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RESEARCH ARTICLE

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Investigating the combination of single-pass tangential flow filtration and anion exchange chromatography for intensified mAb polishing

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Abstract

There is growing interest within the biopharmaceutical industry to improve manufacturing efficiency through process intensification, with the goal of generating more product in less time with smaller equipment. In monoclonal antibody (mAb) purification, a unit operation that can benefit from intensification is anion exchange (AEX) polishing chromatography. Single-pass tangential flow filtration (SPTFF) technology offers an opportunity for process intensification by reducing intermediate pool volumes and increasing product concentration without recirculation. This study evaluated the performance of an AEX resin, both in terms of host cell protein (HCP) purification and viral clearance, following concentration of a mAb feed using SPTFF. Results show that preconcentration of AEX feed material improved isotherm conditions for HCP binding, resulting in a fourfold increase in resin mAb loading at the target HCP clearance level. Excellent clearance of minute virus of mouse and xenotropic murine virus was maintained at this higher load level. The increased mAb loading enabled by SPTFF preconcentration effectively reduced AEX column volume and buffer requirements, shrinking the overall size of the polishing step. In addition, the suitability of SPTFF for extended processing time operation was demonstrated, indicating that this approach can be implemented for continuous biomanufacturing. The combination of SPTFF concentration and AEX chromatography for an intensified mAb polishing step which improves both manufacturing flexibility and process productivity is supported.

KEYWORDS

anion exchange chromatography, monoclonal antibody, process intensification, single-pass tangential flow filtration

1 | INTRODUCTION

Process intensification is an approach to improve manufacturing efficiency by decreasing factors such as processing cost, energy consumption, and facility size.[1,2] Examples of intensified processes have been adopted by steel, petrochemical, food, dairy, pharmaceutical, textile, and wastewater industries.[3-10] Within the biopharmaceutical industry, however, the production of monoclonal antibody (mAb) therapeutics has largely relied on batch processing techniques since commercialization in the 1980s.[11] Because of early investment in large-scale batch processing equipment and the benefit of economies of scale, this approach has maintained viability from a cost of goods perspective.[12] More recently, however, changes in the industry landscape have challenged this approach. Patent expirations have led to growing competition from biosimilars, creating a strong need to reduce manufacturing costs.[13,14] Furthermore, producers of orphan drugs and personalized medicines require increased operational flexibility to maintain a diverse molecule pipeline in multiproduct facilities.[15,16] To address these rising

demands, the biopharmaceutical industry is moving toward intensified processing solutions.

Anion exchange (AEX) chromatography, often a final impurity adsorption polishing step in mAb processing, can benefit from process intensification.[17] Because most mAbs feature basic isoelectric points (pIs), AEX chromatography can be operated near neutral pH to bind acidic host cell protein (HCP), virus, DNA, and leached protein A impurities, while allowing the positively charged mAb to flow through unbound.[18] Typical mAb loadings for AEX polishing resins are between 50 and 200 g mAb/L resin,[19] which allow for acceptable impurity clearance with >95% yield.[20]

Although AEX chromatography is a fixture in many commercial mAb processes,[21] two inefficiencies could be improved with intensification. First, most AEX resins employ quaternary ammonium ligands, which typically require conductivities below 10 mS/cm for proper binding.[22] To overcome this, a dilution or buffer exchange step is often employed to reduce conductivity, creating tank size and productivity bottlenecks along with increased water and buffer consumption.[23] Second, since AEX polishing is used to bind dilute impurities, it is possible that the column may be operated under unfavorable isotherm conditions, resulting in loadings below the resin's maximum binding capacity. Previous literature has utilized the Langmuir isotherm model to describe a correlation between increasing albumin protein feed concentration and increasing AEX resin binding capacity, up to the point of resin saturation.[24-26] Another study noted poor correlation between HCP binding and bovine serum albumin (BSA) binding for an AEX device, concluding that HCP capacity was controlled by binding energy in the linear Langmuir isotherm portion, while BSA capacity was controlled by ligand density in the saturated isotherm portion at the tested feed concentrations.[27] These findings suggest that feed concentration is an important parameter for optimized impurity binding in ion exchange adsorption. However, AEX polishing process development typically focuses on pH and conductivity parameters rather than concentration dependent isotherm effects.[22] As a result, the inefficiencies of using a dilute feed to load an AEX resin are not fully understood.

One approach to intensify the AEX polishing step is to preconcentrate the feed. The use of a concentration step before chromatography has been described to reduce pool volumes and product load times for improved productivity and facility fit.[28,29] In cases where conductivity reduction is required, concentration can help limit the volume expansion associated with dilution. Additionally, if impurity proteins are co-concentrated with the mAb, then improvements in resin impurity binding capacity (and thus resin mAb loading) may be achieved due to the impurity's adsorption isotherm. Although the idea of concentrating an impurity protein to facilitate its improved chromatographic removal runs contrary to current practice, this approach offers an opportunity to improve efficiency of the mAb AEX polishing step in a manner not previously described.

Recent advances in single-pass tangential flow filtration (SPTFF) technology enable flow through product concentration without sacrificing the concentration benefits of traditional recirculated batch TFF.[30,31] The absence of recirculation tanks in an SPTFF process simplifies implementation and reduces footprint, making it an attractive option for connected processing, intensified processing, and debottlenecking applications.[29,32] In this work, SPTFF was investigated for inline concentration of mAb feed prior to an AEX polishing step. Experiments evaluated SPTFF and AEX as decoupled operations to assess feasibility and individual sizing parameters for each step. After SPTFF concentration of both mAb product and HCP impurities, concentrated protein solutions were processed over a commercially available AEX resin to determine the impact of feed concentration on HCP clearance. Virus spiking experiments were subsequently performed to ensure that viral clearance across AEX was maintained for an SPTFF concentrated feed stream. This article demonstrates the benefits of incorporating SPTFF concentration prior to AEX polishing chromatography and discusses strategies for implementing this approach in a linked intensified polishing process.

2 | MATERIALS AND METHODS

2.1 | Monoclonal antibody feed preparation

Monoclonal antibody A (mAb A, pI 8.1–8.4) was harvested from Chinese hamster ovary (CHO) cell culture and clarified by acid precipitation at pH 4.5 followed by depth filtration with a Clarisolve 20MS filter (MilliporeSigma, Burlington, MA). Depth filter effluent was subsequently adjusted to pH 7.4 and sterile filtered with an Express SHC membrane (MilliporeSigma). The clarified, sterile filtered harvest was purified by protein A capture chromatography using ProSep Ultra Plus resin (MilliporeSigma). The protein A purification procedure consisted of resin equilibration with 50 mM Tris pH 7.5, load with clarified sterile filtered harvest, postload wash with 50 mM Tris + 0.5 M NaCl pH 7.5, equilibration with 50 mM Tris pH 7.5, elution with 50 mM acetate pH 3.0, and regeneration with 150 mM phosphoric acid. The protein A eluate was adjusted to pH 5.3 ± 0.3 and purified by either Eshmuno S or Fractogel COO (M) cation exchange (CEX) chromatography resin (MilliporeSigma). The CEX eluate was adjusted to pH 8.4 and diluted to 5.1 ± 0.6 mS/cm conductivity for use as SPTFF feed material.

2.2 | SPTFF concentration of monoclonal antibody

Pellicon 3 cassettes with 30 kDa Ultracel membrane and C screen (MilliporeSigma) and either 88 cm^2 or 0.11 m^2 filtration area were used for SPTFF concentration. The SPTFF assembly consisted of three cassettes assembled in a lab-scale Pellicon mini cassette holder with Pellicon SPTFF cassette diverter plates (MilliporeSigma), as shown in Figure 1. Four diverter plates were used to direct fluid flow in series through the cassettes: one diverter plate was used to seal the holder's retentate port, and the remaining diverter plates were placed after each cassette. This provided a three-section configuration, with one cassette per section. A peristaltic pump was used to drive fluid flow to the SPTFF assembly. All membranes were flushed with Milli-Q water, sanitized with 0.1 N NaOH, and equilibrated to the target pH and conductivity prior to protein concentration. A data acquisition system was used to monitor flow rates and pressures for the feed, retentate, and permeate lines.

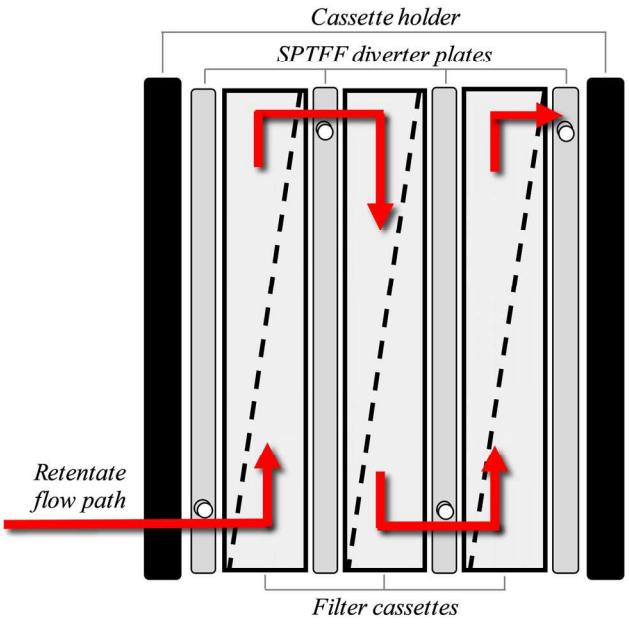

FIGURE 1 Side-view of a single-pass tangential flow filtration assembly containing three filter cassette sections separated by flow path diverter plates. Concentrated retentate is collected at the final diverter plate outlet. Permeate flow is directed to diverter plate outlets after each cassette section

The SPTFF assembly was challenged with mAb A feed at six different crossflow rates, ranging from 0.3 L/min/m^2 (LMM) to 1.6 LMM, where m^2 represents the total filtration area in the system. A backpressure valve on the final retentate port was adjusted to maintain constant pressure of 10 psi to overcome osmotic pressure and prevent reverse flow. For all crossflow rates, the following procedure was used to generate SPTFF sizing data and concentrated mAb A for use in AEX experiments. First, membrane polarization was established by initially operating the SPTFF assembly in a recirculation loop, where the concentrated retentate material was directed back to a well-mixed feed vessel. Recirculation continued for 10–15 min, during which time the feed and retentate pressures were monitored and the retentate concentration was sampled. Once the system pressures and retentate concentration stabilized, the polarization region was assumed to be established. Next, the flow path was reconfigured to achieve single-pass concentration into a retentate collection vessel. During single-pass concentration, flow rates for the retentate and all three permeate lines were measured, permeate samples were collected for protein analysis, and a retentate sample was collected for use in AEX HCP clearance experiments. Finally, the SPTFF system was returned to full recirculation mode, and the pump was adjusted to accommodate the next feed crossflow rate. Sequential experiments were conducted from highest to lowest crossflow rate.

Feed and permeate flow rates were used to calculate the cumulative conversion ratio at each cassette section, according to Eq. 1.

$$\text{Conversion ratio} = Q_{permeate}/Q_{feed} \quad (1)$$

2.3 | AEX HCP clearance evaluations

All AEX experiments were performed with Eshmuno Q resin, a strong AEX resin featuring trimethyl ammonium ethyl functional groups and an 85 μm average particle size (MilliporeSigma). All AEX experiments were challenged with mAb A feed at pH 8.4 and 5.1 ± 0.6 mS/cm. Seven feed concentrations were prepared for HCP clearance evaluations: Eshmuno S eluate which was not concentrated by SPTFF, and six SPTFF retentate samples containing concentrated Eshmuno S eluate (collected from each of the six feed crossflow rates described in the section entitled SPTFF Concentration of Monoclonal Antibody). The AEX resin was evaluated in both loose resin and packed column format. Six of the seven feed concentrations were evaluated in loose resin format, while five were evaluated in packed column format.

Loose resin HCP experiments utilized 20 μL of Eshmuno Q resin equilibrated to pH 8.4 and conductivity 5.1 ± 0.6 mS/cm. The resin was challenged with 1.19 ± 0.15 g mAb/mL resin and 640 ± 90 μg HCP/mL resin at each feed concentration. Experiments were conducted in 2 mL microcentrifuge tubes at room temperature. The tubes were rotated end-over-end for 4 h to achieve equilibrium HCP uptake. Stationary and mobile phases were then separated by centrifugation at 13,000*g* for 10 min, and the supernatant was assayed for HCP and mAb content. The amount of HCP bound to the resin at equilibrium (Q_{eq}, μg HCP/mL resin) and the amount of HCP remaining in solution at equilibrium (C_{eq}, μg HCP/mL solution) were calculated by mass balance. Loose resin experiments were conducted in duplicate, and the relative standard deviation was used to assess replicate error.

Column scale HCP clearance experiments utilized Eshmuno Q resin in 200 μL packed column RoboColumn devices, which feature a 0.5 cm inner diameter and 1 cm bed height (Repligen Corp., Waltham, MA). RoboColumn experiments were performed with a Tecan Freedom EVO (Tecan US, Inc., Morrisville, NC) automated LHS equipped with eight stainless steel pipette tips, a Te-Chrom device for load flow through fractionation, and a robotic arm to transport fraction collection plates. RoboColumns were first equilibrated with 10 column volumes (CVs) of 25 mM Tris, pH 8.4, 5 mS/cm conductivity buffer. Antibody load flow through was fractionated in intervals of either 50 or 100 g mAb/L resin. RoboColumn experiments were operated at a residence time of 3.8 min (16 cm/h), and each mAb A feed condition was evaluated as a single test.

2.4 | Viral clearance experiments

A separate batch of mAb A clarified harvest was purified by ProSep Ultra Plus protein A and Fractogel COO (M) CEX chromatography resins for viral clearance challenge studies. The HCP content in the Fractogel COO (M) eluate was approximately 125 ppm. The CEX elution pool was adjusted to pH 8.4, conductivity 5.1 ± 0.6 mS/cm, and concentrated to 79 g/L with a three-section SPTFF assembly featuring 0.11 m^2 filtration area per section. Minute virus of mouse (MVM) and xenotropic murine virus (XMuLV) were then spiked into separate pools of concentrated mAb feed to target titers of 2.0×10^6 $TCID_{50}$/mL (MVM) or 1.0×10^6 $TCID_{50}$/mL (XMuLV). To ensure mono-dispersity

TABLE 1 Chromatographic Procedure for Viral Clearance Experiments with 79 g/L mAb A Feed

Step	Solution	Flow Rate (mL/min)	Volume (CV)
Sanitization	0.5 N NaOH	0.50	5.0
Strip	25 mM Tris + 1 M NaCl, pH 8.4	0.50	5.0
Equilibration	25 mM Tris, pH 8.4	0.50	to reach pH 8.4
Load	79 g/L mAb A, with virus spike	0.12	7.6
Wash	25 mM Tris pH 8.4	0.33	4.0

Load flow-through was fractionated in 1.9 CV (150 g/L resin) increments. Postload wash was collected as a single pool.

of virus spike, the virus spiked feeds were filtered through a 0.22 μm (for MVM) or 0.45 μm (for XMuLV) membrane prior to testing.

Virus spiked 79 g/L mAb A feed solutions at pH 8.4 and conductivity 5.1 ± 0.6 mS/cm were loaded onto Eshmuno Q resin in 1 mL prepacked column format (0.8 cm diameter × 2 cm bed height) using procedures described in Table 1. Viral clearance tests for the 79 g/L feed were conducted in duplicate, and a new prepacked column was used for each test. The results were compared to control MVM and XMuLV clearance data using a non-SPTFF concentrated 11 g/L mAb A feed. In control experiments, either MVM or XMuLV were spiked into separate pools of 11 g/L mAb A feed, and the two feed streams were used to challenge separate 1 mL prepacked Eshmuno Q columns. Control viral clearance experiments were conducted as a single test, loaded to 500 g/L resin at a 2-min residence time.

Additionally, a single experiment challenged a 1 mL prepacked Eshmuno Q column with 79 g/L mAb A in the absence of virus; this condition was loaded to a slightly lower challenge level than that shown in Table 1 (530 g mAb/L resin), and four fractions were collected in increments of 133 g mAb/L resin. All Eshmuno Q experiments at 1 mL prepacked column scale were operated with a multichannel peristaltic pump.

2.5 | Evaluating SPTFF for extended processing times

Lyophilized polyclonal human immunoglobulin G (IgG; SeraCare Life Sciences, Milford, MA) was used as feed material for SPTFF stability testing over a 24-h period. The IgG feed was formulated to 8.6 g/L in 25 mM MES (pH 6.8, 5.1 mS/cm conductivity), and sterile filtered using an Express SHF membrane (MilliporeSigma). After establishing SPTFF membrane polarization, the feed was concentrated with a three-section assembly of 88 cm^2 cassettes operated at 0.3 LMM feed crossflow rate for 24 continuous hours. Fifteen retentate samples were collected over the course of processing and measured for IgG concentration.

2.6 | Analytical tools

Concentrations of mAb and polyclonal IgG were measured by SoloVPE slope spectroscopy (C Technologies, Bridgewater, NJ). HCP content was measured with a commercially available CHO HCP ELISA kit (Cygnus Technologies, Southport, NC). All HCP samples were assayed at two different dilutions, and each dilution was assayed in duplicate. Assay error for HCP analysis was 21%, based on both duplicate variability and dilution recovery. Aggregate content was measured by SEC-HPLC using a Tosoh TSKgel Super SW3000 column (Minato, Japan). Viscosity was measured using a RheoSense microVISC viscometer (San Ramon, CA). Titers of MVM and XMuLV were measured by $TCID_{50}$ infectivity assay.

3 | RESULTS AND DISCUSSION

3.1 | SPTFF concentration data

Cumulative conversion ratios for each SPTFF cassette section obtained at six different feed crossflow rates are shown in Figure 2. Conversion was observed to increase with longer SPTFF residence times, either by decreasing feed crossflow rate or increasing the number of cassette sections in series.

Feed and retentate concentrations for each of the six feed crossflow rates are shown in Figure 3. Antibody concentration aligned with the three-section conversion ratios shown in Figure 2 for all crossflow rates, except 0.3 LMM (96% conversion in Figure 2, 89% conversion in Figure 3). It is possible that given the low volumetric feed flow rate employed for this condition (5.3 mL/min), the equilibration time of 10–15 min was insufficient in allowing high concentration product to displace the hold-up volume in the retentate outlet tubing, resulting in a discrepancy between conversion ratio and concentration.

Retentate HCP and mAb data showed similar trends, as concentration increased with reduced crossflow rate in both cases. Permeate samples collected and assayed at each condition did not show measurable HCP or mAb content (data not shown). The HCP content for the feed and all retentate samples was consistent, ranging from 590–662 ppm. The retention of HCP with a 30 kDa membrane is consistent with other reports.[29]

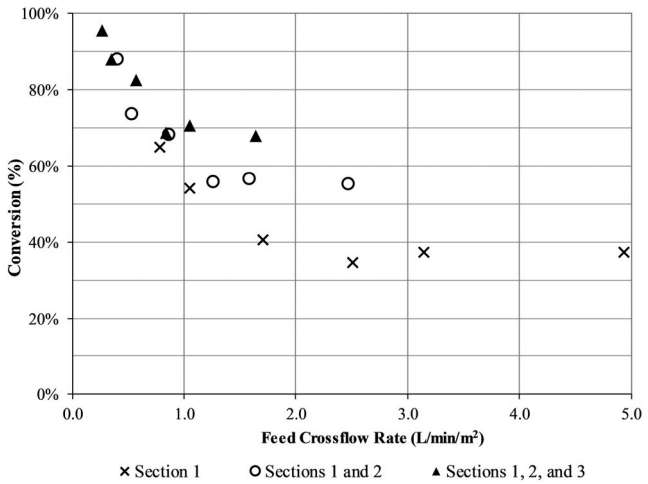

FIGURE 2 Cumulative percent conversion as a function of feed crossflow rate for each evaluated cassette section

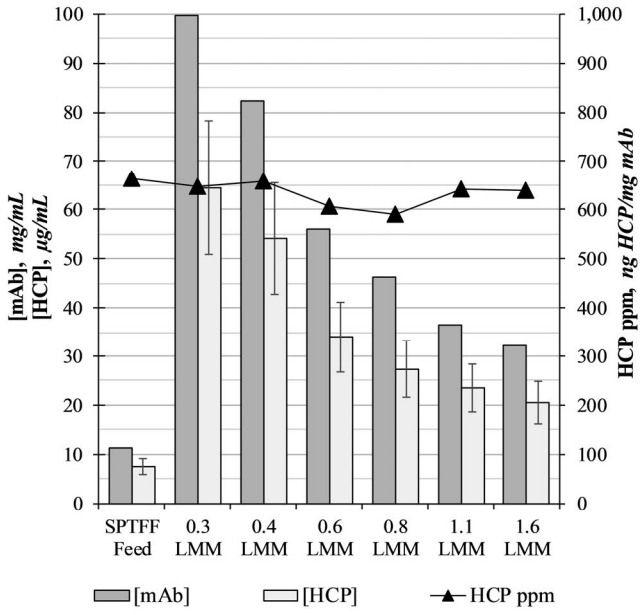

FIGURE 3 Single-pass tangential flow filtration concentrations for mAb, host cell protein (HCP), and the ratio of HCP to mAb, expressed as ppm. Error bars indicate HCP assay error

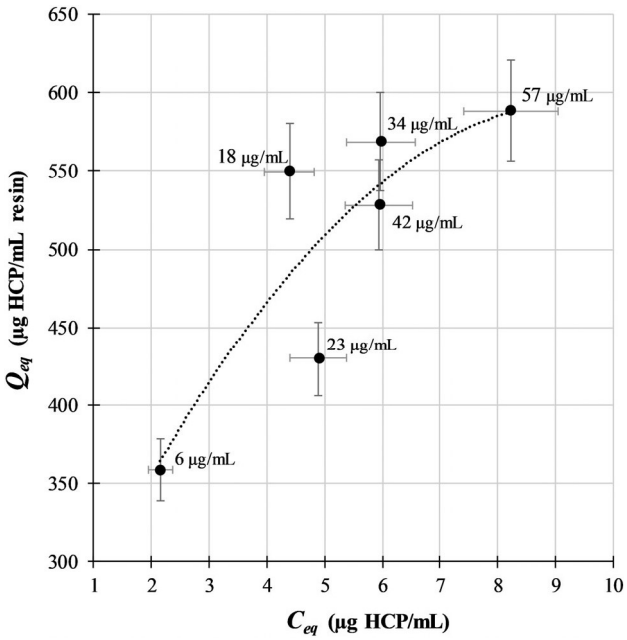

FIGURE 4 Loose resin host cell protein (HCP) equilibrium adsorption data plotted as a Langmuir isotherm. Marker labels indicate HCP feed concentration (C_{feed}). Error bars represent the relative standard deviation of replicate conditions

For some mAb molecules, high viscosity (>10 cP) can create processing issues when operating at concentrations greater than 70–120 g/L.[33,34] However, mAb A showed no significant viscosity increase with concentration; at approximately 100 g/L mAb A, the viscosity was 3.3 cP.

3.2 | Loose resin HCP uptake experiments

Loose resin experiments were used to evaluate HCP uptake at various feed concentrations, and the data were plotted as a Langmuir isotherm (Figure 4). Since the Langmuir isotherm is appropriate for single component feeds, this analysis assumed that the heterogeneous CHO HCP population behaved as a single species. This assumption is supported by previous work which characterized HCP breakthrough on an AEX adsorber and noted similar isotherm slopes along the breakthrough front, suggesting that the heterogeneous HCP species may behave in a similar manner.[27] Although complicating factors such as steric hindrance or diffusional resistance may contribute to some disagreement between Figure 4 experimental data and the Langmuir fit, conclusions can be drawn from the trends observed. Results indicated that for the nonconcentrated control condition, HCP binding to Eshmuno Q resin fell below the saturated portion of the isotherm. Increasing HCP feed concentration (C_{feed}) correlated with an increase in HCP mass bound to the resin (Q_{eq}). While this relationship between C_{feed} and Q_{eq} is consistent with ion exchange protein adsorption behavior described in the literature,[35] the practice of concentrating undesired proteins to facilitate their improved chromatographic removal is a counter-intuitive approach not commonly employed.

The trends observed in Figure 4 may apply to a broad range of mAb purification processes. Protein A capture chromatography is reported to remove >90% of HCP impurities in a single step,[36] and CEX, commonly used after Protein A and before the AEX column,[17] can reduce HCP to levels as low as 25–200 ppm.[20,37] This, combined with the need to dilute AEX load material for conductivity reduction,[38] creates a low concentration of HCP impurities in the AEX feed. As a result, the AEX step is commonly operated in the linear portion of the HCP isotherm, well below the resin's maximum binding capacity. Figure 4 shows that increasing the concentration of HCP in solution from approximately 6 µg/mL to 57 µg/mL boosted Q_{eq} by a factor of roughly 1.6, suggesting significant opportunity to improve the AEX polishing step mass loading by incorporating precolumn concentration.

3.3 | Packed column HCP breakthrough experiments

Flow through fractions collected during Eshmuno Q RoboColumn scale experiments were assayed for HCP content, and the cumulative data was plotted as a function of resin mAb loading (Figure 5). For this analysis, the target HCP endpoint was 10 ppm, a common HCP clearance level for AEX polishing.[39] In the absence of SPTFF concentration, the pH and conductivity adjusted CEX pool reached a 10 ppm HCP endpoint at approximately 140 g/L resin mAb loading. The data showed that as HCP and mAb were increasingly concentrated, the HCP breakthrough curve became shallower, resulting in improved resin mAb loading. The loading was maximized at an 82 g/L mAb feed concentration, which enabled loading to 600 g/L resin at the target HCP endpoint, a fourfold improvement over the control condition. This significant resin mAb loading improvement reduces the AEX CV required for processing, since a fourfold increase in mAb loading decreases the resin volume needed to purify a given antibody mass by

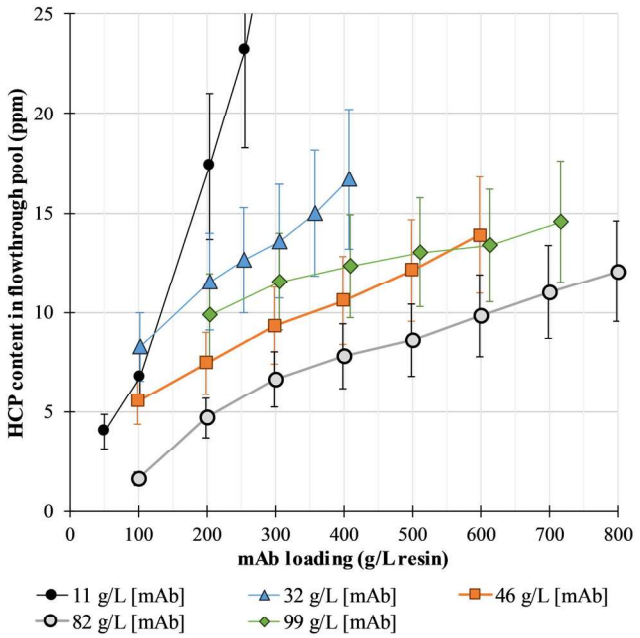

FIGURE 5 Host cell protein (HCP) breakthrough as a function of mass loading for a range of feed concentrations. Error bars indicate HCP assay error

a corresponding factor of four. Beyond the resin cost savings, operational benefits associated with a smaller AEX column include reduced process buffer volumes (e.g., equilibration, cleaning, and storage buffers), as well as reductions in pump flow rate, tank size, and chromatography skid requirements. Taken together, implementation of SPTFF concentration before AEX polishing was found to improve resin mAb loading and improve manufacturing flexibility by reducing these facility fit parameters.

Alternatively, rather than maximizing resin mAb loading, one could utilize the shallow AEX breakthrough profile achieved with SPTFF preconcentration to reduce HCP content below 10 ppm at loadings which exceed the control condition. For example, a 300 g/L resin mAb loading using the 82 g/L feed condition reduced HCP content to 6 ppm, while still providing a twofold load increase as compared to the control condition. This approach could be useful in applications where product quality is paramount.

Figure 5 data suggests that there may be an upper limit to the resin mAb loading benefits provided by SPTFF preconcentration. Although the 82 g/L concentration improved loading to 600 g/L resin, the 99 g/L feed condition was only loaded to 200 g/L resin at the 10 ppm HCP target. This was likely due to the negatively charged HCP species binding to the positively charged mAb, an effect that became more pronounced at high concentrations for this feed. Although similar behavior has been described in the literature, it is acknowledged that the extent of interaction between HCPs and mAb product is molecule specific.[40] Figure 5 shows that operating Eshmuno Q at approximately 80 g/L feed concentration provided optimal mAb loadings for this feed stream.

By enabling both feed volume reduction and increased resin mAb loading, SPTFF preconcentration significantly improved AEX

TABLE 2 Resin Productivity Data

mAb Feed Conc. (g/L)	mAb Loading (g/L resin)	Load Time (h)	Nonload time (h)	Productivity (g/L/h)
11	140	0.8	1	77
32	160	0.3	1	122
46	350	0.5	1	236
82	600	0.5	1	410
99	205	0.1	1	181

A 3.8-min residence time was used to calculate load time. Nonload time is based on 20 total CV at 3-min residence time for postload wash, strip, sanitization, and equilibration.

productivity, defined as the mass of mAb processed per resin volume and cycle time (Eq. 2)

$$\text{Productivity}\,(\text{g/L/h}) = \frac{M_{protein}}{V_{resin} \cdot t_{cycle}} \quad (2)$$

Table 2 details the productivity for different feed concentrations when loaded to the 10 ppm HCP endpoint identified in Figure 5. Concentrating the feed from 11 g/L to 82 g/L provided greater than fivefold improvement in resin productivity when using identical AEX load flow rates and nonload process times.

3.4 | Viral clearance experiments

Based on the optimal conditions identified for HCP clearance, viral clearance across Eshmuno Q was evaluated at 600 g/L resin loading using mAb A concentrated to 79 g/L by SPTFF. During SPTFF concentration of mAb A viral clearance feed material, samples were collected at various concentrations to assess aggregate profile. The SPTFF feed material contained 1.0% aggregate, while material concentrated to 72 g/L and 100 g/L contained 1.3% and 1.4% aggregate, respectively. Like the previously mentioned viscosity and mAb-HCP interaction effects, the effect of feed concentration on aggregation is likely molecule and process specific.[41]

Viral clearance results for MVM and XMuLV are shown in Figure 6. At 600 g/L mAb loading level, more than 5 logs of MVM and 4 logs of XMuLV reduction were achieved for all experimental replicates. In comparing results of SPTFF concentrated mAb A feeds to those of the non-SPTFF treated mAb A control condition, concentration did not negatively affect AEX virus clearance, even at high mAb load levels. An 8-min residence time was selected for SPTFF concentrated viral clearance testing to align with proposed residence times for a linked SPTFF and AEX process (further detailed in section entitled Considerations for Implementing Linked SPTFF and AEX Processing). Previous literature comparing AEX viral clearance at both 4- and 8-min residence times concluded that residence time was not a significant factor in viral clearance, and instead physiochemical conditions such as pH and conductivity were more important.[42] This conclusion is consistent with the observations in Figure 6, where the 2-min residence time control data show comparable performance to the 8-min residence time

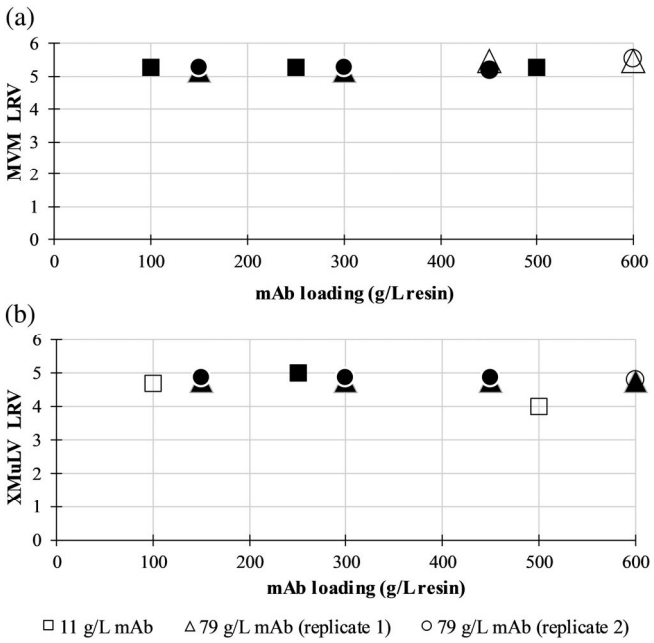

FIGURE 6 Minute virus of mouse (a) and xenotropic murine virus (b) clearance results for control (11 g/L mAb, 2-min residence time) and single-pass tangential flow filtration concentrated (79 g/L mAb, 8-min residence time) experiments. Filled symbols indicate virus content was below assay detection limit

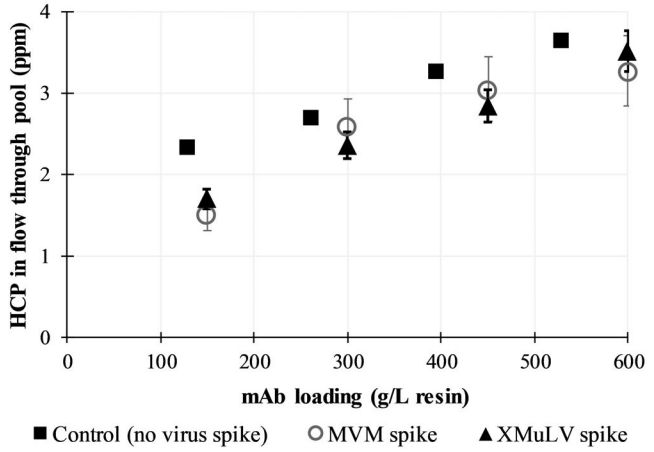

FIGURE 7 Host cell protein breakthrough profiles for single-pass tangential flow filtration concentrated mAb A with and without virus spike (1 mL CV scale). Error bars represent the relative standard deviation of replicate experiments

experimental data. As a result, similar viral clearance levels would be expected for an SPTFF concentrated mAb A feed loaded on Eshmuno Q at shorter residence times.

Flow through fractions collected during SPTFF concentrated viral clearance experiments were also assayed for HCP content. Excellent HCP clearance was maintained in the presence of virus spike, as less than 5 ppm HCP was measured in the flow through pool at 600 g/L resin loading (Figure 7). The results indicate that the virus and HCP species did not compete for binding sites, and clearance of both impurities can be achieved simultaneously. After loading, columns were subjected to a 4 CV wash with equilibration buffer. No virus was detected in the wash pool for any of the replicates, and the combined wash and load flow through pool contained less than 5 ppm HCP. For the control condition evaluated in the absence of virus, the wash effluent was fractionated in 1 CV increments. Flushing the Eshmuno Q column with 1 CV of wash buffer provided 95% product yield, and a 4 CV wash provided 97% yield (data not shown).

3.5 | Considerations for implementing linked SPTFF and AEX processing

Since the AEX step is often placed downstream of a CEX column eluted at high salt, it is important to consider the impact of conductivity dilution prior to AEX polishing.[20] For the SPTFF experiments described in Figures 2 and 3, permeate and retentate conductivities were comparable to the feed solution at all crossflow rates (in the range of 5.1 ± 0.6 mS/cm). This indicates that while SPTFF does not eliminate the need for conductivity adjustment, the 30 kDa membrane effectively permeates excess buffer salts to enable conductivity reduction with minimal volume expansion. Moving the dilution step downstream of SPTFF provides an opportunity for buffer savings, since less diluent is required to adjust concentrated retentate as compared to the nonconcentrated feed. In this case, SPTFF can be designed to over-concentrate the product so that the target AEX feed concentration is achieved after conductivity dilution. Recent advancements in inline pool adjustment technology can enable pH and/or conductivity manipulation between SPTFF and AEX operations without requiring a dedicated mixing tank.[43,44] Integrating SPTFF and inline pool adjustment before AEX polishing reduces dilution buffer volumes, thus minimizing buffer tank requirements for smaller process footprint.

The product flow through operation of both SPTFF and AEX chromatography make it convenient to connect these steps for intensified polishing. Given the relatively low feed crossflow rates used for SPTFF operation (approximately 1/10th that of batch TFF[34]), feed channel pressure drops are not limiting. As a result, a single pump can be used to operate the SPTFF membrane and AEX column as a linked operation, while a secondary pump is required to control dilution and/or pH adjustment.

The linked intensified polishing setup can also be integrated with upstream or downstream operations as part of a continuous manufacturing process, so long as a strategy for continuous AEX resin regeneration is in place, such as a two-column cycling approach. In the mAb study above where the feed was concentrated to 80 g/L by SPTFF and loaded to 600 g/L resin on Eshmuno Q, loading Column 1 at an 8-min residence time provided a load time of approximately 1 h/cycle, allowing sufficient time to wash, sanitize, and regenerate Column 2. While this multicolumn approach can maximize continuous processing efficiency, it requires the use of specialized equipment for automated column switching. Alternatively, one could employ standard chromatography equipment for single column operation, using a surge vessel between the SPTFF membrane and AEX column

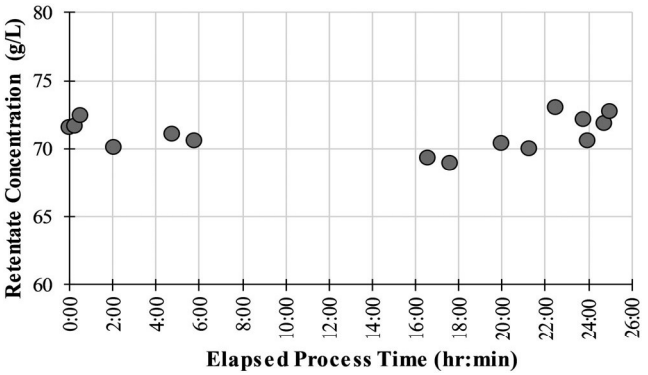

FIGURE 8 Retentate concentration during extended single-pass tangential flow filtration operation

to collect concentrated retentate while the column is washed, regenerated, and sanitized.

Unlike the AEX resin, which requires frequent regeneration, the SPTFF membrane can be operated continuously for extended processing times with consistent conversion. Figure 8 shows data for a three-section SPTFF assembly challenged with 8.6 g/L polyclonal IgG for 24 h at 0.3 LMM crossflow rate. Fifteen retentate samples collected during the operation showed an average concentration of 71 g/L, with a range of ±4%. These results indicate that a single SPTFF assembly can provide consistent performance for continuous processing over extended time periods.

Extended SPTFF operation can be used to overcome challenges associated with recovery flush of linked SPTFF and AEX steps. For high concentration applications, a buffer flush through a linked SPTFF and AEX system may be unable to displace the membrane polarization layer, resulting in 100% conversion and loss of residual product in the retentate channel. To maximize yield, it is best to minimize SPTFF recovery flush frequency. Based on typical mAb concentration parameters and the known hold-up volumes of Pellicon TFF assemblies, extended SPTFF operation may render the recovery flush unnecessary. For example, consider a three-section SPTFF assembly with 0.33 m^2 total membrane area operated at 0.2 LMM feed crossflow rate to concentrate mAb feed from 10 g/L to 120 g/L. Membrane polarization concentration is typically near 1.5 g/m^2, and the hold-up volume within the cassette and holder is about 0.17 L/m^2.[45] These mass hold-up parameters were used to estimate product yield for the SPTFF process at various timepoints without including a recovery flush: 4 h provided >95% yield, 8 h provided >97% yield, and 24 h provided >99% yield. These estimates suggest that an SPTFF recovery flush may not be required for acceptable yield when using the membrane for extended processing times.

4 | CONCLUSIONS

SPTFF is a useful tool for modifying process intermediate volumes and concentrations without recirculation. Previous literature has utilized this technology for pool volume reduction prior to chromatographic purification. In this work, additional benefits were realized when applying SPTFF concentration prior to a flow through AEX column for intensified mAb polishing. Concentrating mAb and HCP content by a factor of approximately eight increased resin mAb loading to 600 g/L at a 10 ppm HCP endpoint, a fourfold improvement over the nonconcentrated control condition. Excellent viral clearance was also maintained at the increased load level. Results demonstrated more than 5 logs MVM and more than 4 logs XMuLV clearance on Eshmuno Q at 600 g/L resin loading, in line with typical clearance for AEX operations. The increased mass loading enables volume reductions in both AEX resin and process buffers for equilibration, cleaning, and storage, thus shrinking the overall size of the polishing step for improved facility fit and manufacturing flexibility. Since AEX polishing is commonly used as a final adsorption step to bind low-level impurities below the saturated isotherm region, the benefits demonstrated in this work likely apply to a broader range of molecules and processes. In addition to confirming these results for other antibody feeds, further work to characterize any effects that increased resin loading may have on resin lifetime would be useful for proper design of column size and resin cleaning strategy.

Given that both the SPTFF and AEX chromatography steps are operated in product flow through mode without recirculation, it is natural to link these unit operations for intensified polishing. Compared to batch TFF, pressure drop across SPTFF is low, so a single pump can drive mAb product through an SPTFF membrane linked directly to an AEX column. Results demonstrated consistent SPTFF performance over a 24-h period, suggesting that continuous operation of a linked intensified polishing process is feasible. Overall, incorporating Pellicon SPTFF concentration before Eshmuno Q polishing chromatography was found to increase resin mAb loading, enabling reductions in column size, buffer, and tank volume requirements. The results support implementing these technologies in an intensified mAb polishing process for improved manufacturing productivity and flexibility.

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