A flexible antibody purification process based on ReadyToProcess products

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A flexible antibody purification process based on ReadyToProcess™ products

We have developed and used an efficient monoclonal antibody (MAb) purification protocol to reduce the level of aggregates from 10% to 0.4% in a two-step chromatography process giving a monomer yield of 81%. The process involved the use of MabSelect SuRe™ for the capture step and Capto™ adhere for the polishing step. In addition, we describe the transfer of a small-scale process based on prepacked HiScreen™ columns (4.7 mL) to a ReadyToProcess format based on prepacked and prequalified columns and filters suitable for clinical phase I/II production for increased speed and process flexibility.

Introduction

In the biopharmaceutical industry, the need to reduce cost while increasing the success rate of drug development requires effective screening tools to produce robust and high yield purification processes. In this work, we describe the scaling up of a two-step antibody purification process (described in GE Healthcare application notes 28-9468-58 and 28-9509-60) using ReadyToProcess products to achieve shorter time-to-clinic and cost savings. The results from the scaled-up process using the ReadyToProcess platform were compared with those from the scaled-up process using the AxiChrom™ column format. The overall schematic process is shown in Figure 1.

This is the fourth application note in a set of four with the overall theme of providing you with efficient tools and methods to enhance process development and production of monoclonal antibodies—from exploratory stages through laboratory and clinical trials to full-scale production. The other application notes are:

- High-throughput screening and optimization of a protein A capture step in a monoclonal antibody purification process (28-9468-58)
- High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process (28-9509-60)
- Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom columns (28-9403-49)

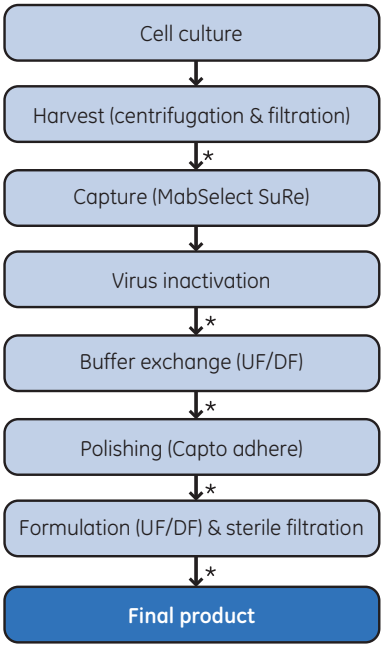

Fig 1. Flow scheme of the purification process. In-process filtration of the sample to reduce bioburden is indicated with asterisks.

Materials and methods

PreDictor™ 96-well filter plates were used for the initial screening of the chromatography medium and operating conditions (1, 2). HiScreen columns were then used to verify and optimize these intial conditions (1, 2).

Cell culture

Chinese hamster ovary (CHO) cells expressing the target IgG were cultured in WAVE Bioreactor™ systems 20/50 and 200, with working volumes of 10 L and 100 L, respectively. Process parameters for 100 L culture were: 18 rpm (rocks per min), an angle of 8°, temperature 36.8°C, pH 7.1 to 6.7 and dissolved oxygen (DO) ≥ 50%. During the culture pH was controlled with CO_2 and sodium bicarbonate, and DO with air and O_2. The culture was fed with Lucratone Yeast UF8804 hydrolysate, glucose, and glutamine. Culture duration was 16 d, peak cell density 3.7×10^6 viable cells/mL and harvest viability 52%.

Cell harvest

Cells were harvested by calcium phosphate flocculation followed by centrifugation. Briefly, 4 L of calcium chloride (0.8 M) was mixed in line with 4 L of 0.5 M sodium phosphate and the mixture was added directly to the WAVE Bioreactor. After incubation for 20 min, the feed was centrifuged at 4000 × g for 20 min at room temperature. The supernatant was filtered through an ULTA™ Pure HC (sterilizing-grade membrane 0.6/0.2 µm, 10" capsule). The filtrate was stored in sterile containers.

Normal flow filtration (NFF)

After each process step, material was filtered through a ReadyToProcess ULTA Pure HC capsule into a disposable bag for storage until further processing. This was performed to control bioburden and ensure product stability.

Chromatography

The columns used for the scaled-up process were ReadyToProcess 1 L columns. These are prepacked, prequalified columns with a bed height of 20 cm. The scale-up was performed on an ÄKTA™ ready system with a disposable flow path (low flow kit).

Capture with MabSelect SuRe

The MabSelect SuRe steps and buffers are described in Table 1.

The quality of all buffers and salts was of analytical purity (p.a.) and the water used was purified water.

Virus inactivation

Virus inactivation was performed by incubation of the elution pool from the capture step at pH 3.8 for 40 min. The pool was then adjusted to pH 7.0 by the addition of 0.5 M Na_2HPO_4.

Table 1. Process description of the MabSelect Sure capture step

Step	Duration	Buffer	Comment
Equilibration	1 CV	20 mM sodium phosphate, pH 7.2	
Load	23 L	N/A	Residence time: 4 min. Load: 30 g/L
Wash 1	5 CV	35 mM sodium phosphate, 500 mM NaCl, pH 7.2	Wash with high salt to remove HCP
Wash 2	1 CV	20 mM sodium phosphate, pH 7.2	Wash without salt for salt-free elution
Elution	5 CV	20 mM sodium citrate, pH 3.6	Typically, elution is finished in 1.5 to 2.0 CV's
CIP	3 CV	500 mM NaOH	Contact time: 15 min
Re-equilibration	5 CV	20 mM sodium phosphate, pH 7.2	Until stable pH is reached

Buffer exchange, cross flow filtration

Concentration/diafiltration was performed using a 60 cm hollow fiber filter cartridge with 30 kD nominal molecular weight cut-off (NMWCO), 0.5 mm lumen i.d., and a total membrane area of 0.48 m^2. The filtration system was a fully disposable setup based on ReadyCircuit assemblies, assembled using ReadyMate™ aseptic connectors. The feed and retentate pressures were controlled to 1.5 bar (22 psi) and 0.5 bar (7 psi), respectively, resulting in a transmembrane pressure (TMP) of 1 bar (15 psi). The sample was introduced into the system and then continuously diafiltered with 6 volumes of 50 mM phosphate, 50 mM NaCl pH 7.0. The system was drained and flushed once with 1000 mL of diafiltration buffer to maximize product recovery.

Polishing with Capto adhere

The Capto adhere step was run in combined flow-through elution mode. This means that flowthrough, wash and elution were collected in one pool. The Capto adhere polishing steps and buffers are described in Table 2.

The quality of all buffers and salts was of analytical purity (p.a.) and the water used was purified water.

Formulation, cross flow filtration

The filtration system was the same as that used for the buffer exchange step. The feed and retentate pressures were controlled to 1.5 bar (22 psi) and 1 bar (15 psi), respectively, resulting in a transmembrane pressure (TMP) of 1.25 bar (18 psi). The sample was concentrated 6 times, continuously diafiltered with 6 volumes 20 mM sodium phosphate, 150 mM NaCl pH 7.0, and then concentrated an additional 3-fold. The system was drained and flushed once with 1000 mL of diafiltration buffer to maximize product recovery.

Table 2. Process description of the Capto adhere polishing step

Step	Duration	Buffer	Comment
Equilibration	1 CV	50 mM phosphate, 50 mM NaCl, pH 7.0	
Load	12.06 L	N/A	Residence time: 5 min. Load: 60 g/L. Concentration: 5 g/L
Wash	5 CV	50 mM phosphate, 50 mM NaCl, pH 7.0	Wash to elute product
Elution	20 CV	50 mM phosphate, 250 mM NaCl, pH 6.1	Step to specifically elute bound monomer
Strip	3 CV	100 mM phosphate, pH 3.0	Remaining proteins are eluted from the media
CIP	3 CV	1 M NaOH	Contact time: 15 min
Re-equilibration	5 CV	50 mM phosphate, 50 mM NaCl, pH 7.0	Until stable pH is reached

Analytical methods

Total IgG concentration was measured by analytical Protein A chromatography. Briefly, 50 µl of the sample was adsorbed to a 1 mL HiTrap™ MabSelect SuRe column, washed, and finally eluted with 100 mM sodium phosphate, pH 3.0. The elution peak was integrated and compared to a standard curve to obtain the concentration value.

Monomer purity was assessed by size exclusion chromatography (SEC) using two Superdex™ 200 5/150 GL columns connected in series to achieve optimal peak separation. The mobile phase was phosphate buffered saline (PBS) and the flow rate was 0.35 mL/min for 15 min. The sample (10 µL) was applied to the column.

Host cell protein (HCP) levels were measured using commercial anti-CHO HCP antibodies (Cygnus Technologies). Essentially, an ELISA methodology was adapted to a Gyrolab™ Workstation LIF using Gyrolab Bioaffy™ 200 HC microlaboratory discs.

Ligand leakage measurements were performed using a commercial ELISA kit (Repligen Corp., USA) with a slightly modified protocol compared to the one supplied by the manufacturer.

Results and discussion

The aim of this study was to use single-use products in all unit operations from upstream to downstream, which meant that several steps could be eliminated; for example, column packing and cleaning steps. The MAb purification process was scaled up using equipment from the ReadyToProcess platform. The process included a WAVE Bioreactor System 200 for mammalian cell culture at 100 L scale, an ÄKTA ready chromatography system with a disposable flow path, ReadyToProcess chromatography columns (1 L) and filters for downstream operations. It also included the ReadyCircuit bags and assemblies for the crossflow filtration steps.

Cell culture and harvest

A CHO cell line, expressing monoclonal IgG, was cultured in a WAVE Bioreactor at the 100 L scale. The expression level after 16 d was 0.93 mg/mL. Harvest was performed by calcium phosphate flocculation followed by centrifugation. After centrifugation the supernatant was filtered through an ULTA Pure HC sterilizing-grade filter. Due to the flocculation step no prefilter was required after centrifugation. After harvest, 90 g of IgG was available for further processing.

Capture on MabSelect SuRe

In the scaled-up process runs, the harvested sample was applied directly onto a MabSelect SuRe ReadyToProcess 1 L column (load approximately 30 g/L) in 3 cycles. The average yield was 96% and the eluted pool contained 10% aggregates. The HCP content was reduced from approximately 37 500 ppm to 15 ppm, a reduction factor of 2500. Ligand leakage was low, 9 ppm on average.

Virus inactivation

Directly after each cycle of the capture step virus inactivation was performed by lowering the pH to 3.8 and incubating for 40 min at room temperature. The pH was then adjusted to 7.0 by the addition of phosphate buffer followed by filtration using a 2" ULTA Pure HC capsule sterilizing-grade filter.

Buffer exchange, cross flow filtration

The elution pool from MabSelect SuRe was buffer exchanged by continuous diafiltration with 6 volumes 50 mM phosphate, 50 mM NaCl pH 7.0. A stable flux with an average of 14 LMH (expected for ultrafiltration operations with hollow fiber filters) was observed throughout the process. The outcome was a 160 min process with a recovery of 98% (including sterile filtration), Table 3. No impact on product quality could be detected by SEC analysis and SDS-PAGE. Finally, the sample was diluted to a concentration of 5 g/L by the addition of equilibration/wash buffer for the Capto adhere step.

Table 3. Process data for the buffer exchange

Unit operation	buffer exchange
Start volume (L)	3.0
Final volume(L)	3.8
Diafiltration volume (times)	6
Start concentration (mg/mL)	19
Final concentration (mg/mL)	15
Load (g/m^2)/(L/m^2)	116/6
Filtration time (min)	158

Polishing on Capto adhere

The buffer-exchanged sample was loaded in one cycle onto a 1 L ReadyToProcess Capto adhere column. The load was 60 g/L. The flowthrough, wash, and elution fractions were collected in one pool. The starting aggregate concentration of 10% was reduced to 0.4% in this single step (Fig 2). The monomer yield was 89%, which was judged to be good considering the high aggregate content at start.

Column: Two Superdex 200 5/150 GL connected in series
Sample: 10 µl of IgG
Mobile phase: PBS, pH 7.0
Flow rate: 0.35 ml/min
System: ÄKTAexplorer™ 10

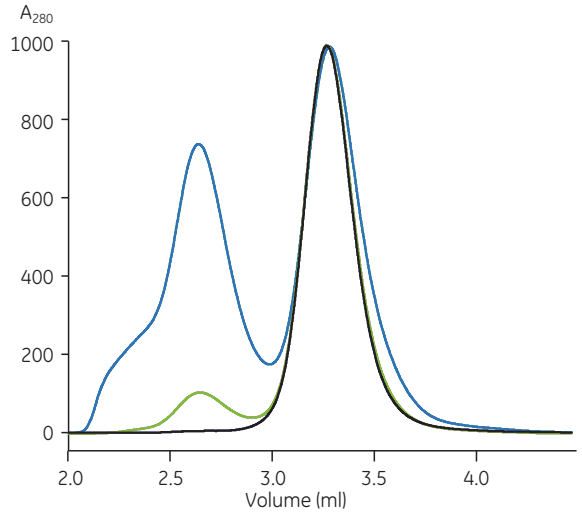

Fig 2. SEC analysis of the MAb in the Capto adhere step—sample before purification (green), purified fraction (black) and strip fraction (blue). The curves were normalized with respect to the monomer peak of the purified fraction.

Formulation, cross flow filtration

After the Capto adhere step, formulation was performed. Diafiltration was performed at a concentration factor of 6.5. The product was continuously diafiltered with 6 volumes of formulation buffer (20 mM sodium phosphate, 150 mM NaCl pH 7.0) and finally concentrated to give a total concentration factor of 10 after the addition of flush volume. The process resulted in an average flux of 19 LMH (expected for an ultrafiltration operation with hollow fiber filters). As expected, the observed flux was highly dependent on the product concentration but stable during diafiltration. The recovered pool showed a yield of approximately 98% (including sterile filtration) and no increase in aggregate level was seen.

Table 4. Data from the formulation

Unit operation	Formulation
Start volume (L)	18.3
Final pool volume(L)	1.8
Diafiltration volume (times)	6
Start concentration (mg/mL)	2.16
Final concentration (mg/mL)	21.0
Load (g/m2)/(L/m2)	82/41
Filtration time (min)	225

Scale-up summary

We successfully scaled-up a two-step chromatography purification process to pilot scale using ReadyToProcess products. Chromatography was run using ReadyToProcess columns on an ÄKTA ready system and filtration was performed using ReadyToProcess filters and a fully disposable cross flow filtration system for ReadyToProcess hollow fiber cartridges. The chromatography steps were performed on the same ÄKTA ready system; only the flow kit was changed between the runs. The process, consisting of a capture step on MabSelect SuRe and polishing step on Capto adhere with a buffer exchange step in between, and a formulation step at the end, was able to reduce the HCP concentration from 37 500 ppm to 1.0 ppm (Table 5). In addition, the Capto adhere step removed aggregates from a concentration of 10% down to 0.4% and the Protein A ligand leakage was reduced to below the limit of quantification from 9 ppm. The total yield of the downstream process, including all filtration steps, was 81%.

Table 5. Summary of monomer yield, aggregate content, and HCP reduction in the scale-up

Process step	HCP (ppm)	Ligand (ppm)	Aggregate content (%)	Yield (%)
Fermentation	37 500	Not done	10	
Harvest	37 500	Not done	10	100
Capture, MabSelect SuRe (2 cycles)	19	8.8	10	96.0*
UF/DF 1	12	9.1	10	97.7
Polishing, Capto adhere	<LOQ**	<LOQ**	0.4	89.0
UF/DF 2 & Sterile filtration	1.0	0.1	0.4	97.4
Total yield:				81.3

* Average of 2 cycles
** LOQ = level of quantification (4.6 ng/mL for HCP, 3 ng/mL for ligand)

Process using single-use formats vs process using conventional formats

We scaled up the process using a traditional approach with AxiChrom columns connected to an ÄKTApilot™ system for chromatography and Kvick™ Lab cassettes with a UniFlux 10 system for ultrafiltration (3). The scale-up results from ReadyToProcess column process were similar to the results obtained from the AxiChrom column process. Figures 3 and 4 compare the chromatography steps on MabSelect SuRe (Figures 3A and 3B) and Capto adhere (Figures 4A and 4B) in the two different formats. The comparable performance of the ReadyToProcess and AxiChrom columns was confirmed by the analytical results (Table 6). Yield and contaminants levels were practically identical during all steps, demonstrating the equivalent performance of the column types. The overall yield also demonstrated the similarity between the processes. The yield for both the ReadyToProcess process and AxiChrom process was 81%.

A comparative performance evaluation between the standard setup with Kvick cassettes and the setup with ReadyToProcess hollow fibers and ReadyCircuit™ assemblies (3) is difficult since the formats are different. From a product quality perspective, the analysis methods showed no difference between the two formats. However, due to the inherent nature of the hollow fiber format, the flux is lower, which in turn gives a longer filtration time.

Sample:	30 g IgG/L media
Equilibration buffer:	20 mM sodium phosphate, 50 mM NaCl, pH 7.2
Wash buffer 1:	35 mM sodium phospate, 500 mM NaCl, pH 7.2
Wash buffer 2:	20 mM sodium phospate, pH 7.2
Elution buffer:	20 mM sodium citrate, pH 3.6
Residence time:	4 min

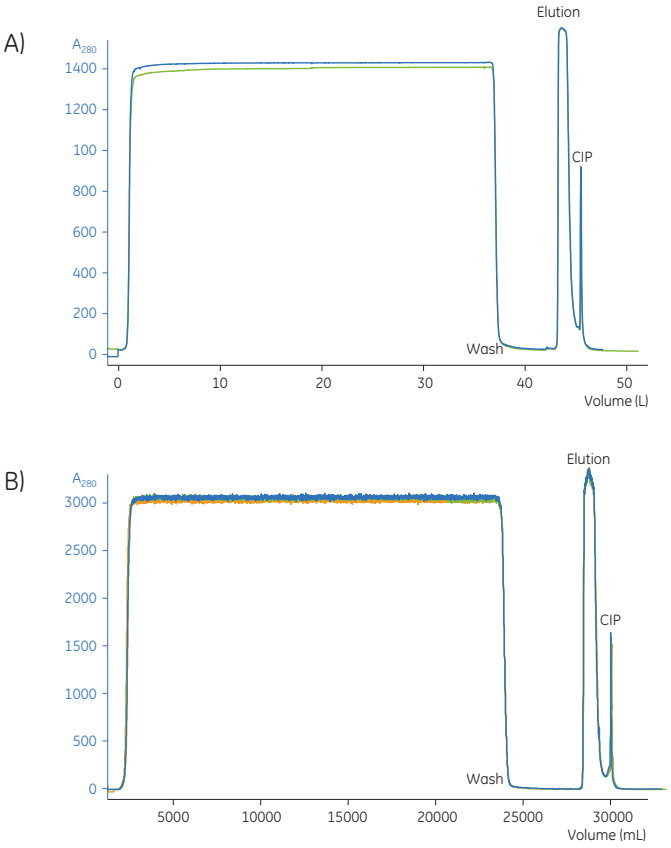

Fig 3. A). Overlaid chromatograms from the first two cycles of the MabSelect SuRe step with ReadyToProcess columns. The load volume was identical in the runs. A_{280} trace is shown in blue (cycle 1) and green (cycle 2); **B).** Overlaid chromatograms from the first three cycles of the MabSelect SuRe step with AxiChrom columns. The load volume was identical in the runs. A_{280} trace is shown in blue (cycle 1), green (cycle 2), and red (cycle 3). The difference in absorbance is due to the difference in path length between the two systems.

Table 6. Comparative evaluations of scaled-up processes with single-use ReadyToProcess columns and traditional AxiChrom columns. The yield is expressed as monomer yield for the Capto adhere step

Process step	Yield (%)		Aggregate content (%)		HCP (ppm)		Protein A (ppm)	
	Single-use	Traditional	Single-use	Traditional	Single-use	Traditional	Single-use	Traditional
Fermentation			10	12	37 500	34500	Not done	Not done
Harvest	100	100	10	12	37 500	34500	Not done	Not done
Capture, MabSelect SuRe	96.0*	96.2*	10	12	19	24	8.8	1.9
UF/DF1	97.7	97.8	10	12	12	25	9.1	1.9
Polishing, Capto adhere	89.0	86.0	0.4	0.6	<LOQ**	<LOQ**	<LOQ**	<LOQ**
UF/DF 1 & sterile filtration	97.4	102***	0.6	0.6	1.0	1.0	0.1	<LOQ**
Overall yield	81	81						

* Average
** LOQ = level of quantification (4.6 ng/mL for HCP, 3 ng/mL for ligand)
*** unit operations with > 100% in yield were calculated as 100%

Sample: 60 g/L of diafiltrated elution pool from the MabSelect SuRe step
Binding buffer: 50 mM sodium phosphate, 50 mM NaCl, pH 7.0
Elution buffer: 50 mM sodium phosphate, 250 mM NaCl, pH 6.1
Residence time: 5 min

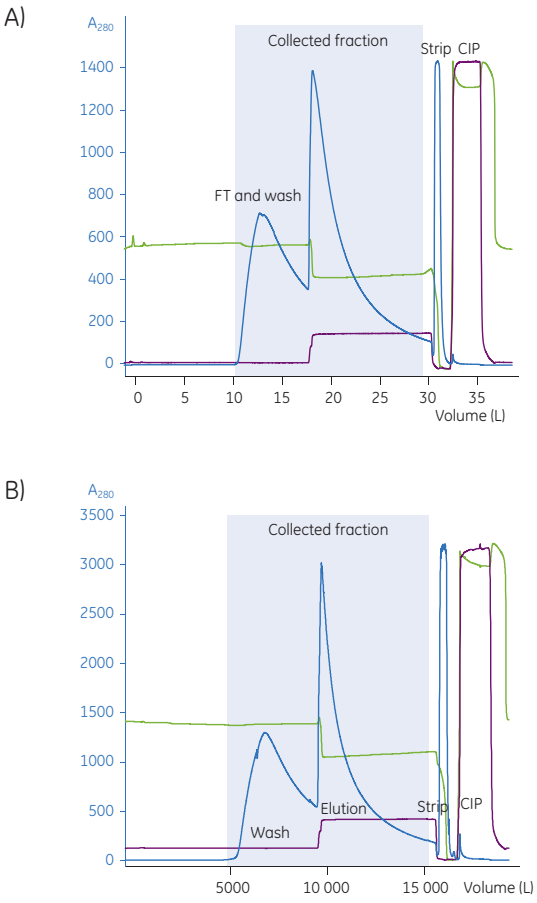

Fig 4. A) Chromatogram from the Capto adhere step with ReadyToProcess columns. A_{280} trace is shown in blue, pH in green and conductivity in lilac; **B)** Chromatogram from the Capto adhere step with AxiChrom columns. A_{280} trace is shown in blue, pH in green, and conductivity in lilac. The difference in absorbance is due to the difference in path length between the two systems.

The flux curve/behavior is dependent on product concentration as noted in the cross flow filtration steps (Figures 5 and 6). Filtration time using the traditional format was between 50 and 60 min, while using the ReadyToProcess format filtration took 160 min for the buffer exchange step and 220 min for the formulation step. However, for a true comparison, the times for setup, membrane rinse and equilibration, filtration, and CIP/storage need to be considered. The ReadyToProcess format only requires time for setup and filtration, so the actual process time is shorter. Comparing times for the chromatography steps showed that the process running times are the same for both approaches. However, time savings are achieved using the ReadyToProcess format due to the elimination of column packing, cleaning protocols, and cleaning validation steps.

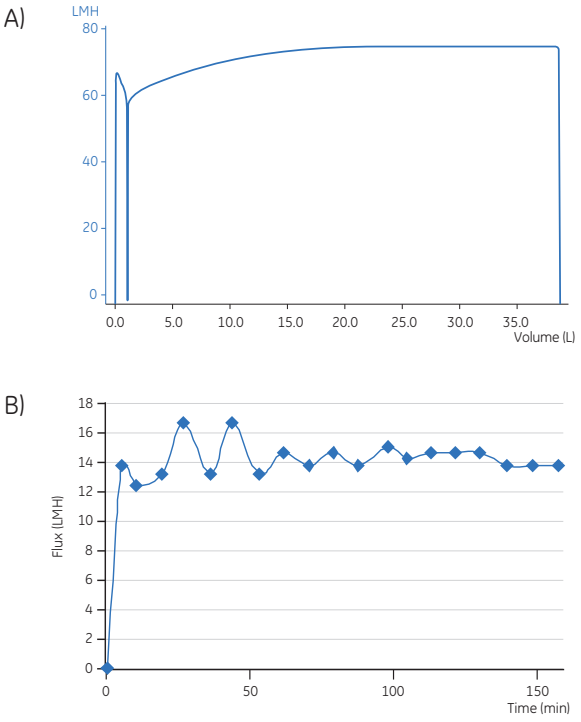

Fig 5. Flux curves from the buffer exchange step. **A)** traditional format with Kvick Lab cassettes; **B)** with ReadyToProcess.

When we examine the duration of the entire downstream process involving ReadyToProcess and AxiChrom formats (Table 7), we see that the process is 50% faster with the ReadyToProcess format.

Table 7. Comparative evaluation of process time for scaled-up processes using ReadyToProcess and AxiChrom columns

Process step	ReadyToProcess	Traditional
Harvest	2 h	2 h
Capture, MabSelect SuRe*	8 h	17 h
Buffer exchange	3.5 h	6 h
Polishing, Capto adhere**	5 h	14 h
Formulation	4.5 h	6.5 h
Total time	23 h	45.5 h

* Two cycles including preparations
** One cycle including preparations

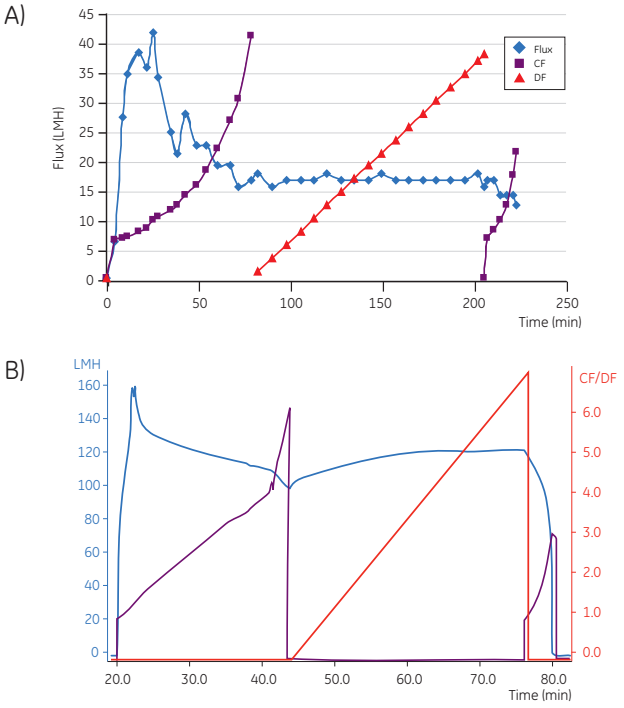

Fig 6. Flux curves from the formulation step. Flux is denoted by blue, concentration factor (CF) by lilac, and diafiltration factor (DF) by red. **A)** traditional format with Kvick Lab cassettes; **B)** with ReadyToProcess.

Conclusions

In this study we scaled up a complete monoclonal antibody purification process to 100 L cell culture volume. The scale-up was performed with ReadyToProcess equipment: WAVE Bioreactor for cell culture, ÄKTA ready system and ReadyToProcess columns for the chromatography steps, and ReadyCircuit assemblies, ReadyToProcess hollow fiber cartridges and single-use capsules for all filtration operations. The chromatography steps were run on one system and the filtration equipment was the same for both ultrafiltration steps. This gave the process a small foot print, and also showed the flexibility that the ReadyToProcess platform offers. The use of ReadyMate disposable aseptic connections, presanitized columns, a disposable flow path in the chromatography system, together with the filtration assemblies contributes to ensuring maximum product safety and to maintaining high process hygiene.

We compared this process with a traditional process originating from a stirred tank reactor and using AxiChrom columns, ÄKTApilot chromatography system and Kvick Lab cassettes. The final product from each process contained the same low amount of impurities (approximately 0.5% aggregates) and the total yield from each process was the same (81%). From a quality point of view there is no difference between the traditional process and the single-use process.

When we compared total process durations, process time for the ReadyToProcess setup was 23 hours and for the traditional setup with AxiChrom columns, 45.5 hours. This represents a reduction in process time by about 50% using the ReadyToProcess platform. The decrease in process time for the ready-to use process is due to the elimination of column packing and testing, system cleaning, cleaning validation, etc. Also, by using presterilized bags instead of traditional stainless steel vessels the ready-to-use process contributes to increased product safety.

References

1. Application note: High-throughput screening and column optimization of a monoclonal antibody capture step, GE Healthcare, 28-9468-58, Edition AA (2009).
2. Application note: Screening and optimization of aggregate reduction in a MAb polishing step, GE Healthcare, 28-9509-60, Edition AA (2009).
3. Application note: Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom columns, GE Healthcare, 28-9403-49, Edition AA (2009).

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