Process-economy simulation of mAb capture step with MabSelect™ PrismA protein A chromatography resin

MabSelect PrismA is a next-generation protein A chromatography resin that offers significantly enhanced alkaline stability and capacity compared with its predecessor products. This application note gives calculation examples that highlight scenarios where the enhanced capacity and alkaline-stability of MabSelect PrismA can provide process economic benefits to large-scale mAb processing. Calculations show that normalized cost per batch can be reduced by 75% using MabSelect PrismA compared with the predecessor MabSelect SuRe™ resin.

Introduction

The choice of chromatography resin is ultimately driven by process-economy, of which key influencing factors often include process time, buffer consumption, resin lifetime, hardware requirements, labor, and facility footprint, to name a few. MabSelect PrismA is a next-generation protein A resin with substantially improved performance compared with its predecessors. A significantly enhanced alkaline-stability offers the possibility of cleaning the resin with high concentrations of low-cost sodium hydroxide, to ensure a better process-economy while meeting the requirements of a stringent bioburden control (1). With enhanced properties of both the agarose base matrix and the protein A ligand, MabSelect PrismA also offers a notedly increased capacity compared with its predecessor resins (2). The improved capacity allows handling of the ever-increasing upstream titers, resolving bottlenecks in downstream mAb processing.

This process-economy simulation demonstrates the influence of dynamic binding capacity (DBC) and alkaline-stability of the resin in the design of a process, focusing on factors directly related to resin volume requirements. The predecessor resins—MabSelect SuRe and MabSelect SuRe LX resins—were included for reference.

Materials and methods

Process-economy simulation

For the process-economy simulation, the following assumptions were made:

- Model process: mAb capture from cell culture harvest at 2000 L bioreactor scale.
- Feed titer: 2.5, 5, and 10 g/L.
- A 10% dilution factor after harvest, with a mAb recovery of 95%.
- Product recovery of the capture step: 95%.
- Process time was limited to a maximum of 12 h. The time required for pre-load preparations and post-load system cleaning and column storage was excluded.
- A minimum load safety factor of 20%, although the used safety factor will generally be higher, assuming the feed volume is evenly split over total required number of cycles.
- Recommended running conditions for each resin were applied, using a sample load residence time of 4 min. The non-loading steps were identical, using the same number of column volumes (CV), differing only in flow velocities (Table 1). The flow velocities used in calculations for the MabSelect SuRe and MabSelect SuRe LX resins were based on those described in procedure 29008129 (3).
- Resin DBC at the given residence time for a generic mAb was calculated from an average DBC at 10% breakthrough (Q_{B10}) for a range of different mAbs, with data originating from internal, customer, and collaboration studies:
  - MabSelect SuRe: 44 g mAb/L resin
  - MabSelect SuRe LX: 51 g mAb/L resin
  - MabSelect PrismA: 66 g mAb/L resin
- Columns with fixed bed height of 200 mm, and i.d. ranging from 400 to 800 mm
- The maximum number of cycles per batch was set to 6.

Table 1. Process conditions

Process step	Buffer	Flow velocity	Residence time
Sample load	Total feed volume was evenly distributed over the calculated required number of cycles	300 cm/h	4 min[1]
Post-load wash	2 CV of phosphate buffered saline (PBS)	300 cm/h	4 min
Wash 1	3 CV of PBS	350 cm/h (MabSelect SuRe and MabSelect SuRe LX) 300 cm/h (MabSelect PrismA)	3.4 min (MabSelect SuRe and MabSelect SuRe LX) 4 min (MabSelect PrismA)
Wash 2	1 CV of 50 mM sodium acetate, pH 6 or appropriate buffer	350 cm/h (MabSelect SuRe and MabSelect SuRe LX) 300 cm/h (MabSelect PrismA)	3.4 min (MabSelect SuRe and MabSelect SuRe LX) 4 min (MabSelect PrismA)
Elution	3 CV of 50 mM sodium acetate, pH 3.5	300 cm/h	4 min
Strip	100 mM acetic acid, pH 2.9	350 cm/h (MabSelect SuRe and MabSelect SuRe LX) 300 cm/h (MabSelect PrismA)	3.4 min (MabSelect SuRe and MabSelect SuRe LX) 4 min (MabSelect PrismA)
Cleaning in place (CIP)	3 CV of 0.5 M NaOH	240 cm/h	5 min (total contact time is 15 min)
Re-equilibration	3 CV of PBS	350 cm/h (MabSelect SuRe and MabSelect SuRe LX) 300 cm/h (MabSelect PrismA)	3.4 min (MabSelect SuRe and MabSelect SuRe LX) 4 min (MabSelect PrismA)

[1] Although a load residence time of 6 min is recommended for MabSelect SuRe LX (3), 4 min was used for all resins to simplify the comparison.

Throughput, productivity, and buffer consumption

Throughput was defined as the amount of recovered target produced per hour (g/h). Productivity was defined as mass of recovered product per resin volume and process time (g/L/h). The buffer consumption was calculated from the total number of CV used per sample load, wash, elution, and regeneration, as well as the number of cycles required to process the total harvest volume.

Lifetime throughput and normalized cost per batch

The resin lifetime throughput shows the relative amount of product in percent (based on DBC) that can be produced with the respective resin under its lifetime normalized against the MabSelect SuRe resin. The lifetime was defined as the number of cycles that could be performed with bioreactor harvest feed until 90% of the initial DBC remained when using the specified cleaning-in-place (CIP) solution (0.5 M NaOH). With this feed, the number of cycles achieved with MabSelect PrismA was 105, 70 for MabSelect SuRe LX, and 38 for MabSelect SuRe resin. The cost per batch was normalized against the cost of the MabSelect SuRe resin and was calculated from the accumulated contribution of cost of resin, buffer and labor, shown both at a fixed number of cycles and when the resin lifetime was accounted for. In the former case, the influence of the CIP conditions was omitted, and consequently, the key cost differentiator will rely on the DBC of the resin. The buffer cost was assumed to be 2.5 USD/L, the labor cost was assumed to be 75 USD/h. For resin cost, list prices were used.

Results

Two of the most important parameters for the productivity outcome in downstream processing are process time and resin DBC. At a fixed process time, the choice of resin (and its DBC) is what sets the requirements for other parameters such as resin volume, column size, and buffer consumption. At equivalent resin volume and load residence time, the resin capacity becomes crucial for the amount of product that can be processed in a certain amount of time. As all process parameters relate to each

other, improvement of one parameter often comes at the cost of another. Hence, the main process priority—whether it is low resin volume, high throughput, or small facility footprint—needs to be defined as a first step in the design of a process. In this process-economy simulation, parameters were selected to simplify comparison. For other scenarios, absolute numbers will differ depending on input factors such as product concentration in the feed, number of cycles, and target mAb. It should be noted that the use of using discrete column dimensions can have a significant impact of the relationship between the resin volume and other process parameters at different scales.

Calculation examples for the selected scenarios are summarized in Table 2, and the 2000 L bioreactor with a 2.5 g/L titer scenario is visualized in Figures 1 to 6. The results show that MabSelect PrismA provides the shortest process time for all included mAb titers when using the same column size for all resins. The higher DBC of MabSelect PrismA also consistently resulted in a lower number of required cycles compared with the MabSelect SuRe and MabSelect SuRe LX.

As the buffer consumption is directly linked to the DBC under the given conditions, the higher DBC of MabSelect PrismA provides a significant reduction in buffer volumes compared with the MabSelect SuRe and MabSelect SuRe LX resins. Similarly, both the throughput and productivity is clearly improved for MabSelect PrismA, and the difference from MabSelect SuRe and MabSelect SuRe LX generally increases with increasing mAb titer.

In addition to its high capacity, another benefit with MabSelect PrismA is its high alkaline-stability that allows for an efficient cleaning, thereby prolonging the lifetime of and the lifetime throughput of purified mAb with the resin. In this calculation example, MabSelect PrismA exhibited a 420% and 217% higher lifetime throughput compared with MabSelect SuRe and MabSelect SuRe LX, respectively (Fig 6).

As shown in Figure 7A, the higher capacity of MabSelect PrismA can be used to increase throughput of an existing facility. At a given mass throughput, the improved capacity of MabSelect PrismA can also reduce the resin volume, thereby reducing column size and ultimately facility footprint (Fig 7B). The combination of a high DBC together with an increased alkaline-stability can also contribute to a significant reduction in the cost per batch (Fig 8). The normalized cost per batch (accumulated cost of resin, buffer, and labor during the resin lifetime) can be reduced by 75% using MabSelect PrismA compared with MabSelect SuRe resin.

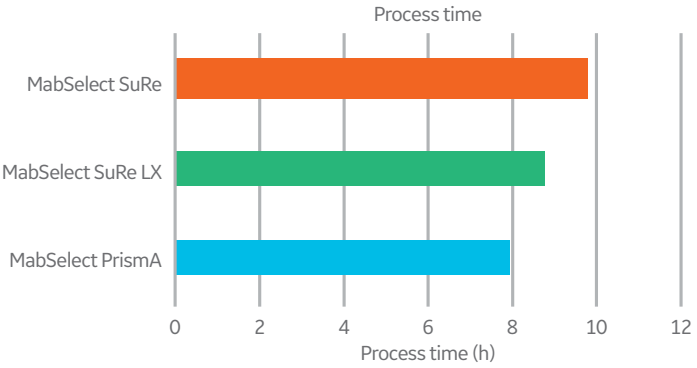

Fig 1. Time required for processing 2000 L of bioreactor feed with a titer of 2.5 g/L in a 450/200 mm column. Process time was reduced with MabSelect PrismA in all titer scenarios. Compared to MabSelect SuRe, the process time was reduced from 9% to 23% for the different titers.

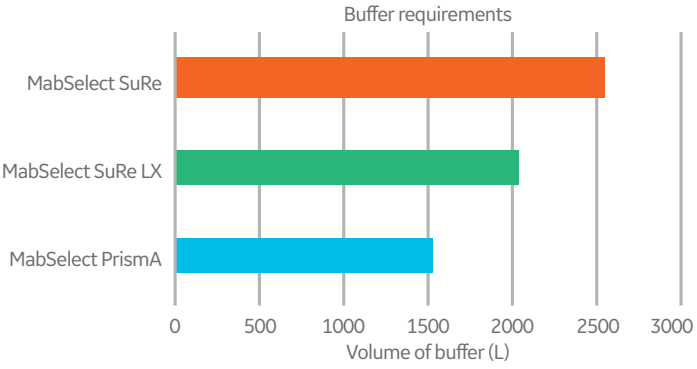

Fig 2. Buffer requirements for processing 2000 L of bioreactor feed with a titer of 2.5 g/L in a 450/200 mm column. Compared to MabSelect SuRe resin, the buffer volume for MabSelect PrismA was reduced from 20% to 40% for the different titers.

Table 2. Throughput and productivity for selected scenarios

Process details	**2000 L at 2.5 g mAb/L**			**2000 L at 5 g mAb/L**			**2000 L at 10 g mAb/L**		
	MabSelect SuRe	MabSelect SuRe LX	MabSelect PrismA	MabSelect SuRe	MabSelect SuRe LX	MabSelect PrismA	MabSelect SuRe	MabSelect SuRe LX	MabSelect PrismA
Cycles per batch	5	4	3	5	5	4	6	5	4
Process time (h)	9.8	8.8	8.0	7.8	7.8	7.1	7.7	6.6	5.9
Column i.d. (mm)	450	450	450	600	600	600	800	800	800
Bed height (mm)	20	20	20	20	20	20	20	20	20
Load (g/L)	28	33	42	28	33	42	28	33	42
Safety factor (%)	31	27	25	23	34	36	28	26	28
Resin volume (L)[1]	36:6	36.6	37.5	65.0	65.0	66.7	115.6	115.6	118.6
Volume buffer/cycle (L)	2543	2035	1526	4522	4522	3617	9646	8038	6431
Throughput (g/h)	485	542	596	1221	1221	1345	2474	2860	3206
Productivity (g/L/h)	12.6	14.1	15.1	17.9	17.9	19.2	20.3	23.5	25.7

[1] Packing factors used: MabSelect SuRe = 1.15, MabSelect SuRe LX = 1.15, MabSelect PrismA = 1.18

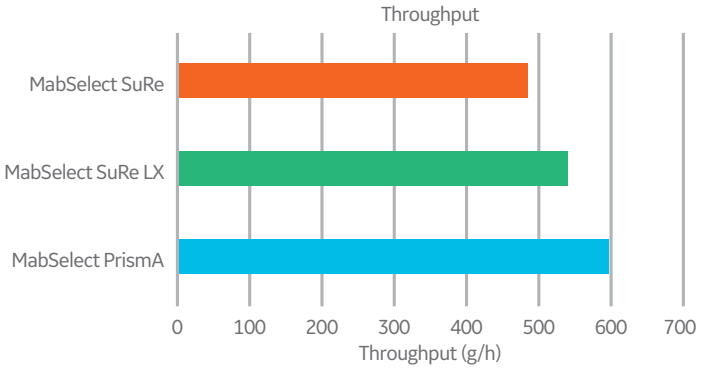

Fig 3. Throughput (g/h) in processing 2000 L of bioreactor feed with a titer of 2.5 g/L in a 450/200 mm column. MabSelect PrismA provides a higher throughput of 10% to 30% compared with MabSelect SuRe resin for the titers investigated in this study.

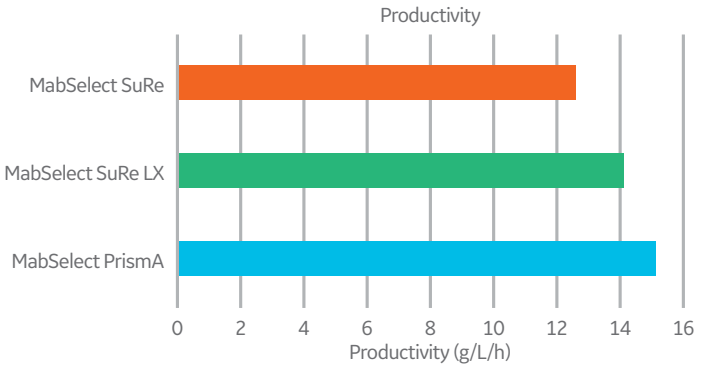

Fig 4. Productivity (g/L/h) in processing 2000 L of bioreactor feed with a titer of 2.5 g/L in a 450/200 mm column. MabSelect PrismA provides a higher productivity of 7% to 26% compared with MabSelect SuRe resin for the titers investigated in this study.

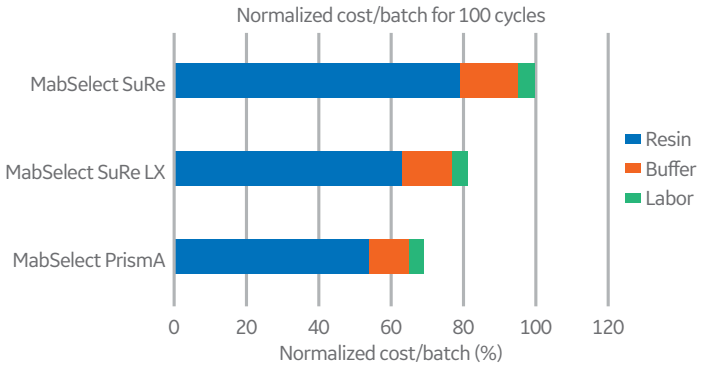

Fig 5. Normalized cost per batch over 100 cycles for processing 2000 L bioreactor batches of mAb with a titer of 2.5 g/L. At equal column volumes, the higher DBC of MabSelect PrismA reduced the cost from 15% to 32% compared with MabSelect SuRe. MabSelect PrismA extends resin lifetime because of enhanced alkaline stability, which could further improve the cost per batch compared with MabSelect SuRe and MabSelect SuRe LX, see Figure 8.

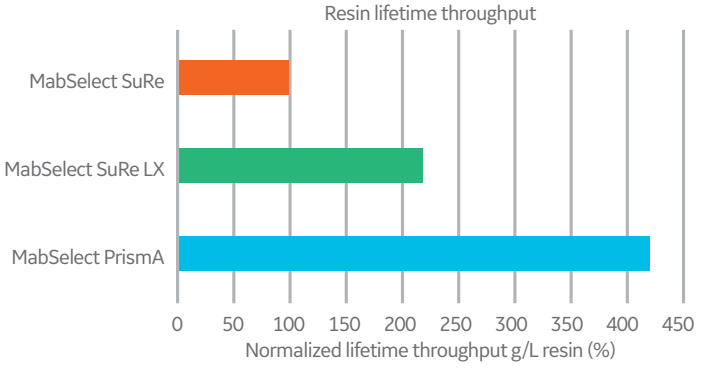

Fig 6. Lifetime throughput for MabSelect PrismA and its predecessor products, showing the relative amount of product that can be processed with the resins under their lifetimes, up to the point where 90% of the initial DBC remains, when cleaning with 0.5 M NaOH was included in each cycle. MabSelect PrismA exhibited 217% and 420% higher lifetime throughput compared with MabSelect SuRe LX and MabSelect SuRe, respectively.

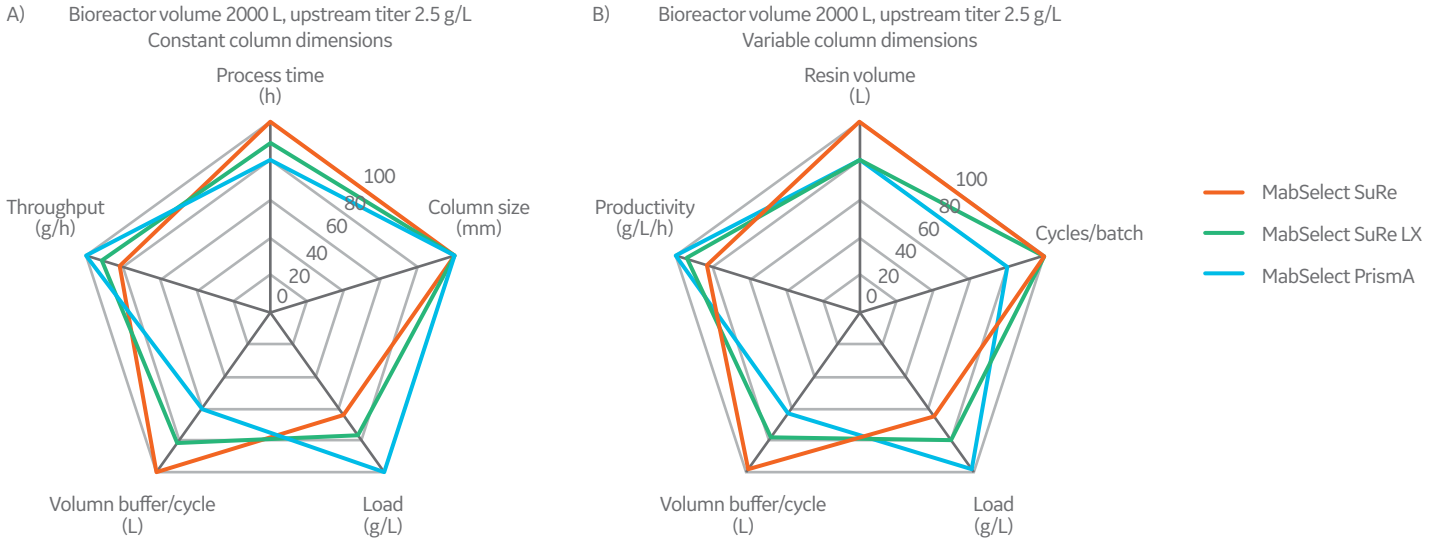

Fig 7. Example using a titer of 2.5 g/L to show the relation between process parameters. As a change in one parameter might influence other parameters, it is crucial to clearly define the main priority when designing a process. For MabSelect PrismA, the high capacity can either be used to (A) increase throughput or reduce process time with existing equipment or to (B) reduce equipment footprint and required resin volumes within a given maximum process time.

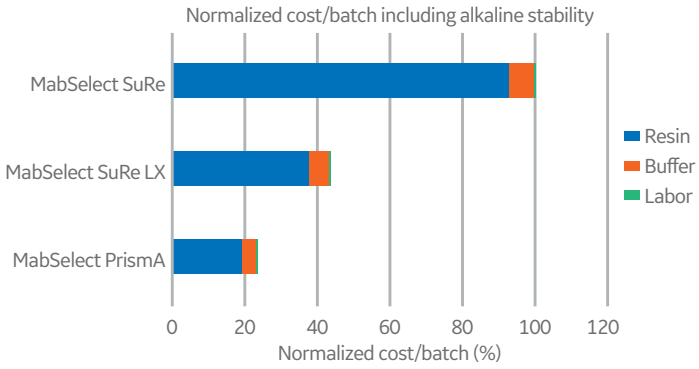

Fig 8. Normalized cost/batch (2000 L bioreactor with titer 2.5 g/L) and resin lifetime for MabSelect PrismA and its predecessor products taking alkaline stability into account. Lifetime measured up to the point where 90% of the initial DBC remains when cleaning with 0.5 M NaOH was included in each cycle. The normalized cost for MabSelect PrismA is reduced with 75% compared to MabSelect SuRe resin during its lifetime.

Discussion

When designing a new process or changing an existing process, there are usually some key parameters that strongly influence the process outcome. In the end, the process design will rely on process-economy, amount of material that is to be processed, and productivity. As every process is unique, the resin benefits will vary with the process scenario, which in turn relates to the desired process outcome: be it shorter processing times, increased productivity and throughput, improved product purity and yield, reduced buffer consumption, or ability to handle increasing titers in the product feed. These factors will vary significantly with the number of batches that are to be produced per year. In the case of clinical production phase, typically only a part of the resin lifetime is utilized. However, in a commercial manufacturing-scale setting, the resin cost will be distributed over the entire lifetime of the resin, in which case the resin lifetime will have a significant impact on the process-economy. As demonstrated here, resins that exhibit high DBC outperform resins with lower DBC, and the impact of resin performance will increase at higher titers. The use of high capacity resins can greatly lower the buffer consumption, which in addition to reducing costs for buffer chemicals and water, also reduces the size of the buffer containers and facility footprint. Combined with improved alkaline-stability, which provides a significantly longer resin lifetime, the impact of resin capacity on process-economy per batch can be significant.

Another aspect in downstream processing is how to handle the increasing titers of modern upstream processes, which can be a challenge for downstream processing unless either replacing hardware or allowing longer process times. A viable solution for new processes with increased titers can be to introduce a resin with improved performance in terms of DBC at similar product purity and yield, which will allow for higher throughput and shorter process times with existing hardware.

A resin with higher alkaline stability means higher remaining DBC over multiple cycles compared with more alkaline-sensitive resins. Higher alkaline stability may also allow the use of lower safety factors without impacting the risk of potentially losing yield, allowing higher loads and additional productivity gains.

Conclusion

MabSelect PrismA offers significantly enhanced alkaline stability and capacity compared with its predecessor products. The higher capacity of MabSelect PrismA can be used to increase the mass throughput per purification cycle using existing equipment to delay capital investment. Alternatively, the increased capacity can be used to decrease the resin volume (and concomitantly the buffer consumption) required to achieve a given mass throughput. Resin lifetime, in combination with an increased capacity, is one of the most important factors for lowering the cost per batch.

Disclaimer

The results and conclusions presented in this process-economy simulation are valid for this specific study. Other study conditions and assumptions could have significant impact on the outcome.

References

1. Application note: Lifetime performance study of MabSelect PrismA during repeated cleaning-in-place cycles. GE Healthcare, KA1061120418AN (2018).
2. Application note: Capacity and performance of MabSelect PrismA protein A chromatography resin. GE Healthcare, KA1965230418AN (2018).
3. Procedure: mAb capture step development using MabSelect SuRe LX. GE Healthcare, 29008129, Edition AC, (2014).

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GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752 USA
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073 Japan
GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA UK
For local office contact information, visit gelifesciences.com/contact.
KA2210051018AN