Integrated process consisting of column chromatography followed by virus filtration for plasma IgG and mAb purification at constant flow rate: analysis of filtration behavior using clogging models and viral clearance tests

Hironobu Shirataki,[1] Yoshiro Yokoyama,[2] Hiroki Taniguchi,[3] Miku Azeyanagi[2]

[1]Scientific Affairs Group, Bioprocess Division, Asahi Kasei Medical Co., Ltd., 1-1-2 Yurakucho, Chiyoda-ku, Tokyo 100-0006, Japan

[2]Technology Development Department, Bioprocess Division, Asahi Kasei Medical Co., Ltd., 5-4960, Nakagawara-machi, Nobeoka-shi, Miyazaki-ken 882-0031, Japan

[3]New Products Development Department, Bioprocess Division, Asahi Kasei Medical Co., Ltd., 2-1 Samejima, Fuji-shi, Shizuoka 416-8501, Japan

Corresponding author: Hironobu Shirataki, Scientific Affairs Group, Bioprocess Division, Asahi Kasei Medical Co., Ltd., 1-1-2 Yurakucho, Chiyoda-ku, Tokyo 100-0006

email: shirataki.hb@om.asahi-kasei.co.jp

Short running title: Integrated process for continuous processing

Abstract

We evaluated filtration behavior and virus removal capability for a mAb and plasma IgG under constant flow rate directly following flow-through column chromatography in an integrated process. For mAb solution with quantified host cell protein (HCP) content processed in flow-through mode on in-series mixed-mode AEX and modified CEX columns connected to the Planova BioEX filter (pool-less), HCP logarithmic reduction value (LRV) of 2.3 and 93.9% protein recovery were achieved for the process. Filtration behavior for 5 to 15 mg/mL plasma IgG run at flux of 10 to 100 LMH to 100 L/m^2 throughput on Planova BioEX filters showed similar behavior across the protein concentrations tested although filtration pressure increased with throughput at 50 LMH and above, indicating the suitability of lower flux processes for continuous processing. Comparing both plasma IgG and mAb filtration behavior to four clogging models showed little difference in fit among the models, but with slightly better fit to the cake filtration model. Viral clearance tested by in-line spiking X-MuLV or MVM into purified plasma IgG showed robust removal at low flux. Integrating the Planova BioEX filter into continuous processes with column chromatography can achieve efficient downstream processing with reduced footprint and process time.

Keywords: chromatography, integrated process, virus filtration

Introduction

The growth of the biologics market since 2000 has been driven by increasing production of monoclonal antibodies (mAb), which is in turn driving the demand for development of more efficient upstream processes (USP) and downstream processes (DSP). Significant progress has been made in USP, resulting in 100-fold improvements in space and time use in the past 10 years (Halan & Minas, 2020; Rader & Langer, 2015). However, the lack of comparable advances in DSP is reported to have caused a bottleneck in production efficiency (Gronemeyer et al., 2014; Konstantinov & Cooney, 2015). One approach to address this bottleneck is to transition production of biologics from batch processes with intermediate pool tanks with process pauses between each step to continuous processes with more efficient and steady production. For example, perfusion cell culture that allows continuous clarification of cell culture (Pollock et al., 2013; Schmidt, 2017; Schmidt & Wieschalka, 2017) can be paired with simulated moving-bed (SMB) technology that allows continuous chromatography (Warikoo et al., 2012). Likewise, low pH virus inactivation is usually conducted in batch mode, but progress has been made to convert this step to a continuous process (Klutz et al., 2016). Despite these developments in biologics production, there are few commercial biologics produced by fully continuous processes due to the extreme difficulty in stabilizing and integrating each step of the process. In addition to process control issues, methodology for confirming the viral clearance for constant flow rate integrated processes should also be considered. Alternatively, hybrid systems that incorporate batch and continuous processes in production steps from cell culture to protein purification have been identified as a practical option and such integrated processes are being evaluated.

While batch processing at constant pressure may be preferred due to ease of control of the process, the final virus filtration can be integrated with column chromatography and operated under constant flow rate. Development of the technology for implementing virus filtration in continuous processes is ongoing. One challenge is balancing the throughput capacity of column

chromatography (column volume) and virus filter (effective surface area), as well as the flow rate across the system. The robustness of the virus filtration including any process pauses that may occur when switching feed stocks must be verified. Additionally, since column chromatography is generally conducted at constant flow rate, it is necessary to conduct long duration constant flow rate virus filtrations. Another challenge to overcome is characterizing filtration stability. Virus filters are generally used in constant pressure mode, so there are concerns about pressure stability in long duration constant flow rate filtrations (Halan & Minas, 2020). Specifically, increased filtration pressure due to pore clogging and virus breakthrough due to low filtration pressures arising from the low flow rates are concerns. In a continuous process with a constant filtration pressure of 0.1 to 0.5 bar for 24 to 96 h and a process pause for a mAb with relatively low concentration (0.3 g/L), stable filtration and virus removal were achieved (Kleindienst et al., 2019). Long duration virus filtration of PP7-spiked IgG for 4 days with Planova 20N and BioEX filters showed robust PP7 removal rates for both filter types even when challenged with a simultaneous spike in protein concentration, conductivity and PP7 concentration (Lute et al., 2020). These studies suggest that although long duration processes under typical process conditions may be difficult to achieve, there are ways to implement virus filtration into continuous processes.

While integrated processes running at constant flow rate are capable of reducing process footprint and processing time, it is necessary to evaluate the stability of virus filtration before adoption in production processes. In this study, we evaluated the possibility of integrating virus filtration with column chromatography in a continuous or hybrid process. HCP removal and mAb recovery were evaluated for the integrated process with AEX and CEX chromatography directly connected to virus filtration. Additionally, a viral clearance test was conducted at constant flow rate by in-line spiking MVM or X-MuLV to plasma IgG in an integrated process with AEX or CEX chromatography in series with virus filtration. The studies included a process pause followed by buffer wash. Virus filtration of MVM-spiked plasma IgG was conducted at

low constant flow rates over long durations with a process pause to evaluate effects of low flow rates and long durations. Finally, filtration behavior at constant flow rate was theoretically investigated using clogging models for plasma IgG and mAb filtration runs.

Materials and Methods

Monoclonal antibody (mAb)

The mAb used in this study was provided by Manufacturing Technology Association of Biologics (MAB), Japan. The mAb (pI 8.2) was grown in CHO cell culture by fed-batch method (mAb concentration, 3.5 mg/mL; HCP concentration, about 80000 ng/mL), separated from cells and applied to a bind-elute affinity chromatography column with Protein A resin (MabSelect SuRe, GE Healthcare). The clarified cell culture fluid (mAb concentration, 30 mg/mL; HCP concentration, about 9000 ng/mL) was diluted with buffer (20 mM Tris-Acetate, 100 mM NaCl; pH 5, 6, 7 or 8) to produce 10 mg/mL mAb solution with HCP concentration of about 1500 or 3000 ng/mL for use in preliminary experiments for chromatography column selection. A 10 mg/mL mAb solution with the same buffer at pH 6.5 with HCP concentration of 3800 ng/mL was prepared for processing in an integrated process with column chromatography and filtration with a virus filter. HCP in the mAb solution was analyzed using CHO Host Cell Protein ELISA 3G Kit (Cygnus Technologies).

Plasma IgG

Venoglobulin IH5 (50 mg/mL) provided by Japan Blood Products Organization (JBPO) was diluted to 5, 10 or 15 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 5.0 and used for filtration experiments. For runs with plasma IgG solution spiked with virus, 5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 with 1% virus spike (MVM or X-MuLV) was used. $TCID_{50}$ assay was conducted to measure the virus titer in the load solution and

processed solution.

Chromatography resins

Preliminary experiments were conducted to evaluate HCP removal and protein recovery for mAb solutions under flow-through mode for each type of AEX and CEX resin. For AEX resins, normal AEX (Cellufine MAX Q-h) and mixed-mode AEX (weak AEX of primary amine in combination with weak HIC of butyl group, Cellufine MAX IB) were evaluated, and for CEX resins, normal CEX (Cellufine MAX S-r), grafted CEX (Cellufine MAX GS) and modified CEX (cross-linked cellulose with dextran sulfate, Cellufine DexS-HbP) (all resins supplied by JNC Corporation) were used.

Virus filter

For filtration of both plasma IgG and mAb solutions, 0.0003 m^2 Planova BioEX filters (Asahi Kasei Medical) were used.

Integrated system control with AKTA

The AKTA pure 25 or the AKTA avant 25 (GE Healthcare) was used to control the filtration and/or chromatography column processes. AKTA pure 25 was used for all studies except the plasma IgG filtration. . The chromatography column(s) were connected to the column valve and the virus filter was connected to the outlet valve. For the integrated mAb process, the filter was positioned after the flow restrictor. AKTA avant 25 was used for plasma IgG filtration by connecting the filter to the column valve. The flow restrictor was removed to ensure that the pressure monitor displays filtration pressure, and pressure was recorded using the PreC pressure monitor on the AKTA avant 25.

Processing mAb solution in an integrated process

In a preliminary study, AEX (normal AEX and mixed-mode AEX) and CEX (normal CEX, grafted CEX and modified CEX) resins were evaluated individually for protein recovery and HCP removal using 10 mL of 10 mg/mL mAb in 20 mM Tris-Acetate, 100 mM NaCl at pH 5, 6, 7 or 8 with HCP concentration of about 3000 ng/mL (for AEX resins) or 1500 ng/mL (for CEX resins) on 0.5 mL columns (0.25 mL/min). The HCP logarithmic reduction value (HCP LRV) and protein recovery were determined, and mixed-mode AEX and modified CEX were selected for use in the integrated process study conducted with buffer at pH 6.5.

Mixed-mode AEX and modified CEX (both with CV of 5 mL) were run in series in a pool-less integrated process with a 0.0003 m^2 Planova BioEX filter on an AKTA pure 25 as shown in Figure 1. Washing and equilibration with the equilibration buffer (20 mM Tris-Acetate, 100 mM NaCl, pH 6.5) were conducted on the chromatography columns and filter independently. The system was filled with equilibration buffer, and 190 mL of 10 mg/mL mAb solution with 3800 ng/mL HCP at 9 mS/cm was loaded on the system at 380 mg mAb/mL-resin and at 633 L/m^2 (6333 g/m^2) to the filter, followed by 50 mL of equilibration buffer. The system ran with a flow rate of 0.2 mL/min (2.4 CV/h) and 40 LMH to the filter. HCP LRV and protein recovery were determined for the mAb flow-through fraction and for the flow-through fraction with the wash flush.

Filtration of plasma IgG solution with a virus filter

After washing and equilibration with 20 mM sodium acetate, 100 mM NaCl, pH 5.0 buffer, plasma IgG solution (5, 10 or 15 mg/mL) in 20 mM sodium acetate, 100 mM NaCl buffer at pH 5.0 was filtered at constant flow rates corresponding to 10, 20, 50 or 100 LMH on a 0.0003 m^2 Planova BioEX filter to a target throughput of 100 L/m^2. Filtration was conducted using AKTA avant 25 (GE Healthcare). After prefiltering the sample solution with a 0.2 μm microfilter (Minisart RC 25 mm, Sartorius), the solution was filled into Superloop 150 mL (GE Healthcare), and the solution was loaded to the virus filter using the system pump.

Virus removal capabilities of column chromatography

Virus removal capabilities of column chromatography was evaluated individually without a virus filtration step using AKTA pure 25. The viral clearance study was conducted by ViruSure (Vienna, Austria). The chromatography columns used were mixed-mode AEX, grafted CEX and modified CEX. The load solution was 30 mL of 5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 with 1% virus spike (MVM or X-MuLV) and was loaded by sample pump.

Viral clearance for integrated system with plasma IgG

The in-line spiking viral clearance study was designed and conducted by ViruSure (Vienna, Austria). The setup and test conditions for loading protein solution from the chromatography column to the virus filter are shown in Figure 2. In this study, 30 mL of plasma IgG solution with a concentration of 5 mg/mL adjusted to 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 buffer condition was used. Two viruses with different sizes, MVM and X-MuLV, were used. AKTA pure 25 was used for this test. The virus filter and 0.5 mL column volume (CV) chromatography column (mixed-mode AEX, grafted CEX or modified CEX) were directly connected without pooling. Before loading the protein solution, the chromatography column and virus filter were independently washed and equilibrated. The non-virus spiked protein solution was connected to the system pump and 10% virus spiked protein solution (MVM or X-MuLV) was connected to the sample pump. The log titers of MVM and X-MuLV loaded onto the virus filter were 6.8 and 4.9, respectively. The non-virus spiked protein solution was pumped at a flow rate of 0.225 mL/ min from the system pump to the chromatography column. After passing through the chromatography column, the non-virus spiked protein solution passed through the UV monitor, conductivity monitor and flow restrictor, and the 10% virus spiked protein solution was added using the sample pump at a flow rate of 0.025 mL/min. The virus spiked protein solution passed

through the pressure monitor and in-line mixer and was fed to the virus filter at constant flow rate. As a result, 1% virus spiked protein solution was fed to the virus filter at a constant flow rate of 0.25 mL/min. For the effective surface area of the Planova BioEX virus filter used (0.0003 m^2), the flux was 50 LMH. At the point when 30 mL of protein solution was fed to the virus filter, both pumps were stopped for a 35 min process pause in order to change the feed solution to the equilibration buffer to wash the chromatography column. Then, only the system pump was turned on and the equilibration buffer was fed to the setup at a flow rate of 0.25 mL/min in order to wash out the residual protein solution from the chromatography column and the virus filter. Using this procedure, a viral clearance test at constant flow rate was conducted, and the effect of a process pause was evaluated. The load was 270 mg plasma IgG/mL-resin or 54 mL/mL-resin for each column and 500 g plasma IgG/m^2 or 100 L/m^2 for the virus filter.

Viral clearance at low flux with plasma IgG

The low flux viral clearance study was conducted by ViruSure (Vienna, Austria). The setup and test conditions are shown in Figure 3. In this study, 30 mL of 5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 solution spiked with 1% MVM was filtered at 5, 10 and 20 LMH. The log titers of MVM were 7.23, 7.38 and 7.56 for the 5, 10 and 20 LMH runs, respectively. Following plasma IgG solution filtration and a 35 min process pause, 5 mL of equilibration buffer wash was conducted. Viral clearance was measured for the plasma IgG solution permeate and buffer wash permeate.

Clogging model analysis

Filtration behavior of the virus filter at constant flow rate was evaluated with the following four clogging models: cake filtration, intermediate blocking, standard blocking and complete blocking. Clogging models assume the filter to be a collection of cylindrical pores with uniform length and inner diameter. In these models, filtration behavior can be expressed theoretically by

calculating the change in flow resistance based on the types of substances causing clogging. Equations expressing the relationship between filtration volume and pressure for constant flow rate filtrations for the four clogging models, which differ by the condition of the retained substances that cause clogging, are shown below (Grace, 1956; Sumiya, 2013).

Cake filtration model

In this clogging model, the substances causing clogging do not block the cylindrical pores but rather adhere to the surface of the filter, causing the formation of flow paths that are new and different from those of the filter material.

$$p = p_0 (1 + kV)$$
(1)

$$\frac{p}{p_0} - 1 = kV$$
(2)

where p_0 is the initial transmembrane pressure, p is the transmembrane pressure at the filtration volume, V, k is the clogging factor specific to the solution being filtered.

Intermediate blocking model

In this clogging model, the substances causing clogging accumulate on already trapped substances and the entrances of the cylindrical pores. The substances causing clogging are distributed between the entrance of the pores and already clogged pores.

$$p = p_0 \exp(kV)$$
(3)

$$\ln\left(\frac{p}{p_0}\right) = ¿ kV ¿$$
(4)

Standard blocking model

In this clogging model, the substances causing clogging are distributed evenly over the inner

surface of the cylindrical pores, and the inner diameter of the pores gradually becomes smaller.

$$p = \frac{p_0}{(1 - kV/2)^2}$$

(5)

$$2 \left\{ 1 - \left(\frac{\Delta p_0}{\Delta p} \right)^{1/2} \right\} = kV$$

(6)

Complete blocking model

In this clogging model, the substances causing clogging accumulate at the entrance of the pores, and accumulation continues until all cylindrical pores become completely clogged.

$$p = \frac{p_0}{(1 - kV)}$$

(7)

$$1 - \frac{p_0}{p} = kV$$

(8)

Applying Equations 2, 4, 6 and 8 that represent each clogging model for the filtration volume and pressure obtained from filtration experiments, the slope of the left side of each equation plotted against V was used to determine k, the clogging factor, and the experimental results and calculated filtration behavior were compared. Equation 9 was used to calculate the average pressure difference (Δp) between the experimental value of the filtration pressure obtained from filtration experiments and the filtration pressure obtained from each clogging model.

$$average \, \Delta p = \frac{1}{N} \sum_{i=1}^{N} \sqrt{(p_{exp,i} - p_{cal,i})^2}$$

(9)

where $p_{exp,i}$ and $p_{cal,i}$ are the ith filtration behavior measurement for experimental and calculated

pressure values and N is the number of experimental measurements for each solution.

Results and Discussion

Integration of flow-through chromatography and filtration in a mAb process

Chromatography processing with AEX (normal AEX and mixed-mode AEX) and CEX (normal CEX, grafted CEX and modified CEX) resins used separately at various pH values in a preliminary study shows a trade-off between HCP removal and protein recovery (Figure 4). The optimal resin types and pH values were selected based on results obtained separately for each resin type. For 10 mg/mL mAb with a starting HCP concentration of about 3000 ng/mL for AEX column chromatography runs and about 1500 ng/mL for CEX column chromatography runs. For AEX resins, HCP LRV on mixed-mode AEX was around 1 at pH 5 and around 2 at pH 7, which is markedly higher than for normal AEX, which had a maximum HCP LRV of around 1. For CEX resins, modified CEX showed the highest HCP removal rate, followed by grafted CEX and normal CEX, and these differences were more pronounced at lower pH. For modified CEX, HCP LRV was 1.5 at pH 5 but decreased to around 0.5 at pH 7. While AEX resins showed high protein recovery (95% at pH 5 to 7), protein recovery for CEX was around 70% or less at pH 5 and reached 80% to 90% at pH 6 to 8.

Considering the trade-off between HCP removal and protein recovery for these two resin types along with the dependence on pH, mixed-mode AEX and modified CEX were selected for use in series with 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 buffer at constant flow rate on the set-up shown in Figure 1. The profiles of UV absorbance, filtration pressure and conductivity during the process (Figure 5) show an increase in pressure when the protein solution reaches the virus filter after displacing equilibration buffer from the system piping and both chromatography columns. Thereafter, UV absorbance and filtration pressure remained stable, and after switching back to equilibration buffer following the predetermined load of protein,

there was a momentary pressure drop at 200 mL. As protein was pushed out from the column by the equilibration buffer wash (50 mL), the protein concentration decreased and the filtration pressure also gradually decreased. For the total collected volume of 250 mL, good protein recovery of 93.9% and good HCP removal to 17.7 ng/mL (2.94 ppm, HCP LRV of 2.3) were achieved. Although HCP increases due to including of the buffer wash, the improvement in protein recovery is significant.

These results demonstrate that columns in series can be effectively integrated with virus filtration. Constant flow rate protein solution loading with an integrated process consisting of column chromatography and virus filtration connected directly without pooling is an efficient process in which the columns effectively remove impurities, allowing stable filtration pressure for the integrated virus filtration.

Characterization of pressure in filtration of plasma IgG

The filtration behavior (pressure) for 5, 10 and 15 mg/mL plasma IgG solution on a virus filter under constant flow rate corresponding to 10, 20, 50 and 100 LMH is shown in Figure 6. Following a pressure dip at 0 L/m^2 due to switching from equilibration buffer (20 mM sodium acetate, 100 mM NaCl, pH 5.0) to the protein solution, the filtration pressure was stable throughout the protein filtration, followed by a decrease in pressure at 100 L/m^2 when the feed solution was changed to the equilibration buffer. The slight pressure increases were mostly proportional and were higher with higher flux and protein concentration. For filtrations conducted at 20 LMH, the filtration pressure was extremely low and almost no pressure increase during the run was observed.

For all plasma IgG concentrations and flow rates, the filtration pressure was stable, and there were no irregular pressure changes during protein filtration in this test, demonstrating the robustness of the virus filter used for this test.

Viral clearance for integrated systems with plasma IgG

The in-line spiking methods that have been demonstrated as being suitable for constant pressure filtrations (Genest et al., 2013; Wu et al., 2008) may also be applicable to integrated processes with column chromatography. To conduct viral clearance tests for continuous constant flow rate processes, some adjustments from a constant pressure setup are needed. Following the strategy for constant pressure processing with in-line spiking, a pump was used to supply a concentrated virus spike (X-MuLV or MVM) in plasma IgG to the feed stream after the chromatography column (mixed-mode AEX, grafted CEX and modified CEX) and before the filter without pooling (Figure 2). X-MuLV and MVM removal by the Planova BioEX filter following processing in an integrated column chromatography process shows complete removal (no virus was detected in the $TCID_{50}$ assay) for all three resins with virus LRV greater than 3 for X-MuLV and greater than 5 for MVM for both the flow-through fraction and flow-through with wash (**Table 1**). These results demonstrate that under the test conditions in this study, the virus removal capability of Planova BioEX filter connected to the chromatography column is robust. Additionally, no virus was detected in the permeate collected after the 35 min process pause, confirming that the virus filter used in the test has a very robust virus removal capability despite having a process pause.

To confirm the virus removal capability of the chromatography column, a viral clearance test was conducted with the same load to each of the mixed-mode AEX, grafted CEX and modified CEX chromatography columns. The X-MuLV LRV of the flow-through fraction excluding the equilibration buffer fraction was 1.96, 0.21 and 0.38, respectively, and that for MVM LRV was 2.99, -0.16 and 0.45, respectively. These results show that for the mixed-mode AEX flow-through chromatography process, some viral clearance is achieved, while the CEX resins show hardly any removal. Thus, virus filtration is necessary in these processes.

Viral clearance at low flux with plasma IgG

Considering the possibility of a continuous process that connects perfusion cell culture, multi-column chromatography systems and continuous low pH virus inactivation over long durations, virus filtration conducted over a long duration at low flow rates with stable pressure is required. To evaluate the effects of constant and low flow rate filtration with a process pause on virus removal, we used plasma IgG spiked with MVM at flow rates corresponding to 5, 10 and 20 LMH and a 35 min process pause followed by a wash with 5 mL of equilibration buffer as shown in Figure 3. MVM was used for the test because it has a small size, and there is a concern for virus breakthrough with low flow rate filtrations. The volume of protein solution fed to the filter was 30 mL for all runs, and filtration duration was 1200, 600 and 300 min respectively. All runs, including the wash after the process pause, showed good viral clearance with MMV LRV of 5 or higher, and no virus was detected from the filtrate of any of the runs (**Table 2**). Planova BioEX filters used in this study showed robust virus removal capabilities even with a process pause and the low flux and long duration conditions expected in continuous processes.

Clogging model analysis of integrated process with plasma IgG and mAb

Evaluation of filtration behavior with clogging models allows characterization of the process for further process development. In these plasma IgG and mAb processes controlled with constant flow rate, plotting the experimental and calculated filtration pressure against the throughput gives insights into the clogging mechanism and the potential capacity for processing at larger scales. Experimental pressure profiles for the 10 mg/mL plasma IgG filtrations shown in Figure 6 are plotted with the calculated pressure profiles for the clogging models (cake, intermediate, standard and complete) individually for the 10, 20, 50 and 100 LMH runs in Figure 7. The calculated filtration pressure profiles for all clogging models were nearly identical up to 100 L/m^2 throughput. For the 10 LMH run, the pressure increase was very small, and experimental values showed good agreement with all clogging models. For 20, 50 and 100 LMH runs, the experimental pressure is higher than the calculated pressure at the start of the run and then

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switches to being lower than the calculated pressure after about 70 L/m^2. Clogging model results for all flux conditions showed good agreement with experimental results, which suggests that the clogging models are capable of reproducing experimental results for practical pressure ranges. However, because the filtration pressure is markedly lower than the maximum allowable pressure for the virus filter, pressure data from even larger throughputs will be needed in order to evaluate the predictive ability of these clogging models for larger constant flow rate processes.

Clogging factor was shown to be proportional to volume of the substances retained by the filter (Grace, 1956; Sumiya, 2013), and a larger variety of substances are expected to be retained by a filter operated with higher flux. The relationship with flux for clogging factors obtained for 10 mg/mL runs shows a roughly proportionally increasing trend with higher flux (Figure 8a). The calculated pressure profiles did not differ with clogging model, likely due to the very minimal clogging that occurred in these runs. Plotting the difference in filtration pressure between experimental values and modeling results for the 10 to 100 LMH runs calculated using Equation 9 reveals the slight differences among the clogging models (Figure 8b), showing that the cake filtration model has the closest fit with the experimental values and that the largest differences between models are at higher fluxes. The cake filtration model was found to be the best representation of virus filtration behavior for constant pressure virus filtration runs (Shirataki, in preparation), and determining the best fit model for each process is important for evaluating virus filtration behavior with protein solutions.

Clogging model analysis can also be used to plan the scale up of processes. For the integrated mixed-mode AEX and modified CEX mAb process with a Planova BioEX filter, clogging model analysis was applied to the 540 L/m^2 portion of the mAb filtration (up to 200 mL shown in Figure 5). As shown in Figure 9a, the average difference in filtration pressure between experimental values and modeling results was smallest for the cake filtration model. Even for this much larger throughput of 540 L/m^2 for the mAb process compared to 100 L/m^2 for the

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plasma IgG process, the clogging factor, k, for each of the clogging models for the mAb was almost one order of magnitude smaller than for plasma IgG (k is about 0.001 for 50 LMH as shown in Figure 8a), and all were similar with 0.00023 for cake filtration, 0.00022 for intermediate blocking, and 0.00021 for standard blocking and complete blocking models. Thus, the mAb solution processed by mixed-mode AEX and modified CEX column chromatography had a markedly higher filterability than the plasma IgG applied directly to the virus filter used in this study.

While the clogging models were not differentiated for the experimental results up to 540 L/m^2, applying each of the clogging models to extrapolation of this run to 3500 L/m^2 shows the range of scenarios should the process follow any of these clogging models. For the complete blocking model, the maximum allowable filtration pressure of 0.35 MPa is reached at this throughput, but the rise in filtration pressure is expected to be less drastic for the standard blocking model, intermediate blocking model, and cake filtration models, in that order, over this throughput range as shown in Figure 9b. The cake filtration model, which was identified as best matching experimental data, predicts that filtration pressure will remain below 0.2 MPa at 3500 L/m^2, suggesting that a markedly larger throughput can be achieved for these constant flow rate filtration conditions. However, these clogging model predictions are based on experimental results for less than 600 L/m^2, and the accuracy of these predictions for higher throughput has not been verified. It is also worth considering whether combined clogging models suggested for constant pressure filtration processes (Bolton and Apostolidis, 2017; Bolton et al., 2006; Ho & Zydney, 2000) are also applicable to constant flow rate conditions.

We confirmed that integrating a chromatography step without pooling before the virus filtration step can be used to effectively improve the filterability of the protein solution in a virus filtration operated at low flux in constant flow rate mode. In a mAb process the combination of a mixed-mode AEX and modified CEX column used in series effectively removed HCP. Even for long duration filtration at low flux with a process pause, a high virus removal capability was

confirmed. Thus, the Planova BioEX filter is highly applicable for continuous processing. This integration could lead to large reduction in footprint and process time in DSP processes, and is significant for realizing an efficient continuous process.

Acknowledgements

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Tables

Table 1 X-MuLV and MVM clearance for constant flow rate in-line spiking test of 5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 for integrated processes consisting of chromatography and virus filtration

		Virus LRV[†] (log $TCID_{50}$/mL)		
Virus spike		Mixed-mode AEX⇨Planova BioEX	Grafted CEX⇨Planova BioEX	Modified CEX⇨Planova BioEX
X-MuLV	Flow-through fraction	≥3.75	≥3.62	≥3.50
	Flow-through + Wash	≥3.39	≥3.26	≥3.12
MMV	Flow-through fraction	≥5.56	≥5.50	≥5.56
	Flow-through	≥5.19	≥5.13	≥5.19

+ Wash	

†Log titers of X-MuLV and MVM loaded onto the virus filter were 4.9 and 6.8, respectively.

Table 2 MVM clearance of 30 mL of 5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5 for virus filtration at various constant flow rates

Flow rate	MMV LRV† (log $TCID_{50}$/mL) at various flow rates		
	0.025 mL/min (5 LMH)	0.05 mL/min (10 LMH)	0.1 mL/min (20 LMH)
Filtrate sample	≥5.27	≥5.40	≥5.59
Filtrate sample + Wash	≥5.13	≥5.24	≥5.43

†The log titers of MVM loaded onto the virus filter were 7.23, 7.38 and 7.56 for the 5, 10 and 20 LMH runs, respectively.

Figure legends

Figure 1 Setup for integrated process consisting of mixed-mode AEX, modified CEX and a virus filter connected in series for processing of mAb solution (10 mg/mL mAb in 20 mM Tris-Acetate, 9 mS/cm, pH 6.5). AKTA pure 25 was used for this setup.

Figure 2 Setup for viral clearance test using an integrated process with in-line spiking with virus (MVM or X-MuLV) consisting of one IEX chromatography column (mixed-mode AEX, grafted CEX or modified CEX) and a virus filter connected in series for processing of plasma IgG solution (5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5). AKTA pure 25 was used for this setup.

Figure 3 Setup for the constant flow rate MVM clearance test with plasma IgG (5 mg/mL plasma IgG in 20 mM Tris-Acetate, 100 mM NaCl, pH 6.5). Filtration runs were conducted at 5, 10 and 20 LMH.

Figure 4 HCP reduction and protein recovery for flow-through processing of mAb solution with 0.5 mL CV AEX and CEX column chromatography. (a) HCP reduction with AEX. (b) HCP reduction with CEX. (c) protein recovery with AEX. (d) protein recovery with CEX. For all runs, mAb at 10 mg/mL in 20 mM Tris-Acetate, 100 mM NaCl, pH 5, 6, 7 or 8 was processed at 0.25 mL/min (0.5 CV/min) with 200 mg/mL-resin load. HCP concentration in the load solution was about 3000 ng/mL for AEX column chromatography runs and 1500 ng/mL for CEX column chromatography runs.

Figure 5 Absorbance of UV at 280 nm, filtration pressure and conductivity profiles obtained from the mAb processing setup shown in Figure 1. Protein recovery and HCP concentration are

noted in the figure. mAb at 10 mg/mL in 20 mM Tris-Acetate, 9 mS/cm, pH 6.5 with 3800 ng/mL HCP and a buffer wash were processed.

Figure 6 Transmembrane pressure during filtration of plasma IgG solution shown as (a) 5 mg/mL plasma IgG, (b) 10 mg/mL plasma IgG and (c) 15 mg/mL plasma IgG. For all filtrations, 100 L/m^2 of plasma IgG at 5, 10 or 15 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 5.0 was filtered at 10, 20, 50 or 100 LMH.

Figure 7 Comparison of pressure profiles for experimental values and calculated values using each clogging model for filtration of plasma IgG solution with a virus filter at various flux shown as (a) 10 LMH, (b) 20 LMH, (c) 50 LMH, (d) 100 LMH and (e) Combination of plots (a) through (d). Because the pressure fluctuation was small, the Y-axes of plots (a) through (d) are scaled appropriately for each flux condition. For all filtrations, plasma IgG at 10 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 5.0 was filtered. Clogging models: cake filtration, intermediate blocking, standard blocking and complete blocking.

Figure 8 Clogging model analysis for filtration of plasma IgG solution with a virus filter shown as (a) Clogging factor, k, of each clogging model for 10, 20, 50 and 100 LMH runs and (b) Average pressure difference between experimental values and calculated values using Equation 9 for 10, 20, 50 and 100 LMH runs. Clogging models are noted in each panel. For all filtrations, plasma IgG at 10 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 5.0 was filtered. Clogging models: cake filtration, intermediate blocking, standard blocking and complete blocking.

Figure 9 Clogging model analysis of mAb solution processing with a throughput of 540 L/m^2 using clogging models. (a) Average pressure difference between experimental values and

calculated values for each clogging model (calculated using Equation 9). (b) Extrapolation of experimental values based on calculated values for each clogging model. Calculated pressure profiles are extended to 3500 L/m^2. mAb at 10 mg/mL in 20 mM Tris-Acetate, 9 mS/cm, pH 6.5 was processed. Clogging models: cake filtration, intermediate blocking, standard blocking and complete blocking.