.

MAb concentration vs DBC and residence time

DBC, 10% breakthrough for MabSelect SuRe LX was determined with clarified CHO cell culture at three different MAb concentrations. Residence time was varied between 2.4 and 10 min. Results are shown in Table 2 and Figure 3. As expected, DBC increases with increased residence time, while the MAb concentration in the clarified CHO cell culture supernatant does not have any significant influence on DBC.

Table 2. DBC of MabSelect SuRe LX at three residence times and three MAb concentrations

Residence time (min)	DBC, 10% breakthough (mg/ml)		
	1 g/l MAb	5 g/l MAb	10 g/l MAb
2.4	39	39	40
6	63	61	62
10	68	69	69

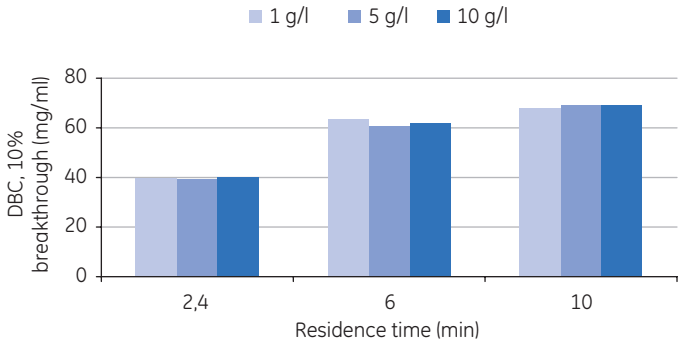

Fig 3. MAb concentration vs DBC, 10% breakthrough and residence time. The different concentrations do not markedly affect the DBC of MabSelect SuRe LX.

Dynamic capacity of different antibodies

DBC, 10% breakthrough for MabSelect SuRe LX and MabSelect SuRe was determined at 6 min residence time for seven different MAbs. Table 3 and Figure 4 show the results. As can be seen, DBC was 20% to 46% higher for MabSelect SuRe LX.

Table 3. DBC, 10% breakthrough for seven different MAbs at three residence times

MAb	MabSelect SuRe	MabSelect SuRe LX	Increase in DBC (%)
MAb 1	46	57	24
MAb 2	42	54	24
MAb 3	49	63	29
MAb 4	50	60	20
MAb 5	45	61	38
MAb 6	38	55	45
MAb 7	35	51	46

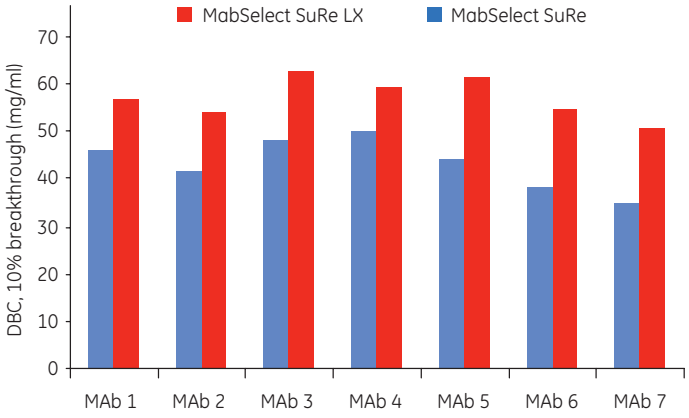

Fig 4. Significantly increased DBC of MabSelect SuRe LX compared with MabSelect SuRe at a residence time of 6 min.

Productivity analyses

The gains in throughput and robustness that originate from its exceptional binding capacity, plus the proven mechanical stability of high-flow agarose, mean that MabSelect SuRe LX can capture MAbs at a broad range of flow velocities and sample loads. This translates into better processing productivity compared with MabSelect SuRe.

Figure 5 shows the results of measuring the productivity ($g_{protein}/l_{medium}/h$) of both media while processing progressively higher titer clarified CHO cell culture supernatant (1, 5, and 10 g/l). Highest gains were achieved when using MabSelect SuRe LX to process large amounts of antibody at loading velocities that ensure optimal use of the medium's full binding capacity.

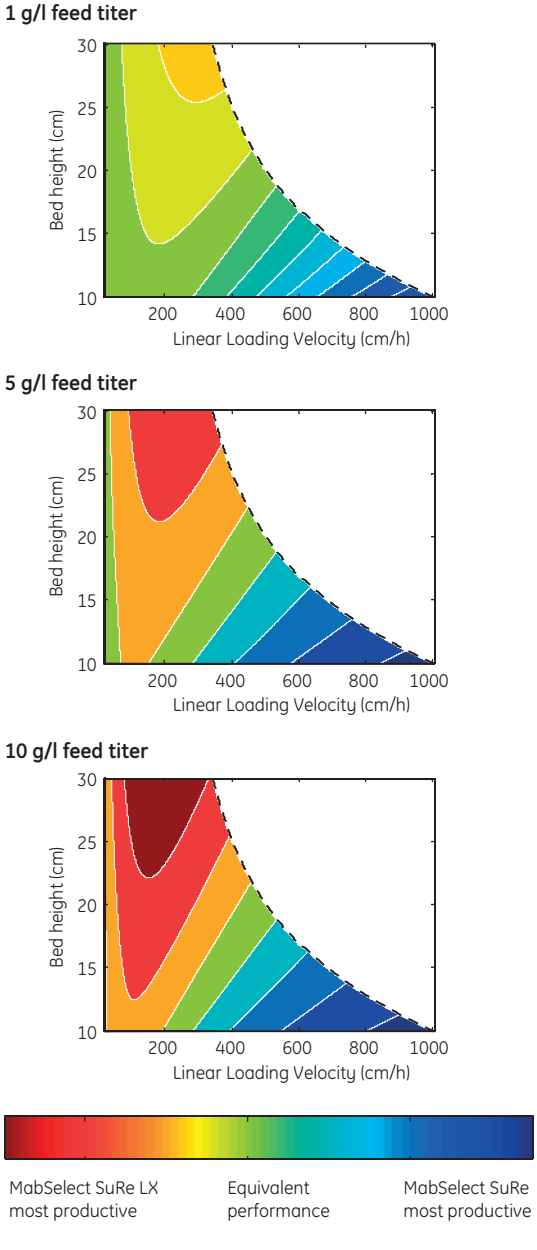

Fig 5. Contour plots showing the productivity difference (ΔProd, $g_{protein}/l_{medium}/h$) between MabSelect SuRe LX and MabSelect SuRe at different column bed heights and loading velocities. Maximum linear loading velocity is constrained by column pressure drop limitations as shown by the dashed lines. All column cycle operations excluding loading were run at the maximum flow rate as determined by the pressure drop constraint.

Media utilization in manufacturing

The high binding capacity of MabSelect SuRe LX allows large amounts of sample to be loaded onto the column. This can reduce processing times and/or allow current columns and hardware to be used instead of having to invest in new equipment when processes are scaled up. Incorporating the purification process into existing facilities should also be easier. In some cases, the volume of medium required to process a given mass of antibody can be reduced.

In-house optimization calculations were conducted to identify performance gains using MabSelect SuRe LX compared with MabSelect SuRe within a high-throughput, large-scale MAb manufacturing environment. The aim was to process 10 000 l of high-titer fermentation broth within an 8 h working shift. Table 4 shows the results.

The performance benefit of MabSelect SuRe LX is evident from an accompanying reduction in the quantity of medium and number of cycles required to purify the mass of antibody. Furthermore, these reductions also decrease the overall buffer consumption of the purification step.

Table 4. Analysis data of an optimized process for processing a 50 kg batch of antibody on MabSelect SuRe LX and MabSelect SuRe

Selected input parameter	Set values
Batch volume (l)	10 000
Loading concentration (g/l)	5
Total loading per day (kg)	50
Max batch processing time (h)	8
Loading per cycle	80% of breakthrough capacity at DBC 10%

Selected output parameter	Optimized values	
	MabSelect SuRe LX	MabSelect SuRe
Column diameter (cm)	140	160
Bed height (cm)	16	13
Number of cycles, $N_{cycles/batch}$	5	6
Process time (h)	7.8	7.9
Column volume CV (l)	246	261
Linear loading velocity (cm/h)	227	254
Max linear flow velocity (cm/h)	639	792
Loading per cycle (g/l)	41	32
Productivity ($g_{protein}/l_{medium}/h$)	25.9	24.1
Total buffer consumption (l)	24 581	31 329

Note: A process cycle is assumed to comprise column equilibration (3 CV), loading, two post-load wash steps (3 CV and 2 CV), elution (4 CV), regeneration (3 CV) and CIP. Equilibration, post-load washing, elution, and regeneration were run at a fixed residence time of 3 min. Column CIP is assumed to have a contact time of 15 min after every cycle of operation.

Table 5 shows the results of a purification potential analysis applied to the case described in Table 4. Assuming that the lifetime (N_{life}) of both media is equivalent, MabSelect SuRe LX will enable approximately 30% more mass of antibody to be purified over its lifetime than MabSelect SuRe (1).

In many cases, MabSelect SuRe LX demonstrates an increased lifetime compared to MabSelect SuRe. Data from a lifetime performance study examining the effect of repeated cleaning-in-place (CIP) with 0.5 M NaOH on DBC show that MabSelect SuRe LX retains a greater percentage of its DBC than MabSelect SuRe over the same number of CIP cycles (1). A 20% longer lifetime, for example, plus 30% increased load, would result in > 50% more purified antibody per liter of medium over its lifetime.

Table 5. Productive efficiency analysis based on results outlined in Table 4 when purifying a 50 kg batch of antibody

Parameters	Output values	
	MabSelect SuRe LX	MabSelect SuRe
Mass of antibody to be purified per batch (kg)	50	50
Volume of medium required, CV (l)	246	261
Number of cycles, $N_{cycles/batch}$	5	6
Medium lifetime, N_{life} (cycles)	200	200
Purification potential, $P_{potential}$ (kg/l_{medium})	8.13	6.39

Conclusions

As expected, DBC increases with increased residence time, while MAb concentration in the clarified CHO cell culture supernatant does not have any significant influence on DBC. At 6 min residence time, MabSelect SuRe LX gives ≥ 20% higher DBC compared to MabSelect SuRe, while the capacity is almost equivalent at residence times below 3 min. Adding the increase in lifetime of around 20%, MabSelect SuRe LX could purify up to 50% more antibody than MabSelect SuRe over its lifetime.

Using MabSelect SuRe LX reduces processing times and allows the dimensions of columns and other equipment hardware to be reduced. As Table 4 shows, total buffer consumption can be decreased significantly, which should improve process economy by saving on preparation and handling costs. The overall result should be a much-improved facility fit, that is, higher titer cultures can be processed using current equipment in existing facilities.

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

Reference

1. Application note: Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place, GE Healthcare, 28-9872-96, Edition AA (2011).

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