Evaluation of performance of a disposable mAb affinity chromatography column used over multiple process cycles

Presanitized and prequalified disposable chromatography columns are widely used to save time in downstream processes. However, reuse of such columns could positively impact process economy. This application note demonstrates cycling of ReadyToProcess™ MabSelect SuRe™ LX columns in multiple process runs. Analysis of mAb purity and recovery as well as column bed integrity testing show that the column can be used with maintained performance for at least 50 cycles, providing significant process economic benefits.

Introduction

The use of presanitized and prequalified disposable chromatography columns has increased significantly over the last years, as they allow for improved flexibility in facility design and quick startup by omitting the need for column packing and bed integrity testing (Fig 1). The possibility to dispose the column after use eliminates time-consuming and costly cleaning and unpacking operations between production batches or campaigns. Disposable columns, however, can be perceived as costly compared with clean-and-reuse columns, and a large part of the column cost can be attributed to the included chromatography resin. As resins are designed for up to several hundreds of cycles, justifying their cost per volume, the possibility to cycle the disposable column could significantly improve process economy (1). Reuse of the disposable chromatography column would, however, require a stable and validated column bed to ensure a consistent performance over multiple process cycles.

In a lifetime study with purified protein, it has been demonstrated that MabSelect SuRe LX maintains its dynamic binding capacity for over 100 purification cycles (2). In addition, product purity and yield remained high, while leached protein A and host cell protein (HCP) were consistently low over the runs. In this work, performance of the prepacked ReadyToProcess MabSelect SuRe LX 1 L column was evaluated over 50 process cycles using mAb-containing feedstock to be more similar to a real production situation.

Fig 1. ReadyToProcess columns are validated high-performance bioprocessing columns that are supplied prepacked and ready for use.

Materials and methods

Sample preparation

Target mAb was produced from Chinese hamster ovary (CHO) cells grown in HyClone™ ActiPro™ medium supplemented with 6 mM L-glutamine and fed daily from day 3 and onwards with 3% HyClone Cell Boost™ 7a and 0.3% Cell Boost 7b (of starting culture volume). Cell culturing was performed using the Xcellerex™ XDR-200 bioreactor system, with a starting volume of 140 L. The culture harvest was clarified using the Zeta Plus™ Encapsulated System equipped with a 1.6 m^2 filter capsule (3M Purification Inc.) and thereafter filtered through an ULTA™ Pure HC 0.6/0.2. To prepare the sample for the capture step, the sample was filtered through an ULTA Pure HC 0.2 µm filter. Concentration of mAb in the final sample was 1.2 g/L.

Chromatography method

A prepacked ReadyToProcess MabSelect SuRe LX 1 L column was used for mAb capture from clarified cell culture harvest. Sample load was 15 g mAb/L resin, which can be considered low with regard to the high dynamic binding capacity of MabSelect SuRe LX of up to 60 g/L.

Table 1. Chromatography capture step

Step	CV	Flow velocity (cm/h)	Buffer	Residence time (min)
Equilibration	3	300	20 mM sodium phosphate, pH 7.0 + 500 mM NaCl	4
Sample	12.5	200	1.2 g/L in culture medium	6
Wash 1	5	200	20 mM sodium phosphate, pH 7.0 + 500 mM NaCl	4
Wash 2	1	200	50 mM sodium acetate, pH 5.0	4
Elution	3	200	50 mM sodium acetate, pH 3.5	4
CIP	3	240	0.1 M NaOH (0.5 M every 10th cycle)	5
Re-equilibration	3	300	20 mM sodium phosphate, pH 7.0 + 500 mM NaCl	4

The sample load was constrained in this study due to limited supply of mAb cell culture supernatant. Hence, prepared mAb-containing sample was only used each 10th cycle, whereas for intermediate cycles, passed material was recycled.

The method outlined in Table 1 was performed over 50 cycles, with a cleaning-in-place (CIP) step using 0.1 M NaOH in each cycle. Every 10th cycle, CIP was conducted using 0.5 M NaOH, and the column was removed from the chromatography system and stored for a minimum of two days with 20% ethanol in cold room to mimic real use.

Before start of the study, a blank run (sample load step omitted) was performed, which is recommended to remove non-covalently immobilized ligand. The column was operated on the ÄKTA™ ready chromatography system installed with the Low Flow kit.

Bed integrity tests

Bed integrity testing was performed in 20% ethanol as described earlier (3). Tests were performed every 10th cycle both before as well as after cold room storage in ethanol, to evaluate if the column was affected by the chromatography runs or the storage conditions. As acceptance criteria, the specifications for ReadyToProcess MabSelect SuRe LX 1 L column, > 1800 plates/m and an asymmetry factor (A_S) of 0.80–1.80, was used.

Analyses

HCP concentration in the eluate pool was analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies) and Gyrolab™ workstation. To determine mAb recovery during the study, selected eluates were analyzed for mAb content using the Biacore™ T200 system. Size exclusion chromatography (SEC) was used to analyze monomer content.

Results and discussion

An overlay of chromatograms from selected cycles is shown in Figure 2. No significant difference could be observed between runs. Also, mAb purity and recovery were comparable between eluates from the selected cycles, with a mAb recovery of ≥ 90% at a HCP content of < 4 µg HCP/mg mAb (Table 2).

As ReadyToProcess MabSelect LX columns are used in the first capture step, they are exposed to complex cell harvest loads. The suboptimal harvest/clarification conditions used in this study contribute to the presence of high amount of impurities (fouling agents) in the starting material, which in combination with the relatively low mAb load, contribute to the somewhat high HCP content in the eluates. Impurities that are not removed in the harvest/clarification step can build up on the chromatography column and cause

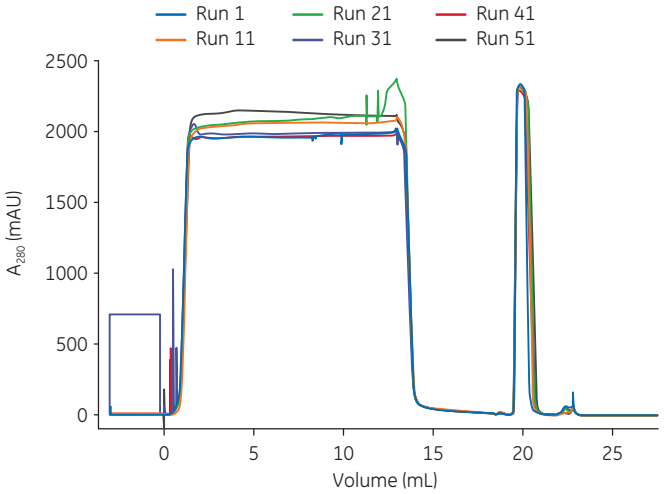

Fig 2. Overlay of chromatograms from Runs 1, 11, 21, 31, 41, 51 of repeated mAb capture cycles using the ReadyToProcess MabSelect SuRe LX 1 L column. Note! In run 21 and 31, the method was paused and restarted during sample load, causing spikes and a drifting UV curve.

Table 2. Analysis results from repeated mAb capture cycles using the ReadyToProcess MabSelect SuRe LX 1 L column

	Eluate volume (L)	mAb conc (g/L)	Tot mAb content (g)	Recovery (%)*	Aggregate (%)	Monomer (%)	HCP mg/g mAb
Start material		1.20	15.00	100			
Run 1	0.95	15.6	14.84	99	N/A	N/A	N/A
Run 11	1.06	13.7	14.52	97	0.99	99.01	3.1
Run 21	1.22	11.6	14.15	94	0.92	99.08	3.8
Run 31	1.15	11.8	13.51	90	1.03	98.97	3.7
Run 41	1.17	11.5	13.50	90	0.76	99.24	3.4
Run 51	1.25	10.9	13.61	91	0.76	99.24	1.9

* Theoretical recovery = 15 g mAb (12.5 L × 1.2 g/L). N/A = not analyzed

fouling of the column. A decrease in resin capacity due to column fouling over time can be prevented by optimization of the precolumn filtration steps as well as the column CIP protocol, ensuring a maintained high recovery for more process cycles. Sample preparation and column CIP protocol should be evaluated and optimized for each specific application.

In column bed integrity testing at start of the study, the ReadyToProcess MabSelect SuRe LX 1 L column exhibited 3242 plates/m and an A_S of 1.14. Plates/m and A_S were maintained within acceptance criteria over 50 cycles. After 50 cycles, the column exhibited 1968 plates/m and an A_S of 1.42. The results summarized in Table 3 show that storage in cold room after every 10th cycle did not affect the column bed integrity.

Table 3. Results from column bed integrity testing in 20% ethanol

Cycle	Plates/m	A_S
After packing	3123	1.12
Before 1	3242	1.14
After 10	3162	1.13
Before 11	3273	1.16
After 20	2966	1.17
Before 21	2883	1.17
After 30	2300	1.28
Before 31	2156	1.31
After 40	1900	1.29
Before 41	2085	1.30
After 50	1873	1.47

Specifications for the ReadyToProcess MabSelect SuRe LX 1 L column: > 1800 plates/m and an A_S of 0.80–1.80. "Before" indicates before storage in cold room. "After" indicates after storage before use in upcoming 10 cycles.

Conclusions

This study aims to demonstrate the performance of ReadyToProcess MabSelect SuRe LX columns when reused over multiple cycles. The study was set up to mimic a real use of the column in purification of therapeutic mAbs. Cycling of the ReadyToProcess MabSelect SuRe LX 1 L column, with necessary CIP procedure included in each run and with a more rigorous CIP protocol as well as cold room storage in 20% ethanol included after every 10th cycle, was evaluated. The results show that column bed integrity, in terms of plates/m and A_S, as well as mAb purity and recovery were maintained over 50 cycles, indicating the possibility of using prepacked ReadyToProcess columns for multiple runs, with a repeated CIP protocol included in each cycle. Compared with limiting their use to only 5 or 6 process cycles, the possibility of using ReadyToProcess MabSelect SuRe LX columns in as many as 50 cycles provides the opportunity for a significantly improved process economy. Depending on the specific application, and with optimized sample preparation and column CIP protocol, the lifetime of the ReadyToProcess columns could be even longer than for 50 cycles.

Disclaimer

The results and conclusions presented in this application note are valid for this specific study. Other study conditions and assumptions could have significant impact on the outcome.

References

1. Case study: Improve process economy by cycling of prepacked chromatography columns GE Healthcare, 29260019, Edition AA (2017).
2. Application note: Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place. GE Healthcare, 28987296, Edition AA (2011).
3. Application note: Efficiency test of ReadyToProcess columns. GE Healthcare, 28919821, Edition AA (2007).

Ordering information

Product	Description	Product code
ReadyToProcess MabSelect SuRe LX 1 L column	Prepacked 1 L column	29026927
ULTA Pure HC 0.6 / 0.2 µm	30 inch capsule filter, terminating with ReadyMate™ connectors	12410099
ULTA Pure HC 0.2 µm	10 inch capsule filter, terminating with TC connectors	KMP-HC9210TT
ÄKTA ready gradient	Chromatography system including Column Trolley	29032038
ÄKTA ready Low Flow Kit	Including inlet manifold, outlet manifold, pump tubing, flow cell for pressure sensor, air trap, air vent tubing, column inlet connector, column outlet connector, 2 × flow cell for pressure sensor, flow meter cell, temperature cell, conductivity censor, UV flow cell, 2 × clamp 25 mm TC, 2 × gasket o.d. 25 mm i.d. 15 mm and product documentation. pH electrode (optional) is ordered separately.	28930182

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