



# Feasibility of using continuous chromatography in downstream processing: Comparison of costs and product quality for a hybrid process vs. a conventional batch process



Ozan Ötes<sup>a,b,\*</sup>, Hendrik Flato<sup>b</sup>, Johannes Winderl<sup>a</sup>, Jürgen Hubbuch<sup>a</sup>, Florian Capito<sup>b,\*</sup>

<sup>a</sup> Institute of Engineering in Life Sciences, Biomolecular Separation Engineering, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

<sup>b</sup> Bioprocess Development, Industriepark Höchst, Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main, Germany

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## ABSTRACT

The protein A capture step is the main cost-driver in downstream processing, with high attrition costs especially when using protein A resin not until end of resin lifetime. Here we describe a feasibility study, transferring a batch downstream process to a hybrid process, aimed at replacing batch protein A capture chromatography with a continuous capture step, while leaving the polishing steps unchanged to minimize required process adaptations compared to a batch process. 35 g of antibody were purified using the hybrid approach, resulting in comparable product quality and step yield compared to the batch process. Productivity for the protein A step could be increased up to 420%, reducing buffer amounts by 30–40% and showing robustness for at least 48 h continuous run time. Additionally, to enable its potential application in a clinical trial manufacturing environment cost of goods were compared for the protein A step between hybrid process and batch process, showing a 300% cost reduction, depending on processed volumes and batch cycles.

## 1. Introduction

Due to improvements in upstream processing (USP) concerning media development, clone selection or host cell engineering, productivity is continuously increasing (Karst et al., 2016) and thereby economic pressure is shifted towards downstream processing (DSP). The enhancements achieved in USP cannot be transferred to downstream directly, because unit operations in DSP depend rather on the mass of product than on its volume. To overcome this increasing demand in space and volume, strategies have been developed, such as flocculation, precipitation, crystallization, aqueous two-phase-systems and chromatographic methods like membrane chromatography and SMB chromatography. The superior goal of these operations is to avoid more complex steps and reduce raw material costs while raising productivity (Low et al., 2007).

Yet, most antibody purification processes follow a similar purification scheme (Fahrner et al., 2001; Shukla et al., 2007), employing protein A chromatography as state of the art operation for the capture step. Most protein A resins exhibit dynamic binding capacities (DBC) > 30 g/L (Hahn et al., 2005, 2006). Interestingly, theoretical calculations have led to the assumption that the maximum DBC of a protein A resin is about 70 g/L (Low et al., 2007), which has still not

been reached but yet displays a certain bottleneck. Using expanded bed chromatography allows for reducing the number of total steps, however fouling of the adsorbent because of cell–cell debris interactions was reported, which becomes even more prevalent as titers increase (Blank, 2005). Simulated moving bed chromatography (SMB) is being used in the petrochemical industry and for separations of binary mixtures and enantiomers (Francotte and Richert, 1997; Schulte and Strube, 2001; Nicoud, 2014). The concept of SMB is based on the simulated movement of the bed in the opposite direction of the eluent flow, which is realized by interconnected columns and valves, respectively. Utilizing the DBC of a column to its maximum, increased productivity and reduced buffer consumption are key advantages of SMB (Low et al., 2007). Pfister et al. (2017) performed extensive modeling of multi-column protein A chromatography and concluded that productivity can be increased when employing additional columns at constant yield. The purification process as a whole benefits from these improvements as reduced equipment and plant size are needed, leading to lower costs and increased flexibility (Pollock et al., 2013). However, implementation into commercial applications has been limited because of concerns regarding the complexity of the system and its scalability (Karst et al., 2016).

Nevertheless, continuous processing offers constant process

\* Corresponding authors at: Bioprocess Development, Industriepark Höchst, Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main, Germany.  
E-mail addresses: [udds@student.kit.edu](mailto:udds@student.kit.edu), [ozan-thomas.oetes@sanofi.com](mailto:ozan-thomas.oetes@sanofi.com) (O. Ötes), [florian.capito@sanofi.com](mailto:florian.capito@sanofi.com) (F. Capito).

performance and quality and is therefore not only desired by manufacturers but also promoted by regulatory authorities (Woodcock, 2014). As most unit operations within a purification process can be performed in continuous mode (Jungbauer, 2013), designing robust continuous chromatography operations is a major goal towards an integrated process stream. Developing continuous processes, it might be advantageous employing hybrid processes as the changes required can be minimized and discontinuous steps in general might serve as holding steps, in case of problems during continuous steps. Recently, capture of mAbs using a two-column setup (Angarita et al., 2015), three columns (Warikoo et al., 2012) and even four columns (Pollock et al., 2013) has been implemented, showing comparable yield and product quality regarding batch mode. Warikoo et al. (2012) connected a perfusion reactor to a continuous capture step, proving the practicality of applying subsequent continuous unit operations. Further, a continuous end-to-end process for the purification of mAbs has been designed, obtaining constant product quality throughout the whole process time, while increasing throughput and reducing the number of unit operations (Godawat et al., 2015).

The studies mentioned above used continuous chromatography systems which can be run using 2–4 columns. In the present work we designed a hybrid process for continuous multi-column capture chromatography using the Cadence BioSMB™ PD system, allowing for the use of up to 16 columns. However, we focused on a four column setup due to reasons of simplicity and comparability to systems of other manufacturers. We focused on comparing data to batch mode experiments, using two mAbs and evaluated the long-term behavior of SMB purification processes during a total process time of 60 cycles, which corresponded to a time of 48 h. Gjoka et al. (2017) performed a three-step transfer from batch to continuous mode using two BioSMB systems.

In contrast, the present study displays a compromise using a hybrid process including continuous and batch mode operations, as a hybrid process would be easier to implement. An enhanced clean-in-place (CIP) strategy was designed to guarantee constant column performance. Additionally, we further purified the product captured by BioSMB in two more batch steps by depth filtration (only mAb 2) and a polishing chromatography step (mAb 1 and mAb 2) in order to examine comparability of product quality after subsequent unit operations. Additionally, we calculated the cost of goods for the protein A step resulting from using continuous protein A capture chromatography in clinical trial manufacturing and at routine production scale in order to evaluate potential benefits.

## 2. Materials and methods

Two monoclonal antibodies (mAb 1 and mAb 2) expressed in Chinese hamster ovary cells were used in this study, showing titers of 1.7 and 2.3 g/L, respectively. The material was obtained sterile filtered as processed bulk harvest (PBH) at pH 7.3, kept stored at  $-80^{\circ}\text{C}$  and was freshly thawed prior to use.

### 2.1. Buffers and stationary phases

Buffers were prepared using purified water taken from a MilliQ Super-Q-Water system, using reagents obtained from Merck KGaA (Darmstadt, Germany). Buffer pH was adjusted using 1 M HCl or 1 M NaOH, respectively. Buffers were sterile filtered using a 0.22  $\mu\text{m}$  membrane filter.

For the protein A step, two washing buffers were applied, containing phosphate and acetate at different molarities and pH values. Washing buffer 2 was also used for equilibration and Na-acetate at low pH served as elution buffer. Solutions of 0.1 M NaOH and 0.5 M NaOH were used for cleaning in place (CIP). Four self-packed columns containing 25 mL of MabSelect Sure™ (GE Healthcare, Uppsala, Sweden) were used for the experiments. Bed height reached 5 cm, while the inner diameter was 2.5 cm.

### 2.2. Batch chromatography

Batch protein A chromatography of mAb 1 and mAb 2 was performed on an Äkta Avant system by GE Healthcare (Uppsala, Sweden). A model process consisting of the following steps was designed for mAb 1: load, wash 1, wash 2, elution, CIP, equilibration. The process for mAb 2 did not include the first washing step. In order to evaluate the binding capacity of the respective columns used, breakthrough curves were generated. Elution was achieved using a buffer at low pH. It was observed that using the same volume in batch and continuous mode led to a different UV peak end level. Therefore, cutting criteria were transferred to the BioSMB process by the volume required to achieve the same UV level, respectively. CIP was applied using 0.1 M NaOH.

### 2.3. Continuous protein A capture chromatography

Continuous protein A capture chromatography was performed on a Cadence BioSMB™ PD system by Pall Corporation (Port Washington, NY, USA). Conductivity, pH and UV detection were monitored online using the programs Tarpon BioSMB Method Control v.11.11 and Tarpon-BioSMB Measurements Main v11.11.

All pumps were purged at 10 mL/min in order to reduce the risk of air bubbles. New columns were equilibrated with at least 5 column volumes (CV) prior to use. BioSMB methods were created using the Tarpon BioSMB Application Software Studio.

The chronogram displayed in Fig. 1 shows two methods employing four columns that were used for continuous protein A chromatography for mAb 1 and mAb 2, respectively. Exemplarily, the composition of the mAb 1 method is described in the following. Within one cycle, the segments can be divided into three different zones. One can be defined as loading zone (lower left), where sterile filtered PBH is loaded onto a column. This step was set as determining step, so only one column was loaded at a time. Within the second zone (upper left), two different washing buffers were used to remove non-specifically bound components, followed by an elution buffer to elute the protein of interest from the column. For regeneration, a CIP buffer was applied to assure proper cleaning of the column. The last step was the equilibration in order to prepare the column for loading again. The third zone (upper and lower right) was covered by two columns in parallel. The loss of product should be minimized by capturing unbound protein of the loading zone. Moreover, this concept reduced system pressure and could therefore be run at higher flowrates. All flowrates were set in a range of 240–799 cm/h. Load was typically performed at 240–350 cm/h. Fractions of the flow through, wash, product and tail of elution were collected cycle-wise.

### 2.4. Preliminary experiments

The overall goal was to assure comparability between methods of batch and continuous mode. An individual method was designed for each mAb using the parameters of the corresponding batch processes. Cutting criteria were adjusted as mentioned above. Elution is controlled by volume while operating on the BioSMB system in contrast to UV-controlled elution during batch chromatography. Therefore, pre-experiments had to be performed in order to define similar cutting criteria for BioSMB and batch mode.

For the reason that a cycle in batch chromatography is longer than in continuous chromatography, the steps within the capture process were transferred by their share time concerning the whole sequence. Cycle time of continuous chromatography was reduced about 3-fold for mAb 1 and 6-fold for mAb 2, respectively. A total cycle time of 45 min was set.

### 2.5. Regeneration

In batch mode, regeneration can take about 25% of the total run

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