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Research Article

Pool-less processing to streamline downstream purification of monoclonal antibodies

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With cell culture titers and productivity increasing in the last few years, pressure has been placed on downstream purification to look at alternative strategies to meet the demand of biotech products with high dose requirements. Even when the upstream process is not continuous (perfusion based), adopting a more productive and/or continuous downstream process can be of significant advantage. Due to the recent trend in exploring continuous processing options for biomolecules, several enabling technologies have been assessed at Biogen. In this paper, we evaluate the capability of one of these technologies to streamline and improve our downstream mAb purification platform. Current conventional downstream polishing steps at Biogen are operated in flow-through mode to achieve higher loadings while maintaining good selectivity. As titers increase, this would result in larger columns and larger intermediate product pool holding tanks. A semicontinuous downstream process linking the second and third chromatography steps in tandem can reduce/eliminate intermediate holding tanks, reduce overall processing time, and combine unit operations to reduce validation burdens. A pool-less processing technology utilizing inline adjustment functionality was evaluated to address facility fit challenges for three high titer mAbs. Two different configurations of polishing steps were examined: (i) anion exchange and hydrophobic interaction and (ii) anion exchange and mixed mode chromatography. Initial laboratory scale proof of concept studies showed comparable performance between the batch purification process and the pool-less process configuration.

Keywords: Continuous processing / Connected processing / Monoclonal antibodies / Pool-less processing / Process development

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1 Introduction

High productivity cell culture for mAbs with titers in the range of 5–10 g/L is the current reality due to significant advances in cell line and media technologies that have been made over the last decade [1–3]. To mitigate the issue of downstream purification becoming the bottleneck in the manufacturing process, new technologies and strategies for purification need to be identified. In the recent years, there has been a growing in-

terest in realizing the benefits of continuous processing in biologics manufacturing [4, 5]. Continuous processing has been a well established paradigm in many industries [6, 7], however its adoption in the biotech industry has been relatively slow. Following up on the work of a few early pioneers [8], an open dialogue is occurring more readily between the academic and industrial community to foster better understanding and growth in this area [9, 10]. Several variations of a continuous process have been proposed ranging all the way from a fully integrated end-to-end continuous process to making certain parts of the manufacturing process (upstream or downstream) either continuous or connected [11]. In response to this emerging trend, vendors are also coming up with new technologies that can support this new niche of continuous processing [12–14]. In the downstream arena, the novel technologies or methodologies reported in the literature have covered a vast array of downstream purposes; from addressing the challenges of the capture

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Abbreviations: AEX, anion exchange chromatography; FT, flowthrough; HIC, hydrophobic interaction chromatography; STP, straight through processing

Figure 1. Schematic of reconfigured AKTA Pure system to enable inline conditioning. The mixer valve (V-9) is reprogrammed to accommodate the addition of a column position valve. Additionally, the pH and conductivity sensors are repositioned before the column 2 position. This allows for measurement and control of physical properties of the FT pool from the column 1 prior to loading onto column 2.

step [15, 16] to the final product concentration/diafiltration step [17].

One of the trends in downstream processing has been to integrate or connect multiple unit operations into a single fluid system. In a typical manufacturing set-up, the product pool from one chromatography step is collected in a product pool tank, adjusted for pH and/or conductivity needed for the subsequent chromatography step and then processed through the next step. As cell culture titers (thereby product mass) increase, the volume of these intermediate pools can be a significant bottleneck in an existing manufacturing facility. Operating the process in a connected or “pool-less” manner can help to overcome this bottleneck by eliminating the need for expensive in-process product hold tanks [18]. Some instances of semicontinuous connected operations do exist in the literature such as the use of tandem depth filtration followed by anion exchange (AEX) membrane chromatography for harvesting of perfusion cell culture [19] and the tandem use of protein A and AEX chromatography [20].

This paper evaluates connecting two polishing chromatography steps in order to eliminate intermediate process pools. The tandem chromatography column steps examined were AEX followed by hydrophobic interaction or AEX followed by mixed mode chromatography. All the polishing steps were operated in a connected fashion by using appropriate inline pH/conductivity adjustment technology. Multiple mAbs were used in this study to test the applicability of this approach.

2 Materials and methods

2.1 Materials

Three mAbs were used as model proteins for the following studies. All three mAbs were produced in Chinese hamster ovary cells at Biogen. The polishing steps of the purification process used for all three molecules had been previously optimized. All process parameters and buffers were kept consistent between the conventional batch process and connected process being evaluated across both polishing steps. As the target objective for this

study was to evaluate the ability to streamline the downstream polishing steps of the conventional process, representative product pools for the three molecules were generated by purification through the protein A step to serve as the starting material for all experiments. All buffering chemical components used were from Sigma (St. Louis, MO, USA) and J.T. baker (Phillipsburg, NJ, USA) unless stated otherwise. POROS HQ AEX resin was obtained from Life Technologies (Carlsbad, CA), Hexyl Toyopearl 650C HIC (HIS is hydrophobic interaction chromatography) resin was obtained from Tosoh Biosciences (Montgomery, PA) and Capto Adhere mixed mode resin was obtained from GE Healthcare (Uppsala, Sweden).

2.2 Equipment

In collaboration with GE Healthcare, an existing AKTA Pure purification system was retrofitted to create a lab-scale version of their straight through processing (STP) skid for the proof-of-concept studies. The modified configuration of the system, as shown in Fig. 1, had the added ability to run two columns in tandem with individual UV sensors for each column to accurately monitor the protein breakthrough across the two steps. The STP skid, designed by GE Healthcare, is a custom-made equipment available for pool-less processing (Fig. 2A and B). In place of the intermediate hold tanks (Fig. 2A), rapid and accurate feedback controls allows for inline adjustment in real-time between tandem processing steps, thus eliminating separate adjustments. It utilizes two positive displacement pumps, A and B, to deliver effluent into a static mixing chamber. The main pump, A, delivers the effluent from the first chromatography step while the secondary pump, B, delivers the titrant needed to adjust that effluent to the desired pH/conductivity for the second chromatography step. This study was focused on evaluating the potential of connecting the polishing chromatography steps and did not extend to connecting these steps with the viral filtration step. Columns used for all experiments were GE Healthcare 1.0 cm id C columns packed to predetermined bed height (~20 cm) based on requirements specific to each process.

Figure 2. (A) Process flow diagram of current limitations facing the downstream manufacturing process. (B) Process flow diagram of the modified inline pool-less process that eliminates the concern for intermediate hold tanks at manufacturing scale.

2.3 Analytical techniques

All samples collected were analyzed to determine cumulative yield, high molecular weight, and host cell protein. Protein concentrations from samples were determined using a SoloVPE UV-Vis spectrometer. High molecular weight levels were measured in-house by the Department of Process Biochemistry by analytical SEC using a TSK gel G3000 SWXL column. A mobile phase of 100 mM sodium phosphate, 200 mM sodium chloride, pH 6.8 and flow rates optimal for each mAb was used. UV absorbance at 280 nm was used to detect and integrate the elution peak. Host cell protein levels were analyzed by an in-house ELISA assay.

2.4 Determination of adjustment ratio

Bulk offline titration experiments were performed to determine the ratio for the inline adjustment set-point for the tandem process. Titration curves were generated using 100 mL of AEX flowthrough (FT) pool and various concentrations of the titration solution to determine the initial volume to volume (v/v) adjustment ratio and solution concentrations needed to meet target pH and/or conductivity set-points of the third polishing step. Once an acceptable solution was identified, further fine tuning of the v/v adjustment was performed during the inline experiments by adjusting the %B gradient concentration based on the real-time inline pH readings. Experiments were run in triplicate and average values were used for the graph.

2.5 Preparative experiments

All lab-scale preparative runs, batch versus pool less, were performed on the same retrofitted AKTA Pure using the same columns. The process conditions and buffered solutions used were specific to the protein and resin combination developed for each mAb process. Since all the columns evaluated in this study were operated in FT mode, they all followed the same general

processing procedure. The columns were first equilibrated before being loaded with protein. The FT peak collection started as the UV₂₈₀ started to rise and product was chased with equilibration solution for a set volume and/or UV₂₈₀ set-point and collection was stopped. The column was then cleaned and regenerated for additional use.

In the control process scenario, the entire AEX column was processed and FT pool collected. The FT pool from the AEX column was then adjusted to the HIC or mixed mode chromatography load condition and 0.2 μ m filtered before being processed. In the tandem process scenario, utilizing the inline adjustment application, the AEX FT effluent from pump A was immediately mixed inline with the appropriate titration solution from pump B in the mixer as the protein breakthrough occurred. This inline adjusted stream served as the load for the subsequent column connected in series.

3 Results and discussion

A platform approach for purification of mAbs is currently an industry standard [21]. The typical mAb purification platform at Biogen consists of protein A capture followed by two polishing steps (usually AEX and HIC) operated in the FT mode. In the Biogen platform, it was most prudent to evaluate the operation of the two polishing steps in a pool-less fashion (as shown in Fig. 2) by using an appropriate inline pH/conductivity adjustment technology.

3.1 Optimization of the inline adjustment solution

In a conventional batch purification process, concentrated solutions are typically utilized for pH/conductivity adjustment of the intermediate product pool to minimize volume expansion and enable facility fit at manufacturing scale. Keeping this rationale in mind, the Biogen platform was designed to achieve target pH adjustments by the addition of $\leq 2\%$ v/v of the titrant.

Figure 3. Comparison of the volume adjustment ratio for mAb A and B in the downstream polishing steps between conventional process and the inline pool-less process. The aim to minimize volume expansion in batch condition for facility fit purposes was not necessary for the inline condition. In inline condition, it was necessary to increase this adjustment ratio in order to accommodate the limitations of pump accuracy.

The straight-through-processing lab-scale unit evaluated in this study achieves inline dilution by using two pumps—a primary pump for the buffered solutions used by the chromatography column and a secondary pump for the titration solution. A $\leq 2\%$ v/v titrant addition translates to a pump operation of $\leq 2\%$ of the total flow rate. This ratio needed to be much closer to $\sim 10\%$ v/v to ensure the accuracy of the volume being delivered. Hence, the titration solutions that were used in the batch purification process had to be reoptimized to achieve the higher volume additions. Since the primary benefit of this technology is elimination of the intermediate hold tanks, minimizing the adjustment volume was no longer a critical parameter. Several dilute variations of the platform process load adjustment solution were examined to find an alternative that would provide $\sim 10\%$ v/v adjustment ratio.

Three of Biogen's high titer mAbs (A–C) were used for this study. The polishing chromatography steps of the process designed for mAbs A and B, AEX followed by HIC, are operated in FT mode. The HIC step is also performed without the addition of a kosmotropic salt [22]. This “no-salt” HIC operation eliminates the product volume increase caused by the addition of kosmotropic salts typically used to achieve selectivity on HIC [23], and thus was an ideal candidate for this application. The AEX FT pool only needed to be pH adjusted (pH 7.0–6.0) by using an appropriate titration solution. Figure 3 shows the titration curve for the control and the new pH adjustment strategy. The volume adjustment was increased from $<2\%$ to $\sim 9\%$ to achieve the same pH endpoint.

On the other hand, the purification process designed for mAb C required the use of a mixed mode resin (Capto Adhere) as the second polishing step to achieve the desired product quality. In this case, the AEX FT pool required adjustment of both the pH and conductivity prior to loading onto the Capto Adhere step. The pH needed to be adjusted from 7.5 to 6.7 and the conductivity needed to be increased to 27 ms/cm. The conventional

process for this mAb employed two separate solutions to adjust the pH (200 mM citric acid) and conductivity (4 M NaCl). Additionally, the v/v ratios for these two solutions were quite different from each other. As shown in Fig. 4A, the conventional process required a 2% v/v adjustment for conductivity and an 8% v/v adjustment for pH. For the pool-less process, this two-step adjustment needed to be combined into one working solution that adjusted for both conductivity and pH simultaneously and at the same v/v ratio. Using offline batch experimentation, an acceptable solution was identified (40 mM citric acid, 2.73 M NaCl) that could adjust for conductivity and pH simultaneously at $\sim 10\%$ v/v ratio (Fig. 4B).

3.2 Comparison of the conventional and pool-less process

Once the inline titration solutions were identified, the conventional and pool-less processes were run side-by-side for all three mAbs keeping other process parameters constant. Table 1 lists the step yields and key product quality data obtained from these runs. As shown in Table 1, comparable process performance and product quality was achieved between the conventional process and the pool-less process for all cases. Furthermore, mAb C demonstrates that this technology was able to handle a more complicated process where a simultaneous pH and conductivity adjustment was needed for the mixed-mode step. Figure 5A shows the typical chromatogram from the two polishing steps (AEX FT and HIC FT) separately while Fig. 5B represents the pool-less process with the two FT columns running in tandem. The product FT peaks from the two individual columns are overlapping in Fig. 5B that translates to a significant reduction (~ 20 – 30%) in overall processing time at manufacturing. This is an additional benefit of this technology apart from process simplification and elimination of product hold tanks. It is

Figure 4. (A) The conventional two-step, two solution adjustment process for mAb C using mixed-mode chromatography as a second polishing step. (B) The optimized adjustment process for the inline pool-less process. A mixture of the two solutions was made in concentrations that would allow for similar addition of both solutions in one single step.

Table 1. Comparison of product quality for batch and pool-less process

Inline adjustment	Chromatography mode	Molecule	Process	Cumulative yield (%)	HMW (%)	HCP (ppm)
pH only	AEX to HIC	A	Batch	91	0.66	2.8
			Pool less	90	0.60	2.2
		B	Batch	82	0.54	0.8
			Pool less	82	0.52	0.8
pH and conductivity	AEX to MMC	C	Batch	78	0.74	<LLOQ
			Pool less	78	0.71	<LLOQ

LLOQ, lower level of quantitation; MMC, mixed-mode chromatography; HMW, high molecular weight; HCP, host cell protein.

important to note that the selectivity of each of the individual polishing steps was not compromised in any way by pool-less processing. Hence, it is expected that this technology will be able

to handle even higher levels of aggregates or other impurities as long as the respective polishing steps are suitably optimized for the separation.

Figure 5. (A) Typical representative chromatograms of conventional batch AEX FT and HIC FT performed separately. FT of AEX is collected separately before offline titration to HIC load conditions. HIC process is then performed and FT collected for final viral filtration step. (B) Representative chromatogram of the tandem process. The FT portion of the AEX and HIC steps are occurring in parallel to each other as a result of using inline adjustment.

3.3 Streamlining column conditions to further simplify processing and buffer preparation

Another potential benefit of this technology is the ability to build more flexibility into the existing manufacturing space. The results presented so far, were based on retrofitting the conventional purification process into a pool-less process frame-

work. As a further extension, it would be ideal if the two FT polishing steps could be designed to operate under identical conditions. This can significantly reduce the number of process buffered solutions and eliminate the need for inline adjustment. Using mAb A, the HIC-FT step was reoptimized to be performed under identical mobile phase conditions as the previous AEX FT step (pH 7.0). The conventional and new process

Table 2. Streamlining process operating pH for pool-less technology

Operation mode	Process condition	Operating pH	Process mode	Cumulative yield (%)	HMW (%)
FT to FT	AEX to HIC	Adjustment from pH 7 to 6	Batch	88	0.5
		7	Pool less	90	0.5

HMW, high molecular weight.

was operated in a pool-less manner and compared to each other (Table 2). Comparable product quality was seen in both cases. In the conventional purification process configuration, operating the HIC step at pH 7.0 was not possible due to product stability concerns at the intermediate product hold points (data not shown). Since, the pool-less processing technology eliminated the need for intermediate product hold, there was less concern of product stability. Achieving the desired selectivity under one mobile phase condition for both polishing steps might not always be possible for all antibodies; however, where applicable, this approach provides an additional avenue for process simplification.

4 Concluding remarks

As the biotechnology industry moves into the new paradigm of high productivity cell cultures, the burden now lies on downstream purification to meet the demands of processing this increased mass load. There is an urgent need to identify ways of making the downstream process more efficient and productive. With the recent renewed interest in continuous processing of biomolecules, several technologies have been developed that can either be an enabler of a fully continuous process or be used by itself in the batch configuration to drive more efficiency. The work presented in this paper demonstrates one such technology in which all the polishing steps are connected in a pool-less manner without using any intermediate product hold tanks. Connecting two FT chromatographic steps is an ideal application of this technology due to the simplicity of operating mode and the ease of load adjustment between them. Several mAbs were used in this study with a variety of purification processes to establish its applicability across an antibody platform. Comparable process performance and product quality was seen between the traditional and pool-less process. This also translates into the possibility of improved product stability due to removal of unnecessary hold steps, reduced processing times by overlapping processing steps, and significant reduction of analytical testing by elimination of extra in-process sampling points. Furthermore, significant (but hard to quantitate) time savings are also expected due to simplification of batch records and sampling operation. In addition, the possibility of further process simplification was explored by reoptimizing the polishing steps to be performed under identical mobile phase conditions. All results presented here were based on lab-scale replica of the STP system, however the successful performance of this skid at scale has been demonstrated elsewhere [24].

A few factors need to be kept in mind while translating a traditional process to a tandem pool-less one. First, an offline batch titration to determine adjustment ratio will not be exactly repre-

sentative of the inline adjustment ratio due to the nature of the protein pool. During an offline batch titration, the adjustment solution is added to a homogenous static pool with a uniform protein concentration. On the other hand, the tandem process experiences a dynamically flowing stream of varying protein concentrations that needs to be accounted for during the titration process. Hence, while the batch off-line titration values provide a good starting point, this technology relies on good feedback control to reach the desired set-point. Second, careful consideration needed to be given to column sizing, loading, and flow rates during process design to ensure the each column was operating within its desired designed space even when they are connected in tandem. With a thoughtful design of a pool-less process, this is a robust and powerful technology that can significantly help in process simplification and improvement of manufacturing efficiency.

Practical application

Applications of continuous downstream processing are becoming more main stream in the biotech industry. Some of the new enabling technologies address the arising concerns resulting from new and productive high titer cell culture processes. These technologies aim to provide more flexibility to downstream processing by mitigating facility fit limitations and the need for increased productivity. This paper aims to understand benefits of an inline conditioning technology that would allow streamlining of downstream purification steps and potentially also eliminate the use of large intermediate hold tanks at manufacturing scale. Adoption of this technology could alleviate some of the facility fit concerns that many in the industry have due to the constraints of existing manufacturing space. This technology has the ability to be used on its own or be linked to other existing continuous technologies to facilitate a fully continuous downstream process.

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5 References

- [1] Huang, Y.-M., Hu, W., Rustandi, E., Chang, K. et al., Maximizing productivity of CHO cell-based fed-batch culture using

- chemically defined media conditions and typical manufacturing equipment. *Biotechnol. Prog.* 2010, 26, 1400–1410.
- [2] Shukla, A., Thömmes, J., Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol.* 2010, 28, 253–261.
- [3] Yang, W. C., Lu, J., Kwiatkowski, C., Yuan, H. et al., Perfusion seed cultures improve biopharmaceutical fed-batch production capacity and product quality. *Biotechnol. Prog.* 2014, 30, 616–625.
- [4] Nicoud, R. M., The amazing ability of continuous chromatography to adapt to a moving environment. *Ind. Eng. Chem.* 2014, 53, 3755–3765.
- [5] Konstantinov, K. B., Cooney, C. C., The Future of industrial bioprocessing: Batch or continuous? *Biotechnol. Bioeng.* 2015, 112, 648–651.
- [6] Tanner, A. H., *Continuous Casting: A Revolution in Steel*. Write Stuff Syndicate, Fort Lauderdale 1998.
- [7] Fletcher, N., Turn batch to continuous processing. *Manuf. Chem.* 2010, June, 24–26.
- [8] Warikoo, V., Godawat, R., Brower, K., Jain, S. et al., Integrated continuous production of recombinant therapeutic proteins. *Biotechnol. Bioeng.* 2012, 109, 3018–3029.
- [9] Badman, C., Trout, B. L., Achieving continuous manufacturing. *J. Pharm. Sci.* 2015, 104, 779–780.
- [10] Baxendale, I. R., Braatz, R. D., Hodnett, B. K., Jensen, K. F. et al., Achieving continuous manufacturing: Technologies and approaches for synthesis, workup, and isolation of drug substance. *J. Pharm. Sci.* 2015, 104, 781–791.
- [11] Konstantinov, K. B., Cooney, C. L., White paper on continuous bioprocessing. May 20–21, 2014 Continuous Manufacturing Symposium. *J. Pharm. Sci.* 2015, 104, 813–820.
- [12] Fonteyne, M., Vercruysse, J., De Leersnyder, F., Van Snick, B. et al., Process analytical technology for continuous manufacturing of solid-dosage forms. *Trends Analyt. Chem.* 2015, 67, 159–166.
- [13] Myerson, A. S., Krumme, M., Nasr, M., Thomas, H. et al., Control systems engineering in continuous pharmaceutical manufacturing. *J. Pharm. Sci.* 2015, 104, 832–839.
- [14] Page, T., Dubina, H., Fillipi, G., Guidat, R. et al., Equipment and analytical companies meeting continuous challenges. *J. Pharm. Sci.* 2015, 104, 821–831.
- [15] Mahajan, E., George, A., Wolk, B., Improving affinity chromatography resin efficiency using semi-continuous chromatography. *J. Chromatogr. A* 2012, 1227, 154–162.
- [16] Zobel, S., Helling, C., Ditz, R., Strube, J., Design and operation of continuous countercurrent chromatography in biotechnology production. *Ind. Eng. Chem.* 2014, 53, 9169–9185.
- [17] Lutz, H., *Ultrafiltration for Bioprocessing*. Elsevier Ltd, Cambridge: Woodhead 2015.
- [18] Schultz, J. E., Yeboah, S., Hart, R. A., Ogez, J. R., Large-scale pool-less purification. *The 2006 Annual Meeting*. AIChE, San Francisco, CA 2006.
- [19] Vogel, J. H., Nguyen, H., Giovannini, R., Ignowski, J. et al., A new large-scale manufacturing platform for complex biopharmaceuticals. *Biotechnol. Bioeng.* 2012, 109, 3049–3058.
- [20] Shamashkin, M., Godavarti, R., Iskra, T., Coffman, J., A tandem laboratory scale protein purification process using protein affinity and anion exchange chromatography operated in a weak partitioning mode. *Biotechnol. Bioeng.* 2013, 110, 2655–2663.
- [21] Shukla, A., Hubbard, B., Tressel, T., Guhan, S., Low, D., Downstream processing of monoclonal antibodies—Application of platform approaches. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 2007, 848, 28–39.
- [22] Ghose, S., Tao, Y., Conley, L., Cecchini, D., Purification of monoclonal antibodies by hydrophobic interaction chromatography under no-salt conditions. *MAbs* 2013, 5, 795–800.
- [23] Melander, W., Corradini, D., Horváth, C., Salt-mediated retention of proteins in hydrophobic-interaction chromatography: Application of solvophobic theory. *J. Chromatogr.* 1984, 317, 67–85.
- [24] Lin, B., Straight through processing (STP) of monoclonal antibody purification. *BioProcess International Conference*, IBC Life Sciences Raleigh, North Carolina 2009.