Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom columns

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Scale-up of a downstream monoclonal antibody purification process using HiScreen™ and AxiChrom™ columns

Key words: *Monoclonal antibody, Monte Carlo simulation, host cell protein, MabSelect SuRe™, Capto™ adhere*

Abstract

We have developed and used a highly efficient monoclonal antibody purification protocol to reduce the level of aggregates in a starting sample from 12% to 0.6% in a two-step chromatography process resulting in a 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 HiScreen prepacked columns (5 mL) to a large-scale process using AxiChrom columns (500 to 600 mL) on an ÄKTApilot™ system. The scale-up protocol was developed with a combination of a Design of Experiments (DoE) approach and Monte Carlo simulation. The results from the DoE studies were used as input for the simulation studies in order to verify the robustness of the scale-up process. The amount of host cell protein (HCP) was reduced from 37 000 to 1.3 ppm and ligand leakage was also reduced from 1.8 ppm to negligible amounts. The filtration steps were also optimized by determining robust parameters that consistently produced high yields.

Introduction

The ability to transfer optimized scouting results from a small-scale study to a pilot and full process scale is a key component of any successful process development project. The inherent challenges lie in the risk that the optimized parameters and results—such as improved yield and purity—may not transfer from small scale to pilot or full-scale production in a predictable manner. To overcome these obstacles and ensure a more predictable and robust outcome in the process development workflow, we have developed a process development workflow focused on maintaining the integrity of optimized parameters (e.g., yield and purity) from small- to pilot-scale studies for the production of biopharmaceuticals.

This is the third application note in a set of four with the overall theme of providing you with efficient tools and methods to enhance the identification and production of efficacious monoclonal antibodies—from the 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)
- A flexible antibody purification process based on ReadyToProcess™ products (28-9403-48)

The main challenge from the monoclonal antibody purification process described here was the high incidence of aggregation (12%) in the starting sample. This antibody feed stream was successfully scaled up more than ten times while maintaining preset criteria for purity and yield from a two-step chromatography process based on MabSelect SuRe and Capto adhere media. The optimal process conditions worked out from small-scale studies were further improved

and tested for robustness using a workflow comprising DoE and Monte Carlo simulation *in silico*. The DoE studies performed at small scale using HiScreen columns generated sweet spot analyses for the capture and polishing steps where the predefined criteria regarding yield and purity where met. The results from the DoE studies then served as input for Monte Carlo simulations to test the robustness of the optimal conditions obtained from the two chromatographic steps. The workflow (Fig 1) allowed for a rapid screening of both chromatographic conditions and process robustness prior to scale-up.

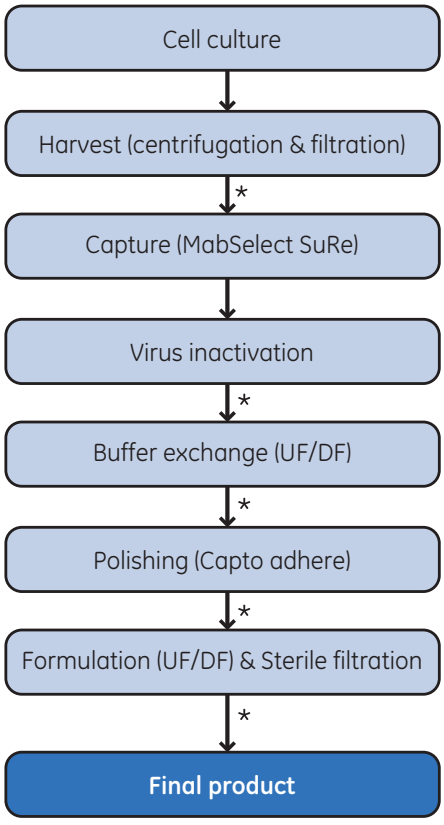

Fig 1. Flow scheme of the purification process in which steps involving in-process filtration of the sample to reduce bioburden are indicated with asterisks (*).

Materials and methods

Screening and optimization of the capture and polishing steps with PreDictor™ prepacked 96-well filter plates were performed as described in the previous application notes (1, 2).

Cell culture

CHO cells expressing the target IgG were cultured in a 120 L stirred tank bioreactor with a working volume of 100 L. The culture parameters were: 25 rpm (double impeller), temperature 36.8°C, pH 7.1 to 6.7, and dissolved oxygen (DO) 50% ± 5%. The pH was controlled during cell culture with CO_2 and sodium bicarbonate. Dissolved oxygen was controlled with air plus O_2. The culture was fed with hydrolysate, glucose, glutamine, and other selected cell culture supplements. Culture duration was 20 d with a peak cell density of 4.5×10^6 viable cells/mL and a final viability of 28%.

Cell harvest

The cells were harvested by centrifugation at 4000 × g for 20 min at room temperature. After centrifugation, the supernatant was filtered through an ULTA™ Prime GF (0.6 µm, 10 in capsule) followed by ULTA Pure HC (0.6/0.2 µm, 6 in capsule). The filtrate was stored in sterile containers until further processing.

Normal flow filtration (NFF)

The material from each stage of the process was filtered through a sterile filter (ULTA Pure HC) for product stability and bioburden control. Filtrates were stored in sterile containers until further processing.

To map the NFF performance throughout the process, a sample of each feed stream was used to challenge 47 mm discs of ULTA Pure HC in a constant pressure capacity test run at 10 psi. Membrane capacity was determined by fitting the filter data to a combined pore plugging model (3) and the required capacity to filter the process feed stream in 30 min was calculated.

Columns and packing

AxiChrom 70/300 columns were packed automatically with an ÄKTApilot system to give a bed height of 20.5 cm and 14.1 cm for the MabSelect SuRe and Capto adhere columns, respectively. The packed columns were tested for HETP and asymmetry in 0.4 M NaCl with 0.8 M NaCl as the test substance (Table 1).

Table 1. Packing parameters for MabSelect SuRe and Capto adhere in AxiChrom 70/300 columns

Chromatography media	Bed height (cm)	Volume (mL)	HETP (N/m)	Asymmetry
MabSelect SuRe	20.5	789	8601	1.12
Capto adhere	14.1	543	7711	1.19

Chromatographic methods and buffers

The capture step was performed with MabSelect SuRe at an approximate load of 31 g/L and a residence time of 4 min. The MabSelect SuRe steps and buffers are described in Table 2.

The Capto adhere process steps and buffers are described in Table 3. The load was approximately 60 g/L with a residence time of 5 min. The loading sample concentration was 5 g/L.

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

Virus inactivation

Virus inactivation was performed by incubating the elution pool from the capture step at a pH of 3.8 for 40 min. The pool was then neutralized by the addition of 0.5 M Na_2HPO_4 until a pH of 7.0 was reached.

Table 2. Process description of the MabSelect Sure capture step

Step	Duration	Buffer	Comment
Equilibration	1 column volume (CV)	20 mM sodium phosphate, pH 7.2	
Load	23 L	N/A	Residence time = 4 min. Load = 31 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 3.7	Typically, elution is finished in 1.5 to 2.0 CV's
CIP	3 CV	500 mM NaOH	Residence time = 15 min
Re-equilibration	5 CV		Until stable pH is reached

Table 3. Process description of the Capto adhere polishing step

Step	Duration	Buffer	Comment
Equilibration	1 CV	50 mM sodium phosphate, 50 mM NaCl, pH 7.0	
Load	6.5 L	N/A	Residence time = 5 min. Load = 60 g/L. Concentration = 5 g/L.
Wash	5 CV	50 mM sodium phosphate, 50 mM NaCl, pH 7.0	Wash to elute product
Elution	20 CV	50 mM sodium phosphate, 250 mM NaCl, pH 6.1	Step to specifically elute bound monomer
Strip	3 CV	100 mM sodium phosphate, pH 3.0	Remaining proteins are eluted from the media
CIP	3 CV	1 M NaOH	Residence time = 15 min
Re-equilibration	5 CV		Until stable pH is reached

Optimization of cross flow filtration unit operations

The cross flow filtration units (UF/DF) were optimized (4) using Kvick™ Lab Packet devices (membrane area of 0.01 m^2) on an ÄKTAcrossflow™ system. A 30 kDa nominal molecular weight cutoff (NMWCO) membrane pore size was chosen to maximize process flux and prevent product loss.

Cross flow filtration—buffer exchange after the capture step (UF/DF 1)

Concentration/diafiltration was performed using three Kvick lab 30 kDa NMWCO devices (total membrane area of 0.33 m^2) and a Uniflux™ 10 cross flow filtration system. Feed pressure drop was controlled to 1.6 bar (23 psi) and the retentate valve was set to fully open. The sample was concentrated 1.2-fold and then continuously diafiltered with 6.5 volumes of equilibration buffer for the Capto adhere unit operation (50 mM phosphate, 50 mM NaCl, pH 7.0). The system was drained and flushed two times with 800 mL of diafiltration buffer to maximize product recovery.

Cross flow filtration—formulation after the polishing step (UF/DF 2)

Concentration/diafiltration was performed using three Kvick lab 30 kDa NMWCO devices (total membrane area of 0.33 m^2) and Uniflux 10 cross flow filtration system. Feed pressure drop was controlled to 1.9 bar (27 psi) and the retentate valve was set to fully open. The sample was concentrated 6-fold, continuously diafiltered with 7 volumes of formulation buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0) and then concentrated further by an additional 3-fold. The system was drained and flushed twice with 250 mL of diafiltration buffer to maximize product recovery.

Analytical methods

Total IgG concentration was measured by analytical protein A chromatography. Briefly, 50 µL of the sample was adsorbed onto a 1 mL HiTrap™ MabSelect SuRe column, washed, and 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. Ten microliters (10 µL) of each sample was applied to the columns.

HCP levels were measured using commercial anti-CHO HCP antibodies (Cygnus Technologies). Essentially, an ELISA method was adapted to a Gyrolab™ Workstation LIF (Gyros AB) using Gyrolab Bioaffy 200 HC microlaboratory discs. Ligand leakage measurements were performed using a commercial ELISA kit (Repligen Corporation) with a slight modification of the manufacturer's protocol.

Monte Carlo simulations

Data obtained from the factorial designs was interpreted with MODDE™ software v8 (Umetrics). The models were then pasted into Crystal Ball™ (Oracle Corporation) software and different levels of robustness were evaluated by running 10 000 iterations. The probability of values for the yield and purity falling within certain predefined parameters were assessed.

Results and discussion

Cell culture and harvest

A CHO cell line expressing monoclonal IgG was cultured in a steel reactor at a volume of 100 L. The expression level after 20 d was 1.06 mg/mL. Cell harvest was performed at approximately 28% cell viability by centrifugation followed by filtration through ULTA Prime GF, 0.6 µm (pre-filter), and ULTA Pure HC (sterilizing-grade filter). The harvest produced 95 g of IgG.

Capture step with MabSelect SuRe

A factorial design (1) was used to optimize the MabSelect SuRe step and the green zones in the sweet spot plot (Fig 2) represent the areas in which all the predefined criteria were met. For the capture step, the set criteria were a yield of > 90%, an aggregate content of < 15%, and a HCP content of < 70 ppm. According to the plot, the criteria were met with the use of a pH of 3.65 to 3.90 and a load of 17 to 34 g/L.

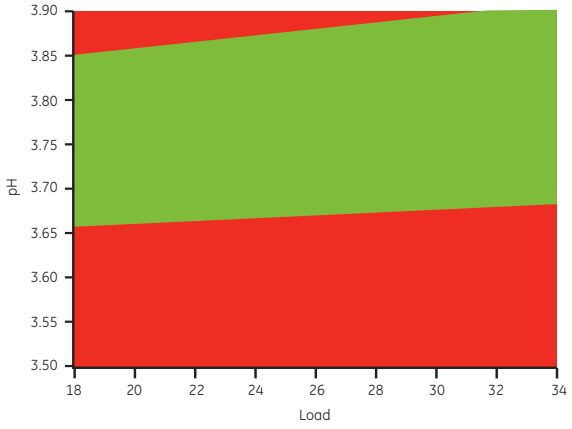

Fig 2. Sweet spot plot of the capture step with the following predefined criteria: yield > 90%, aggregate content < 15%, and HCP content < 70 ppm. Green = all criteria met, red = two criteria met. Load is expressed in g/L.

Monte Carlo simulations were set up with the optimization models obtained from the factorial designs. These simulations allow you to make *in silico* experiments with parameters that vary within defined intervals in a randomized way to imitate natural variations. Monte Carlo simulations also take the standard deviations within the analytical methods into account thus making it a powerful tool for (i) finding robust parametric ranges for each process step; and (ii) predict the outcome of the process.

The most important factor in the capture step was judged to be monomer yield so we set up a Monte Carlo simulation to investigate monomer yield with the following initial ranges:

- Load: 24 to 27.5 g/L
- NaCl concentration in the wash: 430 to 470 mM
- Elution flow rate: 140 to 160 cm/h
- Elution pH: 3.6 to 3.8

The simulation predicted a yield of 92.4 to 95.6% (Fig 3). These results were then optimized *in silico* by increasing the load to a range of 25.7 to 30.0 g/L and decreasing the pH range to 3.5 to 3.7. A new simulation with these new values produced a yield improvement within a narrower range of 94.7% of 96.0%, thereby improving the robustness of the process step.

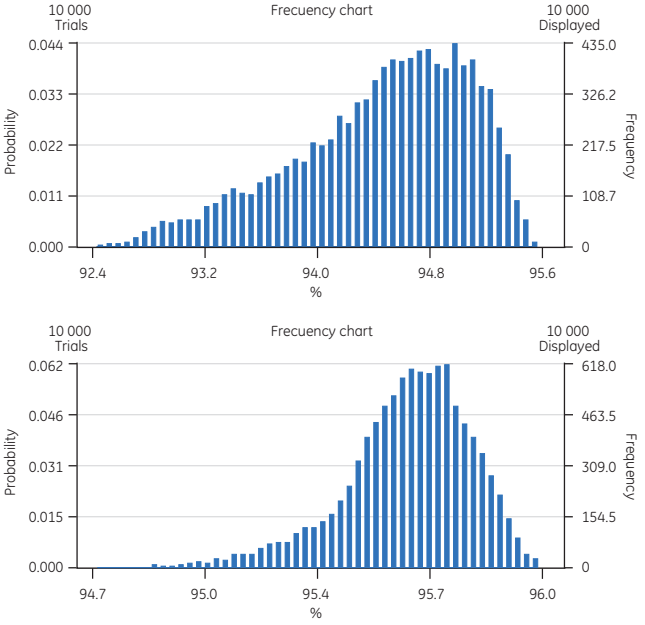

Fig 3. Monte Carlo simulation of the response monomer yield for the capture step before (top) and after (bottom) *in silico* optimization.

The average yield of the scaled process runs was 96.2% and the incidence of protein aggregates in the sample was 12%. The HCP content was reduced by a factor of approximately 1500—from 36 000 to 24. Overlaid chromatograms from the first three cycles, where the load was identical, are shown in Fig 4.

A virus inactivation procedure was performed—immediately after each capture step—by lowering the pH to 3.8 followed by incubation for 40 min at room temperature. The pH was then adjusted to 7.0 by the addition of phosphate buffer and the mixture was filtered.

Column:	AxiChrom 70/300 (20.5 cm bed height)
Sample:	31 g/L of IgG solution after harvest and NFF
Wash buffer 1:	35 mM sodium phosphate, 500 mM NaCl, pH 7.2
Wash buffer 1:	20 mM sodium phosphate, pH 7.2
Elution buffer:	20 mM sodium citrate, pH 3.7
Flow rate:	198 ml/min
System:	ÄKTApilot

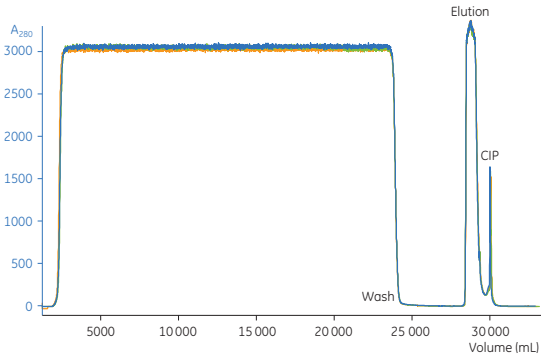

Fig 4. Overlaid chromatograms from the first three cycles of the MabSelect SuRe step. The volume of the load was identical in all three runs.

Buffer exchange after the capture step

The 4.5 L eluate from the MabSelect SuRe step was concentrated to a working volume of 4.0 L followed by continuous diafiltration (6.5 times). A high and stable flux with an average of 73 Liter/m^2/h (LMH) was observed throughout the process (Fig 5).

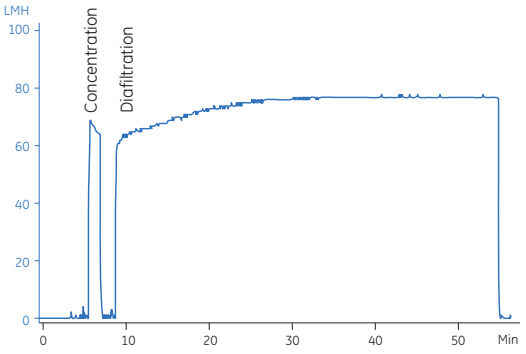

Fig 5. Flux curve from the buffer exchange. After the short concentration time, the inlet was transferred to the diafiltration buffer tank and subjected to diafiltration with 6.5 volumes.

This produced a recovery of 97.8% within 50 min (Table 4). The sample was diluted to a concentration of 5g/L with equilibration/wash buffer for the subsequent Capto adhere step.

Table 4. Process data for the buffer exchange

Unit operation	Buffer exchange
Start volume (L)	4.5
Final volume(L)	5.6
Diafiltration volume (times)	6.5
Start concentration (mg/mL)	20.1
Final concentration (mg/mL)	15.9
Load (g/m^2)/(L/m^2)	275/13.6
Filtration time (min)	50

Polishing with Capto adhere

Optimization of the polishing step was performed as previously described (2). A sweet spot plot was set up with the following criteria: a monomer purity of > 99%; a yield of > 85%; and negligible leves of HCP, and ligand leakage. The sweet spot plot (Fig 6) shows a large operating space in which the predefined criteria were met; hence, suggesting that it was feasible to achieve the desired purity and yield at a starting aggregate concentration of 9% to 12.5%.

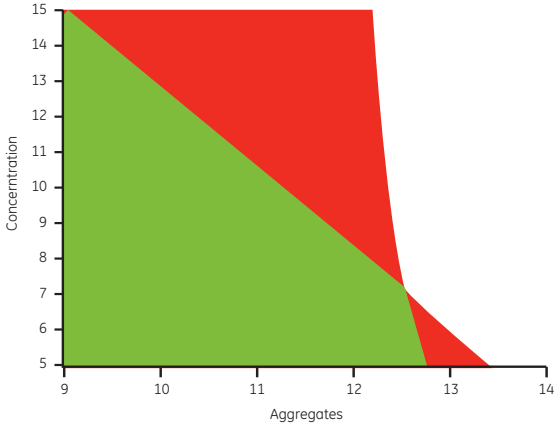

Fig 6. Sweet spot plot of the polishing step with a criteria of yield > 85%; monomer purity > 99% (equivalent to an aggregate content of < 1%). The region within which all the criteria were met is represented in green, red represents a region in which only two of the criteria were met and white represents a region in which only one criterion was met. The other criteria were a load of 60 g/L, an elution pH of 6.1, and an elution NaCl concentration of 300 mM.

Monte Carlo simulations were performed for the polishing step with the following intervals:

- Aggregates: 9% to 12% (uniform distribution)
- IgG start concentration: 4.5 to 5.5 g/L (triangular distribution)
- IgG load: 55 to 65 g/L (triangular distribution)
- Elution pH: 6.0 to 6.2 (triangular distribution)
- NaCl concentration for elution: 230 to 270 mM (triangular distribution)

The Monte Carlo simulation results were a purity of 99.2% to 100.1% (equivalent to an aggregate content of 0.0% to 0.8%) and a monomer yield of 82.5 to 86.7% (Fig 7). This shows that yield was the most important response because the purity was > 99.0% under all the conditions tested.

At this point, parameters such as the load can be increased to produce a corresponding increase in yield but at a cost of a slight decrease in purity. In this particular simulation, increasing the load to an interval of 60 to 70 g/L led to an increase in the yield by approximately 1% (Fig 8). The yield of monomer purity decreased to a range of 98.9% to 99.9%. The probability of achieving a purity of 99.0% or higher was 99.98%; thereby, suggesting that the new range for the load was feasible with regards to the targets for purity and yield.

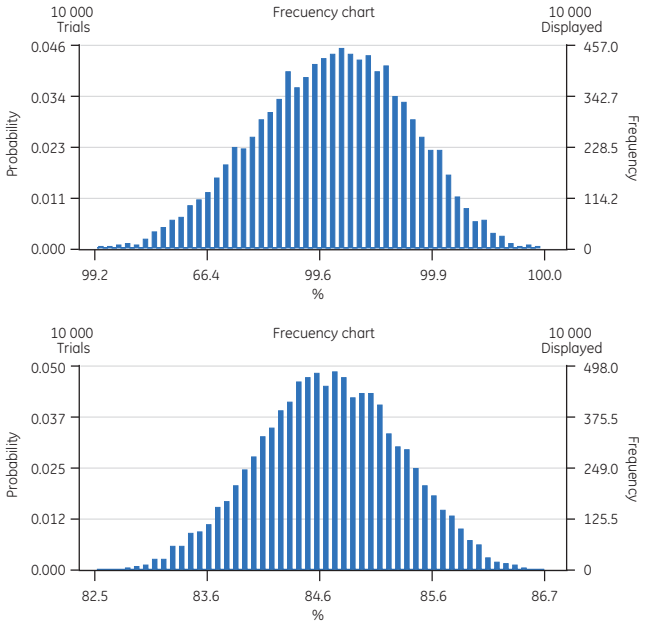

Fig 7. Monte Carlo simulation of the response monomer purity (top) and monomer yield (bottom) for the Capto adhere step before *in silico* optimization.

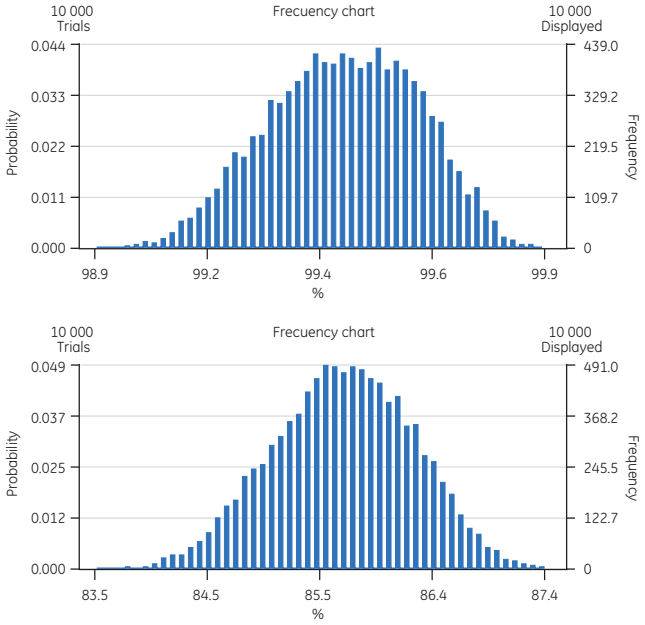

Fig 8. Monte Carlo simulation of the response monomer purity (top) and monomer yield (bottom) for the polishing step after *in silico* optimization.

A representative chromatogram from the Capto adhere step (second cycle) is presented in Fig 9.

In the scaled up process, the starting aggregate concentration of 12% was reduced to 0.6% in a single step (Fig 10). The monomer yield of 86% was relatively high for a sample containing such a high level of aggregates.

Column: AxiChrom 70/300 (14.1 cm bed height)
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
Flow rate: 109 ml/min
System: ÄKTApilot

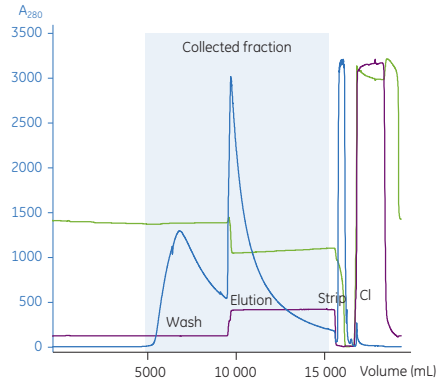

Fig 9. Chromatogram from the Capto adhere step (2nd cycle). A_{280} trace is shown in blue, pH in green, and conductivity in brown.

Column: Two Superdex 200 5/150 GL connected in series
Sample: 10 µl of IgG
Mobile phase buffer: PBS, pH 7.4
Flow rate: 0.35 mL/min
System: ÄKTApilot

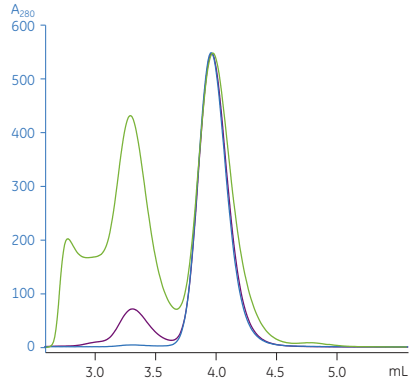

Fig 10. SEC analysis of the monoclonal antibody sample in the Capto adhere step: sample before purification (purple), purified fraction (blue) and strip fraction (green). The curves were normalized with respect to the monomer peak of the purified fraction.

Formulation

The process performance of the formulation step is shown in Figure 11. The diafiltration process was performed at a 6-fold concentration followed by a continuous diafiltration procedure with 7 volumes of the formulation buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0), followed by additional concentration to 9-fold after the addition of flush volumes to the recovered pool. The flux was relatively high (i.e., > 100 LMH) throughout the process.

The recovered pool produced a yield of 99.8% (including sterile filtration of the pool) and there was no significant increase in the proportion of aggregate species. The highly efficient formulation process led to a reduction in the filtration time to less than an hour (Table 5).

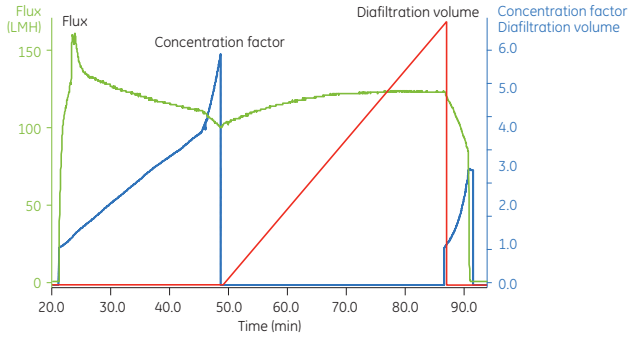

Fig 11. Schematic representation of process performance (during formulation) showing the changes in flux vis-à-vis the concentration factor (CF) and diafiltration volume. The Capto adhere eluate was concentrated 6-fold, diafiltered 7 times, and then concentrated a further 3-fold.

Table 5. Data from the formulation run

Unit operation	Formulation
Start volume (L)	18.3
Final pool volume(L)	2.0
Diafiltration volume (times)	7.0
Start concentration (mg/mL)	2.4
Final concentration (mg/mL)	22.9
Load (g/m^2)/(L/m^2)	133/55
Filtration time (min)	60

Normal flow filtration (NFF)

The small-scale capacity experiments for all the NFF steps and a brief overview of how all the NFF steps can be applied in a biopharmaceutical production process are shown in Figure 12. The process involved volumetric throughput (normalized to the membrane area) for a filtration time of 30 min. Protein concentration and foulant load are among the factors that restrict the capacity of the membrane. At a constant product concentration, the membrane capacity increases throughout the process because the material is being purified away from impurities that can foul the NFF membrane. Conversely, a higher product concentration produces a lower throughput because of the resultant decrease in product flux. The eluate from the Capto adhere column had a low protein concentration but a high membrane capacity.

The purification process involved just two chromatography steps (Table 6) using MabSelect SuRe and Capto adhere media.

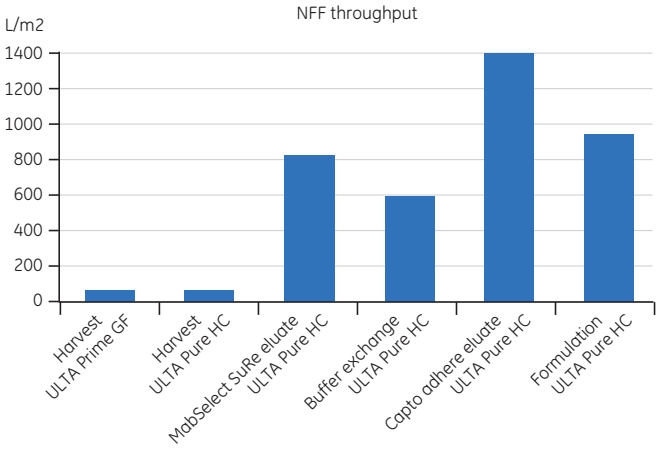

Fig 12. Membrane throughput expressed as volumetric load per membrane area for a filtration step in 30 min. The harvest process produced low membrane capacity because of high particle and impurity levels. The subsequent filtrations steps were limited by product concentration rather than impurities.

Table 6. Summary of monomer yield, aggregate content, HCP reduction, and ligand leakage in the scale up process

Process step	HCP (ppm)	Ligand (ppm)	Aggregate content (%)	Yield (%)
Fermentation	37 000	Not applicable (N/A)	12	
Harvest	37 000	N/A	12	100
MabSelect SuRe (4 cycles)	24*	1.9	12*	96.2*
Buffer exchange	25	1.9	12	97.8
Capto adhere	< LOQ†	< LOQ†	0.6‡	86.0‡
Formulation and sterile filtration	1.0	< LOQ†	0.6	102
Total yield:	80.8			

* Average of 4 cycles
† LOQ = level of quantitiation (4.6 ng/mL for HCP, 3 ng/mL for ligand)
‡ Average of 2 cycles

Table 7. Comparative analysis of the results from the scaled up process, Monte Carlo simulations, and the typical results from reference runs during the optimization processes (1, 2). The percentage yield (Table 7A) and purity (Table 7B) for each step in the process is presented below

A)

Chromatography step (yield %)	MabSelect SuRe	Capto adhere	Overall two-step process
Optimization by DoE	> 95	87.0	
Monte Carlo simulation	95.1 - 96.0	84.0 - 87.2	
Scaled up process	96.2	86.0	82.7

B)

Chromatography step (purity %)	MabSelect SuRe	Capto adhere	Overall two-step process
Optimization by DoE	10 - 15	0.5	
Monte Carlo simulation	9.0 - 14.0	0.1 - 1.0	
Scaled up process	12	0.6	0.6

The total yield of monomeric species—after chromatography and filtration—was 81%. The scaled up results were similar to those obtained in the optimizations runs (Table 7) and Monte Carlo simulations thus attesting to the synergistic benefits of applying such powerful techniques to predict the outcome of the scaled up process.

Conclusions

We have developed and successfully scaled up a monoclonal antibody purification process from a screening phase with PreDictor plates, 6 µl through small scale (4.7 mL HiScreen prepacked) columns to a pilot scale (AxiChrom columns of 790 mL and 543 mL). Despite the high incidence of aggregation (12%) in the starting monoclonal antibody sample, we developed and applied a highly efficient purification scheme that reduced the aggregation content to 0.6% in a single step.

MabSelect SuRe has a high IgG binding capacity, high HCP, and DNA clearance as well as low ligand leakage. Capto adhere allows for the reduction of large amounts of aggregates together with HCP, DNA, ligand leakage, and viruses (5). The combination of PreDictor plates for the screening phase and HiScreen prepacked columns for the optimization phase enabled us to screen a vast experimental space and this provided reliable knowledge of the effect of process parameters; hence, the subsequent development of an efficient scale up protocol.

The use of a two-step chromatography method instead of the traditional three-step process required a buffer exchange procedure prior to loading the sample onto the Capto adhere column. Nevertheless, the two-step protocol described in this study is still more efficient and economical than the traditional three-step purification process. A rapid and efficient cross flow diafiltration step was introduced using Kvick cassettes. In addition, the formulation step was optimized to reduce both the duration of the process and buffer volumes and this resulted in a simple and fast method.

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Ordering information

Product	Quantity	Code no.
PreDictor MabSelect SuRe, 6 µl	4 × 96-well filter plates	28-9258-23
PreDictor MabSelect SuRe, 20 µl	4 × 96-well filter plates	28-9258-24
PreDictor Capto adhere, 6 µl	4 x 96 well filter plates	28-9258-17
PreDictor Capto adhere, 20 µl	4 x 96 well filter plates	28-9258-18
HiTrap Capto adhere	5 × 1 ml	28-4058-44
HiScreen Capto adhere	1 × 4.7 ml	28-9269-81
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-93
HiScreen MabSelect SuRe	1 × 4.7 ml	28-9269-77

Related literature

Data file: MabSelect SuRe	11-0011-65
Data file: Capto adhere	28-9078-88

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/bioprocess

imagination at work

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-7911 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073
Japan

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