Integrated semi-continuous process for mAb production

A complete process for production of a biopharmaceutical consists of a great number of distinct unit operations, for example, cell culture, filtration, and chromatography. Linking these different steps together to a fully continuous process can be a challenging task, and studies in the area of continuous processes typically deals with single unit operations, such as perfusion cell culture or continuous chromatography. Examples where two or more unit operations are connected and integrated are therefore of great interest for understanding the technical solutions as well as the approach for overall control.

This application note demonstrates how the different unit operations in a laboratory-scale monoclonal antibody (mAb) process can be connected and integrated into a semi-continuous process. In brief, the mAb process consists of perfusion cell culture, continuous capture chromatography, viral inactivation (VI), post VI filtration, batch polishing chromatography, and a final pH adjustment step.

Introduction

The focus on process intensification and increased process control continues in the biopharmaceutical industry. However, biomanufacturing has seen a potential bottleneck shift from upstream to downstream over the years. Advances in cell culture technology with highly productive cell culture systems demand increased efficiency in downstream purification. Connected and integrated processes, where two or more unit operations are physically connected and controlled through a distributed control system (DCS) can be an answer. Another key driver for implementing continuous processing is to reduce production costs, while maintaining product quality and throughput in the manufacturing of biopharmaceuticals. The introduction of continuous processing technologies and process analytical technologies has supported the industry in evaluating different approaches for continuous and/or hybrid solutions for up- and downstream processing. A perfusion cell culture setup can reduce the required floor space, as smaller bioreactors can be used. Additionally, continuous chromatography has the potential to increase chromatography resin capacity utilization, and eliminate or minimize the need for intermediate hold steps. By combining and connecting multiple continuous unit operations, the total equipment footprint and need for manual interactions can be reduced. Such benefits can in turn have a positive impact on the process economy and maintaining consistent product quality.

Process operations

In this study, a platform mAb process was integrated, from perfusion cell culture to final purified product. The focus of the work was on automation and the connection of the downstream purification steps to the upstream production bioreactor. For downstream, an ÄKTA™ pcc system, operated in a three-column periodic counter-current chromatography (3C PCC) setup, was controlling the continuous capture chromatography step, and an ÄKTA avant system was controlling all pH adjustments as well as the filtration and the polishing chromatography steps (Fig 1). The complete setup was tested several times with different run times.

The perfusion cell culture system and affinity capture using the ÄKTA pcc chromatography system were connected physically via a small hold-up vessel in between, and the systems were run continuously in parallel after the perfusion cell culture had reached steady state and the affinity capture step was started. The UNICORN™ software of ÄKTA pcc and the ÄKTA avant systems were programmed and connected, so that after 21 elution fractions (i.e., protein A product [PAP] pool) from the ÄKTA pcc system, the ÄKTA avant system was triggered to receive the elution pool from the ÄKTA pcc system and start VI. The subsequent steps were designed to match the volume and the amount of mAb from the PAP pool. After VI and filtration, the filtered-neutralized viral inactivated product (FNVIP) was loaded onto an anion exchange chromatography (AEX) column. The mAb-containing flowthrough was collected and pH-adjusted before final analysis. All steps from VI to final pH-adjusted mAb product were run in batch mode, however, automated and controlled by the ÄKTA avant system. External units for pH adjustments were connected to ÄKTA avant via I/O boxes (1). An example chromatogram from VI through the AEX steps is shown in Figure 2.

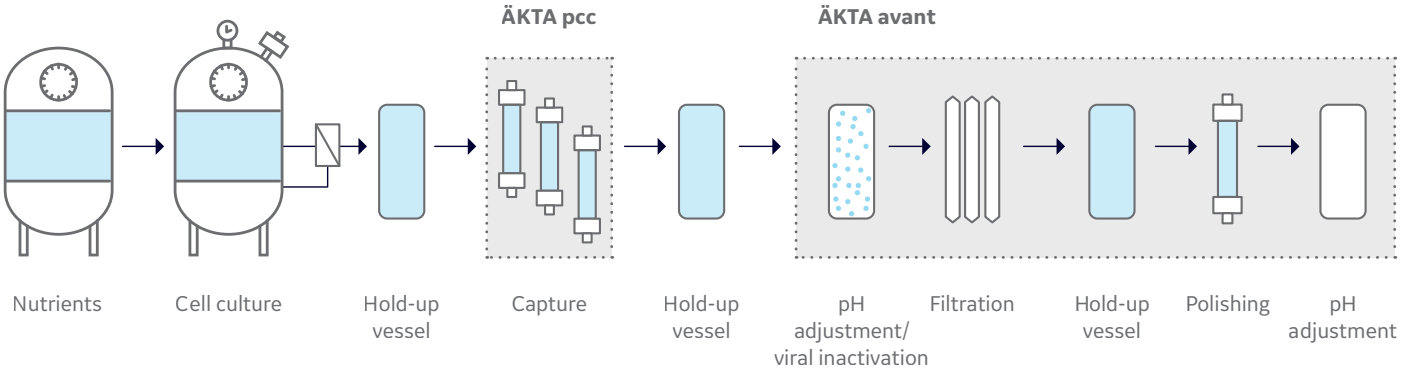

Fig 1. Schematic overview of the connected and integrated process, with hold-up vessels placed between the different unit operations.

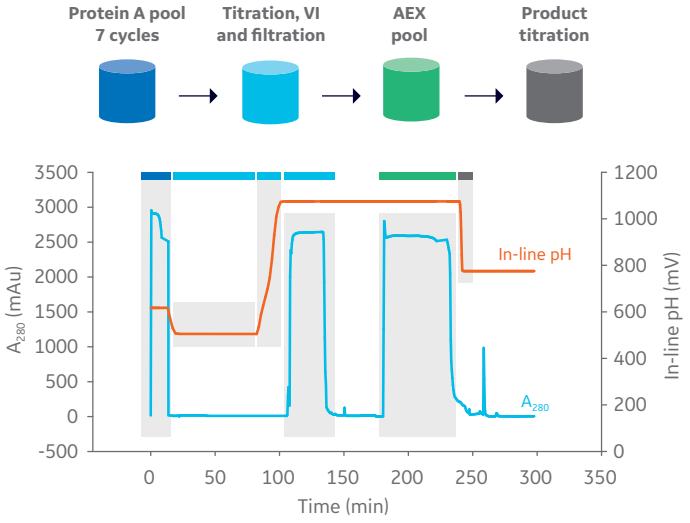

Fig 2. Schematic chromatogram, showing UV and pH from the different unit operations. Once 21 elution fractions from ÄKTA pcc were collected, a signal was sent to ÄKTA avant to start the pH adjustment for viral inactivation (VI). After VI, the pH was adjusted again, after which the material was filtered. The filtrate was applied to an AEX column, and the mAb-containing flowthrough was finally titrated to a pH suitable for the mAb (2).

Materials and methods

Cell culture and harvest

The mAb used in this study was expressed in Chinese hamster ovary (CHO) cells. Collection of perfusion material started when the titer had reached steady state, approximately 9–12 days after start. The perfusion material from one day was collected in a hold-up vessel before the subsequent affinity chromatography capture step was started. The chromatography flow rate was set to match the perfusion cell culture flow rate. The perfusion cell culture continued for different lengths of time in different tests, with a maximum of 35 days.

Protein A affinity capture chromatography

The continuous capture chromatography step was performed using an ÄKTA pcc system in a 3C PCC setup. The columns were packed with MabSelect SuRe™ resin (4 mL column volume [CV]). The columns were loaded to a predefined breakthrough level of the target mAb (typically 60%), which was monitored by the dynamic control functionality of the ÄKTA pcc system (3, 4). The dynamic control is based on the difference in UV signals before and after the column to which the sample is applied, and enables consistent loading of comparable amount of mAb on each column in each run, even with variations in mAb concentration in the feed (e.g., from the perfusion cell culture). One cycle in 3C PCC consists of one complete run on each column, resulting in three elution fractions. The pump usage in loading and non-loading steps were balanced in the method, which is outlined in Table 1. The mAb-containing elution fractions from the MabSelect SuRe columns were directed to and pooled in a hold-up vessel. The subsequent VI and polishing chromatography steps were designed to match the volume and amount of mAb of 21 pooled elution fractions (= 7 cycles × 3 columns).

Table 1. Run conditions for the 3C PCC protein A capture step (4)

Step	Buffer
Equilibration	3 CV of 10 mM sodium phosphate, pH 6.5
Load	Until 70% breakthrough
Post-load wash (Wash 1)	3 CV of 10 mM sodium phosphate, pH 6.5
Wash 2	3 CV of 10 mM sodium phosphate, pH 6.5 + 0.5M NaCl
Wash 3	3 CV of 10 mM sodium phosphate, pH 6.5
Elution	20 mM sodium acetate, pH 3.5 (elution pool was based on UV criteria)
Strip	3 CV of acid or base solution

Viral inactivation and filtration

The 21 elution fractions from the protein A capture step were collected and pooled before the low-pH VI step was started. The mAb-containing pool was transferred to a separate vessel for pH adjustment and VI. The target pH of the VI step was achieved by addition of 1 M acetic acid and by monitoring pH with an external pH electrode, controlled by ÄKTA avant via an I/O box. The low-pH VI continued for 60 min, after which the pH was raised by addition of 1 M Tris base to the target pH value for the polishing chromatography step. Again, the pH adjustment was controlled by the ÄKTA avant system. The pH-adjusted mAb sample was filtered through a 0.65 µm filter followed by a 0.22 µm filter prior to application to the polishing chromatography step.

Anion exchange polishing chromatography and final pH adjustment

The AEX feed was loaded onto the anion exchange polishing chromatography column (17.5 mL CV), and the mAb-containing flowthrough was collected and subjected to a final pH adjustment for mAb stability. AEX and pH adjustment were controlled by the ÄKTA avant system.

Analytical methods

Selected fractions/pools were analyzed for mAb concentration, purity, charge variant distribution, host cell protein (HCP), protein A ligand leaching, and DNA as described previously (4).

Results

Analyses of the final pH-adjusted mAb product showed that recovery was comparable to what can be expected from batch mode operation of the protein A chromatography step for as long as the process lasted (up to 35 days). The overall recoveries were in the range of 84%–87%, as can be compared with 90% for batch mode. The mAb purity was consistent throughout both perfusion runs at about 99% monomer for each AEX product pool (Fig 3). The distribution of charge mAb variants was determined for the first perfusion batch and the main mAb peak was maintained at about 65%–75% throughout the runs. The consistent charge variant profile indicates the ability to pool AEX products from various perfusion days (Fig 4). HCP (Fig 5) and protein A ligand levels (Fig 6) were both below the maximum acceptable level of 100 ppm, and levels were also consistent between perfusion days and runs. DNA levels were below limit of quantification (< 4 ppb) in the final AEX products for each perfusion pool and run.

Conclusions

This work demonstrates the performance of an integrated mAb process, from upstream production to downstream purification. The integrated process was tested for up to 35 days and resulted in an 84%–87% overall mAb recovery at a purity that meets product quality requirements. Product yield and quality were consistent over time, indicating successful performance of the integrated, semi-continuous process.

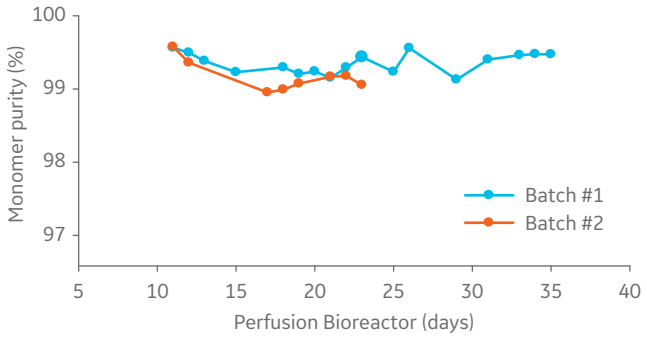

Fig 3. Purity of mAb monomer, analyzed by ultraperformance size exclusion chromatography (UPSEC), in fractions from the AEX step over time.

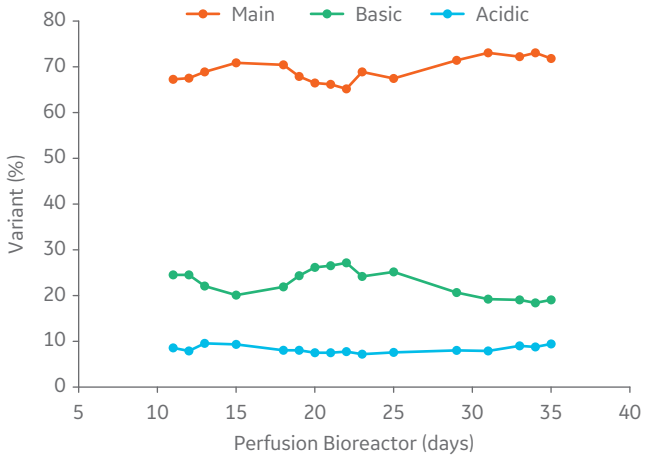

Fig 4. Charged mAb variant distribution, analyzed by high-performance ion exchange chromatography, in fractions from the AEX step over time.

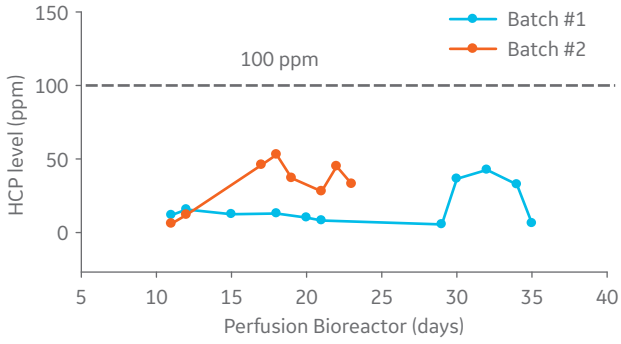

Fig 5. HCP levels (ppm) in fractions from the AEX step over time.

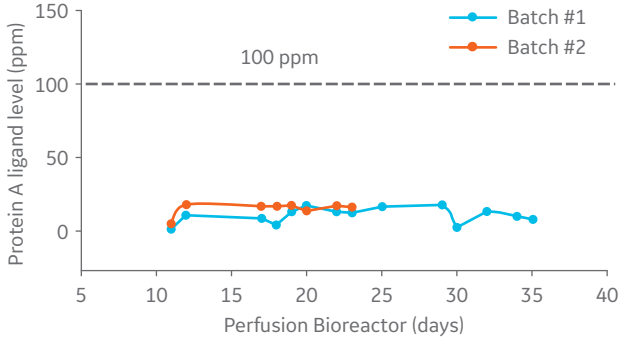

Fig 6. Protein A ligand levels (ppm) in fractions from the AEX step over time.

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