Takamitsu Ichihara¹Takao Ito²Christopher Gillespie³¹Astellas Pharma Inc., Tokyo, Japan²Process Solutions, Merck Ltd., Tokyo, Japan³Process Solutions, MilliporeSigma, Bedford, MA, USA

Technical Report

Polishing approach with fully connected flow-through purification for therapeutic monoclonal antibody

The biopharmaceutical industry is evolving toward process intensification that can offer increased productivity and improved economics without sacrificing process robustness. A semi-continuous downstream process linking purification/polishing unit operations in series can reduce or eliminate intermediate holding tanks and reduce overall processing time. Accordingly, we have developed a therapeutic monoclonal antibody polishing template comprised of a connected flow-through polishing technologies that include activated carbon, cation exchange, and anion-exchange chromatography. In this report, we evaluated fully-connected pool-less polishing with three flow-through technologies, operating as a single skid to streamline and improve an mAb purification platform. Laboratory-scale pool-less processing was achieved without utilizing in-line pH adjustment and conductivity dilution based on the previously optimized single process parameter. Two connected flow-through configurations of polishing steps were evaluated: a two-step process using anion exchange and cation exchange and a three step process using activated carbon, anion exchange and cation exchange chromatography. Laboratory-scale proof of concept studies showed comparable performance between the batch purification process and the pool-less process configuration. Three step polishing highly intensified the processes and provided higher process loading and achieved bulk drug specification with higher impurity clearance (>95%) and high overall mAb yield (>95%).

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

Received: July 28, 2018; *accepted:* October 2, 2018

DOI: 10.1002/elsc.201800123

1 Introduction

Trends of pressure to reduce costs and increase productivity is driving the need for more innovative manufacturing within the biopharmaceutical industry. Significant upstream advances leading to high titer cell culture processes can create a downstream bottleneck requiring increases in chromatography resin volume, intermediate product hold tanks, and operation by large pump systems. Efforts to remove these bottlenecks is one form of process intensification, with an ultimate goal of delivering a less capital

intensive and more cost effective process with a smaller foot-print that can still meet the desired yield and performance [1]. Intensified production includes highly concentrated products and the combination of unit operations, minimized capital investment, streamlined validation process, increased manufacturing flexibility, and decreased total cost of goods while retaining product quality [2].

In a typical downstream process, the product pool between unit operations is collected in product pool tanks, adjusted for pH and/or conductivity, and then processed through the next unit operation. A number of strategies have been employed to intensify processes. Semi-continuous connected operations include direct chromatography column loading with tandem use of protein A elution and AEX chromatography [3].

Multicolumn continuous chromatography systems also allow for downsizing of chromatographic unit operations with continuous feeding and/or elution of cycling batch chromatography [4]. Although the switching operation of multiple pumps and valves is complicated, it can improve productivity with high

Correspondence: Dr. Takao Ito (takao.ito@merckgroup.com). DiverCity Tokyo Office Tower 15F, 1-1-20 Aomi, Koto-ku, Tokyo, 135-0064, Japan

Abbreviations: AC, activated carbon; AEX, anion exchange chromatography; CEX, cation exchange chromatography; HCP, host cell protein; HMW, high molecular weight; LMW, low molecular weight; mAb, monoclonal antibody

flow operation and reduced processing volume with improved binding capacity in bind/elute chromatography.

Fully connected pool-less processing with the combined unit operation for mAb downstream purification can be achieved using a simple single pump skid approach [5]. This concept was adopted to post-virus inactivated mAb recovered by protein A capture chromatography. Two flow-through purification/polishing chromatography steps were successfully combined in a single operation with a pool-less concept without intermediate product hold tanks [6]. The combination of depth filtration with the optimum buffer condition enabled flow-through operation comparable to batch protein A affinity chromatography. The resulting process time achieved from an entire flow-through purification process was estimated to be less than the batch chromatography train itself [7]. Adoption of flow-through polishing with replacement of bind/elute chromatography can significantly reduce the cost of unit operations [8].

In-line pre-concentration by single-pass tangential flow filtration (SPTFF) can improve the productivity of chromatography, that enhances the isotherm binding of chromatography and reduces the volume requirements for pH/conductivity adjustment [9].

The principle of connected flow-through includes the following steps; AC removes lower molecular weight impurities including HCP and DNA; AEX removes negatively charged impurities including acidic HCP, DNA and viruses; and CEX removes larger basic species including product related aggregates [10].

We recently tested a novel antibody purification process that offers significant operational advantages to traditional approaches. The feasibility of an all flow-through polishing process without buffer adjustment was studied [11]. This process template enables the possibility of pool-less processing with a single feed pump skid by utilizing optimum operating windows based on performance characteristics.

In the work described here, we tested applicability of the fully connected approach using three flow-through polishing columns for mAb purification with a single skid. In-series AC, flow-through CEX and AEX were used without inline pH/conductivity adjustment between each column. Laboratory-scale proof of concept studies were conducted to evaluate process loading, overall mAb yield, clearance of HCP, DNA, and product-related impurities.

2 Materials and methods

2.1 Materials

All materials used in this study were the same as the previous feasibility study [11]. AC powder used in Millistak+® CR40 devices was obtained from Merck KGaA (Darmstadt, Germany), HiTrap® Capto™ Q (AEX, cat#: 11-0013-02) was obtained from GE Healthcare (Buckinghamshire, UK) and Eshmuno® CP-FT resin provided by Merck KGaA (Darmstadt, Germany) was used for CEX. The mAb to be purified was a subclass IgG1 antibody with a molecular mass of about 150 kDa (*pI* 7.66), produced

in Chinese hamster ovary cells at Astellas. MAb was obtained as a frozen stock of post Protein A virus inactivation pool. All buffering chemical components were from Wako (Osaka, Japan), Kanto Chemical (Tokyo, Japan), and Merck KGaA (Darmstadt, Germany), unless stated otherwise.

2.2 Equipment

AC and CEX resin were individually packed into a Tricon™ 5 mm diameter x 2.5cmH columns at 0.5 mL (GE Healthcare, Buckinghamshire, UK). AEX was a 1 mL pre-packed column. The flow-through study was performed in-series on the fully automated liquid chromatography system, ÄKTA® explorer 100 (GE Healthcare, Buckinghamshire, UK). Two flow-through trains were tested: AC-AEX-CEX and AEX-CEX. Directly connected columns were installed onto the column position valve of chromatography system.

2.3 Connected flow-through chromatography

All columns were equilibrated using 25 mM sodium acetate buffer (15 mL) at pH 6 and conductivity 1.87 mS/cm. The polishing steps of the purification process had been previously optimized by DOE study [11]. The connected columns were loaded at the flow rate of 0.2 mL/min with a target of >1500 mg mAb loading at 200 mL (133 CV for AEX-CEX, 100 CV for AC-AEX-CEX, as CV=Feed volume/Total resin volume) with fractionation of the effluent every 20 mL (Total 10 fraction: Fr1 – Fr10). Loading conditions were adjusted to pH 6 and 4 mS/cm conductivity by buffer dilution and/or pH adjustment. This conditioning is easily adopted in manufacturing processes as the product of post low-pH virus inactivation is generally denatured. The residence times of columns were: AC = 2.5 min, AEX = 5 min, CEX = 2.5 min. Three cumulative loading results at 60, 120, and 180 mL were evaluated from the mixture of fractions to examine the impact of loading (60 mL loading = Fr1 ~ Fr3, 120 mL loading = Fr1 ~ Fr6, 180 mL loading = Fr1 ~ Fr9). After washing with 25 mM sodium acetate buffer (pH 6, 1.87 mS/cm, 10 CV) at the straightforward run, all columns were eluted using 25 mM sodium acetate buffer with 1M NaCl (pH 6, 83.9 mS/cm, 10 CV).

2.4 Analytical techniques

All samples collected were analyzed to determine cumulative yield, purity, HMW, LMW, DNA, and host cell protein. MAb concentrations were analyzed by HPLC-Protein A affinity chromatography using a POROS® A/20 affinity column (Life Technologies Japan Ltd, Tokyo) with a Shimadzu Prominence system (Shimadzu Corp., Kyoto, Japan). Analytical SEC for HMW and LMW was performed using a TOSOH TSKgel® G3000SWXL column (Tosoh Corp.) with a Shimadzu Prominence/Nexera X2 system. HCP was detected using a commercial microtiter plate ELISA method, CHO HCP ELISA kit (Cygnus Technologies). The residual host cell DNA was measured using quantitative PCR, 7500 fast real-time PCR system (Applied Biosystems).

Figure 1. Typical chromatograms obtained from the in-series, connected flow-through polishing steps. (A) AEX-CEX, (B) AC-AEX-CEX.

3 Results and discussion

Typical chromatograms obtained from the in-series, connected flow-through polishing steps (AEX-CEX and AC-AEX-CEX) are shown in Fig. 1. The product flow-through peak of the connected columns translates to a significant one-third reduction of processing time compared to traditional batch processing. The slight differences of starting flow-through peak between the two chromatograms are due to the hold-up volume (AEX-CEX = 23 min, AC-AEX-CEX = 29 min). Pre-column pressure of the loading step at 0.2 mL/min was quite low and is the pressure available for single-use pump systems at manufacturing scales. However, extremely high elution (stripping) pressure was a result of the in-series connection of the very small column.

Alternate elution methods such as a different buffer and/or single-use operation of resins might be considered to address this.

Breakthrough profiles were evaluated as shown in Fig. 2. A gradual increase of HCP level was detected with increased loading of the AEX-CEX train. Slight leakage of HCP in early loading (<328 mg) was the same level as previously reported for AEX [11]. Early breakthrough DNA was detected at a loading of 656 mg. Addition of AC resulted in a much better

clearance of HCP and DNA. Maximum HCP leakage was reduced to 70 ng/mg IgG, and DNA breakthrough was controlled until 1306 mg loading.

The first front of recovered mAb from the CEX column contains the most weakly retained mAb monomer that is essentially aggregate-free [12]. The mAb concentration of the first fraction was lower due to monomer binding to the CEX column, but rapidly saturated starting with the second fraction (328 mg loading). There were no significant differences in mAb recovery between the AEX-CEX or AC-AEX-CEX trains, indicating that the competitive binding kinetics of CEX is dominant in the connected flow-through train due to the higher selectivity of mAb components by CEX chemistry. The loadings of mAb 100% breakthrough are close to the sum of the required loading at 400 mg, calculated from single unit operation of previous study and resin volume of connected flow-through train (AC = 200 mg/mL resin at 0.5 mL, AEX = 100 mg/mL resin at 1 mL, CEX = 400 mg/mL resin at 0.5 mL). A slight HMW2 removal was provided during the early loading stage (<400 mg loading), however, the effluent concentration of HMW2 exceeded the inlet concentration at later loading. The competitive binding behavior of strongly bound aggregate with the faster breakthrough of monomer was observed for HMW1 but

Figure 2. Comparison of breakthrough profiles with change in impurities concentration. Feed conditions: mAb concentration = 8.2 mg/mL, HCP = 567 ng/mg IgG, DNA = 7276 pg/mg IgG, HMW1 = 0.78%, HMW2 = 1.65%, Monomer = 96.53%, LMW1 = 0.93%, LMW2 = 0.1%.

Figure 3. Analysis of cumulative effluent pools from connected flow-through polishing with different loading volume. (A) Yield, (B) HCP, (C) DNA, (D) Monomer, (E) HMW, (F) LMW. Bold dot line is target bulk drug specification.

not HMW2; 55% HMW1 breakthrough was observed 1500 mg loading. This is expected due to the change of loading conditions from pH 5 (used in the previous study) to pH 6 which might shift CEX selectivity to higher molecular weight aggregates. Analysis of cumulative pools of effluent from connected flow-through polishing with different loading volumes is shown in Fig. 3.

MAb purity with clearance of HCP and DNA was achieved using AC-AEX-CEX with a typical mAb bulk drug specification limit (monomer > 95%, HCP < 100 ng/mg IgG, DNA < 10 pg/mg IgG), even under very high loading conditions of 1500 mg. While loading of AEX-CEX was limited to 490 mg due to the issue of DNA clearance, this is comparable to that of a typical

mAb AEX process. However, when trying to obtain a yield of 95% using AEX-CEX, the target drug specification cannot be achieved. The feed characteristics of the mAb used in this study had a high purification burden of HCP and DNA, while the need for aggregate removal was relatively small. Use of AC intensified the AEX, and the combination of AC and AEX easily achieved the robust clearance of HCP and DNA and delivered a higher product yield. Removal of LMW2, was also achieved by AC.

The results of this connected flow-through polishing approach demonstrate higher process capacity compared with the loading of bind and elute CEX and AEX without AC. Required resin volume of CEX and AEX was reduced by a factor of 75 and 15, respectively. Reduction of the resin volume and single equilibration and elution steps for all connected columns reduced buffer usage by roughly -95% compared with a traditional process (CEX at Bind/Elute e.g. 40 g/L resin, AEX at flow-through e.g. 100 g/L resin). In summary, process compression can be achieved with reduced chromatography media volumes, reduced buffer usage, single system operation and removal of the intermediate hold tank.

4 Concluding remarks

Biopharmaceutical process development and manufacturing will continue to explore and adopt integrated and continuous processing in order to achieve greater efficiencies and productivity. This study demonstrates integrated flow-through polishing of therapeutic mAb purification, in which all steps are connected in-series in a pool-less operation without use of intermediate product hold tanks. Connection of all flow-through polishing steps was successful due to simplicity of the operating mode without the need for in-line pH adjustment and conductivity dilution from the optimal loading conditions.

The principle of connected flow-through reported here was composed of the following steps; AC removal of LMW impurities including HCP and DNA; AEX removal of negatively charged impurities including acidic HCP, DNA; and CEX removal of larger basic species including product related aggregates. This is a robust and powerful tool that can significantly simplify processes, improve process efficiency, and consistently and significantly reduce cost, making it attractive for adoption into current mAb templates. Connected flow-through polishing intensified the processes, providing higher process loading combined with high overall mAb yield and higher impurity clearance. These technologies can be used to replace individual unit operations or be combined to offer a complete process template for antibody purification. The new process offers increased integration between unit operations and has been designed to ultimately enable continuous processing.

Adoption of these new processes will require further engineering and have operational and regulatory challenges that need to be addressed including monitoring, sampling, analytical control, batch definition, validation studies, and documentation. While there is more work to do, the advantages and potential benefits are well-recognized, and must be understood and evaluated

against factors including technology challenges and regulatory guidance.

Practical application

The described connected flow-through technologies train may be used to replace the traditional batch polishing process, resulting in substantial simplification, and reduced chromatography media volume requirements. These technologies can provide significant cost savings compared to current processes and, when applied to future template processes, offer higher productivity through shorter process times with the potential to enable production using a single processing skid. Single skid operations with process integration between unit operations eliminate the need for intermediate tanks, an important benefit for compact facilities.

The author thanks Chihoko Kobayashi and Toshie Katakura for assistance of experimental study, as well as Michael Phillips and Yasuhiko Kurisu for discussions.

The authors have declared no conflict of interest.

5 References

- [1] Montgomery, A., Scott, C., Rios, M., The state of the Industry, Chapter 8, the manufacturing perspective: current approaches to bioprocess intensification. *BioProcess. Int.* 2017, 15, 1–10.
- [2] BioPhorum Operations Group, Biomanufacturing technology roadmap. Executive summary. 2017, July. <https://www.biophorum.com/category/resources/technology-roadmapping-resources/introduction/>
- [3] Shamashkin, M., Godavarti, R., Iska, T., Coffman, J., A tandem laboratory scale protein purification process using protein A affinity and anion exchange chromatography operated in a weak partitioning mode. *Biotechnol. Bioeng.* 2013, 110, 2655–2663.
- [4] Warikoo, V., Godawat, R., Brower, K., Jain, S. et. al., Integrated continuous production of recombinant therapeutic proteins. *Biotechnol. Bioeng.* 2012, 109, 3018–3029.
- [5] Phillips, M., Mann, F., Ribault, S., Continuous mAb processing. *Euro Biotech. News* 2013, 12, 40–41.
- [6] Zhang, J., Conley, L., Pieracci, J., Ghose, S., Pool-less processing to streamline downstream purification of monoclonal antibodies. *Eng. Life Sci.* 2017, 17, 117–124.
- [7] Yamada, T., Yamamoto, K., Ishihara, T., Ohta, S., Purification of monoclonal antibodies entirely in flow-through mode. *J. Chromatogr. B.* 2017, 1061–1062, 110–116.
- [8] Xenopoulos, A., A new, integrated, continuous purification process template for monoclonal antibodies: process

- modeling and cost of goods studies. *J. Biotechnol.* 2015, 213, 42–53.
- [9] Merck KGaA, Intensified polishing using single-pass tangential flow filtration (SPTFF) with anion exchange chromatography. Application note AN5364EN00, 10/2017.
- [10] Gillespie, C., Kozlov, M., Phillips, M., Potty, A. et. al., Integrating continuous and single-use methods to establish a new downstream processing platform for monoclonal antibodies, in: Subramanian, G. (Ed.), *Continuous Processing in Biopharmaceutical Manufacturing*, Wiley-VCH Verlag, Weinheim 2015, 71–95.
- [11] Ichihara, T., ITO, T., Kurisu, Y., Galipeau, K. et. al., Integrated flow-through purification for therapeutic monoclonal antibodies processing. *Mabs* 2018, 10, 325–334.
- [12] Suda, E.J., Thomas, K.E., Pabst, T.M., Mensah, P., et al., Comparison of agarose and dextran-grafted agarose strong ion exchangers for the separation of protein aggregates. *J Chromatogr. A.* 2009, 1216, 5256–5264.