Multidimensional scale-up of a monoclonal antibody capture step, using ÄKTA pilot 600

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Fast and efficient process development and scale-up contributes to a shortened time to market. In this work, a multidimensional scale-up (change of both column diameter and bed height) of a mAb capture step, using the ÄKTA pilot 600 chromatography system, is demonstrated. Column volume per hour (CV/h) was utilized to enable maintaining residence time throughout the scaling process. The process, performed with MabSelect™ PrismA Protein A chromatography resin, was scaled up 20-fold from a 70 mL HiScale™ 26 column to a 1400 mL AxiChrom™ 100 column. Equivalent mAb recovery and purity was achieved between the larger and the smaller scales, showing a robust and scalable process.

Introduction

With the ever-increasing demand for a short time to market, solutions for fast and efficient process development and scaling are gaining interest in the bioprocessing industry. Due to the convenience, time, and cost; the development of a bioprocess is preferably conducted in small scale. The subsequent scale-up is thereafter performed in one or more steps, usually 20- to 30-fold per each step, depending on the final production scale. Equipment that can be adapted to the different stages of the development process facilitates this work. Although process economy is an important parameter; equipment providing simplified bioprocessing, a high level of automation, and compliance with GMP is required to ensure quality of the bioproduct.

ÄKTA pilot 600 is a bench-top chromatography system suited for both GMP and non-GMP applications (Fig 1). Its wide pressure-flow range enables processing of technical batches, as well as small- and intermediate-scale production batches. The modular system design allows flexible exchange of functionalities depending on the requirements. This work demonstrates scaling and modification of a mAb capture step from the smaller HiScale 26 column operated on ÄKTA avant 150 to a larger AxiChrom 100 column operated on ÄKTA pilot 600. Furthermore, the scaled-up process was reconfigured and automated to fit a regulated environment.

Fig 1. ÄKTA pilot 600 bench-top chromatography system can be used in both GMP ("regulatory" version) and non-GMP ("standard" version) environments.

Materials and methods

Feed preparation

CHO cell culture supernatant containing mAb at a concentration of approximately 3 mg/mL was filtrated through a 0.2 µm filter before loaded onto the chromatography column. Sample load was adjusted to 60 mg mAb/mL resin for both column sizes.

Column preparation

The HiScale 26 column was packed with resin to a bed height of 13 cm (70 mL) using ÄKTA avant 150, whereas the AxiChrom 100 column was packed to a bed height of 18 cm (1400 mL) in an automated manner using ÄKTA pilot 600. Results from column performance testing are summarized in Table 1.

Table 1. Column performance test

Column	HETP (cm)	A_s	Plates/ meter	Plate height
AxiChrom 100	0.01334	1.25	7495	2.22
HiScale 26	0.01261	1.36	7931	2.10

HETP = height equivalent to a theoretical plate, A_s = asymmetry factor.

Capture step

The mAb capture step was performed with MabSelect PrismA resin. Both ÄKTA avant 150 and ÄKTA pilot 600 were installed with the UNICORN™ 7.3 system control software, and the created method is outlined in Table 2.

Scale-up procedure

The flow unit CV/h was used to enable a multidimensional scale-up, that is, change of both column diameter and bed height, while keeping retention time during the scale-up process. During development, ÄKTA pilot 600 was equipped with six A inlets, six B inlets, nine outlets, and a mixer.

First, the method developed in small scale on the HiScale 26 column was transferred from ÄKTA avant 150 to ÄKTA pilot 600. Secondly, the method was converted from the small-scale HiScale 26 column to the larger scale AxiChrom 100 column on ÄKTA pilot 600. Finally, the method was adjusted to fit the AxiChrom 100 column, and the system and method were reconfigured to fit a regulated environment. For GMP production, the system was equipped with nine inlets, three outlets, and no mixer, and the eluate was collected in a sterile plastic bag. To allow automating the process, the method was modified so that the feed was loaded via the air-trap, having external air-sensors on the inlet. Cleaning-in-place (CIP) was performed automatically after end of method by using an automated CIP procedure executed using the ***Method Queue*** function of the software.

Analyses

Protein concentration was measured by surface plasmon resonance (SPR) using the Biacore™ T100 system. Aggregate clearance was determined by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column. Peaks were integrated and percentage of aggregates were determined.

Host cell protein (HCP) content was analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies Inc.) and Gyrolab™ workstation (Gyros AB). Protein A content was determined using a commercially available ELISA kit (Repligen Corp.).

Table 2. Method used for mAb capture on MabSelect PrismA

Step	Volume (CV)	Buffer	Flow rate (CV/h)
Equilibration	3	20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	17.5
Load	~ 19	N/A	10
Wash 1a	1.5	20 mM sodium phosphate, pH 7 + 500 mM NaCl	10
Wash 1b	3.5	20 mM sodium phosphate, pH 7 + 500 mM NaCl	17.5
Wash 2	4	50 mM acetate, pH 6	17.5
Elution	5	50 mM acetate, pH 3.5	5
Strip	3	100 mM acetic acid, pH 2.9	17.5
CIP	4	0.5 M NaOH	12
Re-equilibration	5	20 mM sodium phosphate, pH 7.4 + 150 mM NaCl	17.5

Result

Method transfer from ÄKTA avant 150 to ÄKTA pilot 600

The process was transferred to ÄKTA pilot 600 by creating a new method using the same volumetric flow and volumes for each process stage as used in the smaller scale on ÄKTA avant 150. The wide flow rate range of ÄKTA pilot 600 allowed testing the process in the HiScale 26 column to detect possible need for method modifications without wasting a large amount of sample. An overlay of chromatograms from the process run on ÄKTA avant 150 as well as on ÄKTA pilot 600 is shown in Figure 2. The system hold-up volume (in CV) can be seen as the delay from the start of the sample load until the UV signal starts to increase. The difference at the end of the flowthrough is due to that the feed in the run performed on ÄKTA avant 150 was manually chased with equilibration buffer (i.e., added to the sample feed at the end of loading to ensure all sample was loaded).

Scale-up on ÄKTA pilot 600

When scaling using the flow rate unit CV/h, the method can easily be changed in ***Method settings*** of the software by changing column type, and the flow rate will be automatically adjusted for the new column volume. An overlay of chromatograms from the process run on HiScale 26 as well as on AxiChrom 100 using ÄKTA pilot 600 is shown in Figure 3. The observed difference in the flowthrough is due to the use of different feed concentrations and that the sample in the larger scale was manually chased with equilibration buffer.

Modifications to a regulated environment

In a regulated environment, it is necessary to have cleaning procedures in place for both the system and for system components. To simplify operations, it is beneficial if the system can be configured with only the components needed for the process (e.g., number of inlets and outlets, and with or without mixer). ÄKTA pilot 600 can be configured for use in process development (“standard” version) as well as in small-scale GMP production (“regulatory“ version), where its high level of automation ensures a robust production processes.

Once the scaled-up method was established in ÄKTA pilot 600, the method was modified to fit GMP production. An overlay of chromatograms from the process run on ÄKTA pilot 600, configured for a non-regulated as well as a regulated environment, is shown in Figure 4. The observed difference in the flowthrough is due to the difference in feed concentration between the runs.

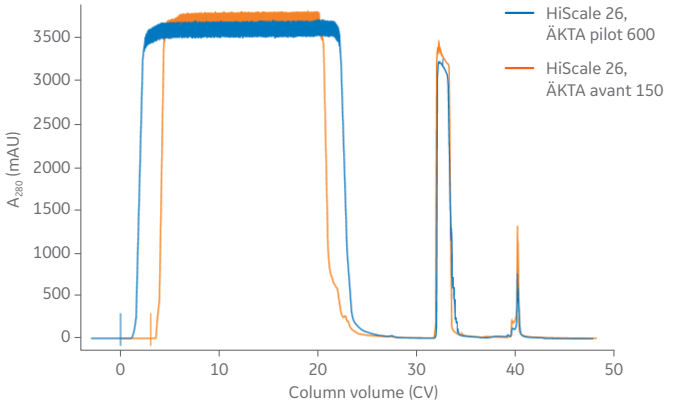

Fig 2. Overlay of chromatograms from mAb capture on the HiScale 26 column using ÄKTA avant 150 and ÄKTA pilot 600. The two curves are aligned from the start of elution. Feed concentrations were 3.5 mg/mL for the ÄKTA avant 150 process and 2.7 mg/mL for the ÄKTA pilot 600 process.

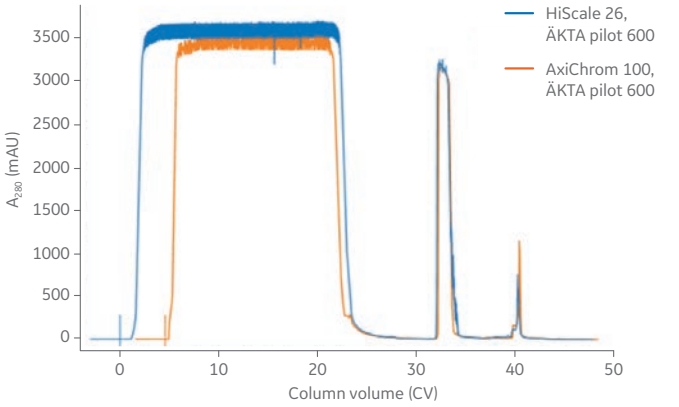

Fig 3. Overlay of chromatograms from mAb capture on the HiScale 26 and AxiChrom 100 columns, both operated on ÄKTA pilot 600. Feed concentrations were 2.7 mg/mL for the HiScale 26 column and 3.0 mg/mL for the AxiChrom 100 column.

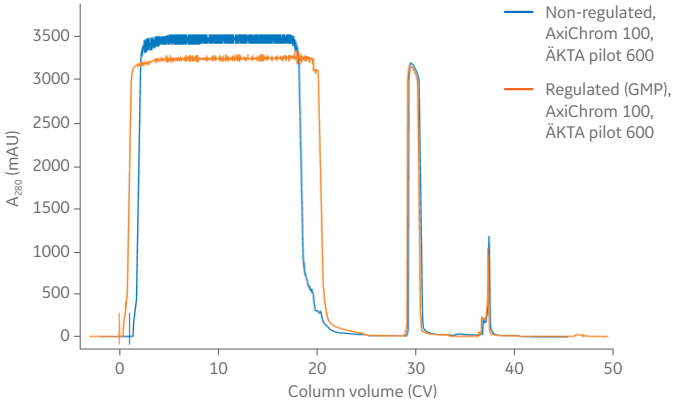

Fig 4. Overlay of chromatograms from mAb capture on AxiChrom 100 using ÄKTA pilot 600 in a non-regulated (non-GMP) and regulated (GMP) environment. Feed concentrations were 3.5 mg/mL for the non-regulated process and 3.0 mg/mL for the regulated process.

Table 3. Recovery and impurity removal on the HiScale 26 column using ÄKTA avant 150 and on the AxiChrom 100 column using ÄKTA pilot 600

Column	Column volume (mL)	mAb load (g)	Recovery by UV, A_{280} (%)	Recovery by SPR (%)	Aggregates (%)	HCP (ppm)	Protein A (ppm)
Feed						173679-184428	
HiScale 1	70	4.2	95.0	96.8	1.14	256	17
HiScale 2	70	4.2	103.0	98.7	1.48	295	12
AxiChrom 1	1400	82	95.8	105.9	1.02	300	13
AxiChrom 2	1400	82	104.4	97.4	1.03	292	8

Analytical results

An overlay of chromatograms from mAb capture on the HiScale 26 column using ÄKTA avant 150 and on the AxiChrom 100 column using ÄKTA pilot 600 is shown in Figure 5. Recovery and purity from duplicate runs in both setups are summarized in Table 3.

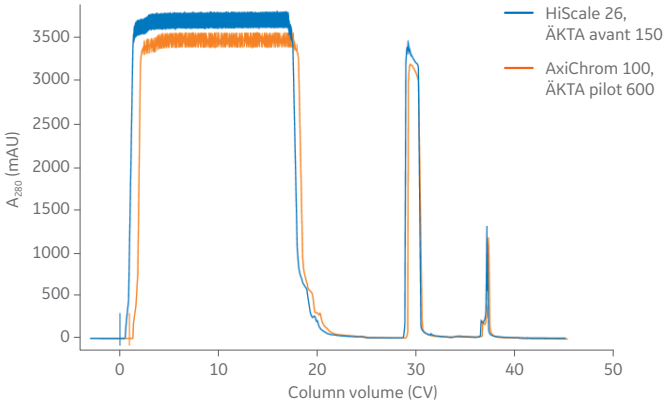

Fig 5. Overlay of chromatograms from mAb capture on the HiScale 26 column using ÄKTA avant 150 and on the AxiChrom 100 column using ÄKTA pilot 600.

Conclusion

A 20-fold multidimensional scale-up was performed, starting from the small-scale process run on the HiScale 26 column operated on ÄKTA avant 150 and scaling up to the AxiChrom 100 column operated on ÄKTA pilot 600. Initially, ÄKTA pilot 600 was configured for process development for verification of consistency between processes run in the small and larger scales. At this stage, the process was performed in a more manual workflow, and the system was configured with extra outlets for collection of flowthrough for subsequent analysis. Similar results in terms of recovery and purity between scales indicate a successful scale-up from HiScale 26 to AxiChrom 100. The process was further modified to allow an automated workflow suitable for production in a regulated (GMP) environment.

This work demonstrates the use of ÄKTA pilot 600 from fast and efficient process development and scale-up (“standard” version) to production in a regulated environment (“regulatory“ version). The use of the same software in ÄKTA pilot 600 simplified method transfer from the smaller ÄKTA avant 150 system. Column volume per hour (CV/h) was utilized to enable maintaining residence time throughout the scaling process.

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