Effect of mixed-mode and surface-modified column chromatography on virus filtration performance

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Abstract

Virus filtration is a critical process in the production of biotherapeutics and drug products derived by plasma fractionation. The processing steps upstream of virus removal filtration impact the filterability (throughput and flux) of process solutions. We processed mAb and plasma IgG spiked with aggregate by chromatography resins and examined the filterability of the output on a virus filter (Planova BioEX). The greatly reduced filterability for protein solutions with aggregate was improved by processing with specific chromatography resins. For mAb, mixed-mode AEX effectively reduced aggregate content and significantly improved filterability. Mixed-mode AEX was also effective for reducing aggregates in plasma IgG but modified CEX showed even greater improvement in filterability. The results clearly show that virus filter performance can be optimized by careful choice of column chromatography. Finally, applying the throughput and flux from the virus filter to four classical clogging models showed that mAb with aggregate was best fit to the standard blocking model and plasma IgG with aggregate was best fit to the complete blocking model, suggesting that differences in solution properties result in different clogging mechanisms.

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Keywords: clogging model, column chromatography, virus filtration

Abbreviations: List here in alphabetical order. HCP, host cell protein; mAb, monoclonal antibody; SEC, size exclusion chromatography; V_{max}, maximum filtration volume

Abstract

Virus filtration is a critical process in the production of biotherapeutics and drug products derived by plasma fractionation. The processing steps upstream of virus removal filtration impact the filterability (throughput and flux) of process solutions. We processed mAb and plasma IgG spiked with aggregate by chromatography

resins and examined the filterability of the output on a virus filter (Planova BioEX). The greatly reduced filterability for protein solutions with aggregate was improved by processing with specific chromatography resins. For mAb, mixed-mode AEX effectively reduced aggregate content and significantly improved filterability. Mixed-mode AEX was also effective for reducing aggregates in plasma IgG but modified CEX showed even greater improvement in filterability. The results clearly show that virus filter performance can be optimized by careful choice of column chromatography. Finally, applying the throughput and flux from the virus filter to four classical clogging models showed that mAb with aggregate was best fit to the standard blocking model and plasma IgG with aggregate was best fit to the complete blocking model, suggesting that differences in solution properties result in different clogging mechanisms.

1. Introduction

Virus filtration is widely used as a necessary virus removal step for biotherapeutics and protein drug products derived by plasma fractionation.[1] The multi-layered membranes of virus filters allow for separating viruses from proteins, despite the small size difference.[2] While virus removal from proteins is possible due to the sensitive separation membrane, larger sized proteins and aggregates in particular have been confirmed to cause clogging in virus filters.[3] To improve the capacity of the virus filtration step, processing the feed solution through one of several commercially available prefilters has been confirmed to be effective in removing substances that cause clogging and has been widely implemented.[4] Column chromatography has also been shown to effectively remove aggregates.[5]

Based on the differences and changes in filtration behavior of solutions with aggregates and following various upstream processing, the impact of protein solution characteristics on filtration capacity can be estimated. The theoretical relationship between filtration volume and filtration throughput for a given feed solution can be fitted to one of four established clogging models for filter membranes:[6] cake filtration, intermediate blocking filtration, standard blocking filtration and complete blocking filtration. Model analysis can inform process development, such as applying the maximum filtration volume (V_{max}) that was theoretically determined using the standard blocking model based on filtrations conducted with a microfilter at constant pressure to facility scale-up recommendations.[7] Following this same strategy, the standard blocking model has been applied in nanofiltration to determine V_{max} of virus filters under constant pressure suggesting that the standard blocking model is the appropriate model for filtration with a virus filter.[8] In more recent studies, there have been attempts to combine several clogging models into a theoretical equation that can be used not only to estimate V_{max} but also to match complex filtration behavior results to theoretical values.[9]

In this report, we demonstrate the improvement in filterability that can be achieved by processing monoclonal antibody (mAb) and plasma IgG, each spiked with aggregates prepared from the respective solution, with various chromatography resins in flow through mode or through a prefilter. Using the filtration volume (throughput) and flow rate (flux) results obtained for constant pressure filtration with Planova BioEX filters, we determine the best fit clogging model and discuss the impact of solution characteristics on clogging behavior. These findings provide a framework for further application of clogging models in process development.

2. Materials and Methods

2.1. Monoclonal antibody

The mAb used in this study was provided by Manufacturing Technology Association of Biologics, Japan (MAB). The mAb (pI 8.2) was grown in CHO cell culture by fed batch method, separated from cells and applied to a two-column process. The first column was a bind-elute affinity chromatography step using Protein A resin (KanCapA, Kaneka Corporation, Tokyo, Japan). The second column consisted of a cation exchange chromatography step using Cellufine MAX S-h resin (JNC Corporation) and yielded 40 mg/mL mAb. Purified mAb was diluted with buffer containing 20 mM Tris-Acetate, pH 7 adjusted to 15 mS/cm with NaCl, which is a relevant column chromatography flow through buffer condition for mAb, to produce 15 mg/mL mAb solution for use in aggregate spiking experiments and 11 mg/mL for use as a reference solution for filtration. Purified mAb solution at 10 mg/mL was used to prepare mAb aggregate solution.

2.2. Plasma IgG

Venoglobulin IH5 (50 mg/mL) provided by Japan Blood Products Organization (JBPO) was diluted with 20 mM sodium acetate, 100 mM NaCl, pH 6 buffer, which is a relevant column chromatography flow through buffer condition for plasma IgG, to produce 15 mg/mL plasma IgG solution for use in aggregate spiking experiments and 11 mg/mL for use as a reference solution for filtration.

2.3. Protein solution characterization

Host cell protein (HCP) in the mAb solution was analyzed using CHO Host Cell Protein ELISA 3G Kit (Cygnus Technologies).

To determine the molecular weight distribution of proteins, size exclusion chromatography (SEC) was conducted. For analysis of mAb solutions, HPLC (Infinity 1260, Agilent) was used with a TSKgel SuperSW mAb HR column and a column guard (Tosoh). For analysis of plasma IgG solutions, HPLC (Acquity UPLC, H-Class Plus System, Waters) was conducted with a TSKgel UltraSW Aggregate column and TSKgel UltraSW Aggregate Guard column (7.8 mm ID, Tosoh BioScience LLC).

mAb and plasma IgG recovery for chromatography and filtration steps was determined by measuring UV absorbance at 280 nm before and after processing steps and calculating recovery as a percentage.

2.4. Aggregate spike preparations

Aggregate solutions for aggregate spike experiments were prepared as follows. mAb aggregate solution was produced by incubating 10 mg/mL mAb in 0.1 M NaCl, pH 4 at 60 for 60 min, which produced a spike stock solution with aggregate content of 11.4% dimer and 33.3% trimer or larger species.

Plasma IgG aggregate solution was prepared by mixing 5.0 mg/mL IgG in 0.1 M glycine, adjusting to pH 2 and incubating at room temperature for 1 h. The produced aggregate spike solution had aggregate content of 14.8% dimer and 57.1% trimer or larger species.

2.5. Column chromatography and nylon prefilter

Protein solutions spiked with aggregate were processed and applied to liquid column chromatography conducted using an AKTA avant 25 or an AKTA pure 25 (GE Healthcare) with the following types of chromatography resins. For mAb solutions, mixed-mode AEX1 (weak AEX of primary amine in combination with weak HIC of butyl group; Cellufine MAX IB), mixed-mode AEX2 (strong AEX with quaternary amine and phenyl group in combination with strong HIC; Capto adhere, GE Healthcare) and normal AEX (Cellufine MAX Q-h) were used. For plasma IgG solutions, mixed-mode AEX1 and normal AEX were used along with modified CEX1 (cross-linked cellulose with dextran sulfate; Cellufine MAX DexS-HbP), and modified CEX2 (sulfated cellulose; Cellufine Sulfate). All Cellufine resins were supplied by JNC Corporation.

Additionally, nylon prefilter Virosart Max Minisart (5 cm^2 surface area, Sartorius), which is considered to be effective for removing aggregates, was used as a treatment for aggregate-spiked mAb solution.

2.6. Virus filter and filtration

The virus filter used was 0.0003 m^2 Planova BioEX filter (Asahi Kasei Medical Co., Ltd., Tokyo, Japan).

To prepare for filtration through the virus filter, the feed solution was filtered through a 0.2 μm microfilter (Minisart RC 25 mm, Sartorius) to remove potential precipitates and placed in a feed vessel. The feed solution was then filtered with the Planova BioEX filter under constant pressure at 0.3 or 0.35 MPa. The mass of permeate was recorded manually during the filtration run to determine flow rate, and throughput (L/m^2) and flux (LMH) were calculated.

2.7. mAb with mAb aggregate spike experiments

Purified mAb solution (40 mg/mL) with 175 ppm HCP and an aggregate composition of 1.4% dimer and 0.2% trimer or larger species as determined by SEC analysis was used to prepare test solutions. mAb solution

(11 mg/mL) in 20 mM Tris-Acetate, pH 7 adjusted to 15 mS/cm with NaCl and with no aggregate spike was prepared as the "reference" solution. mAb solution prepared at 15 mg/mL in the same buffer and spiked with 1.0% mAb aggregate spike was used as the "control" and was processed by one of these methods: column chromatography with an AEX resin (mixed-mode AEX1, mixed-mode AEX2 and normal AEX) or filtration with a nylon filter.

For column chromatography on 2 mL columns operated in flow-through mode, 30 mL of control solution was loaded at a flow rate of 0.25 mL/min (residence time, 8 min), resulting in a mAb loading capacity of 225 mg/mL-resin. Following a 10 mL wash with equilibration buffer (20 mM Tris-Acetate, pH 7 adjusted to 15 mS/cm with NaCl), the entire 40 mL output of 11 mg/mL mAb was collected, and protein recovery by UV absorbance was [?]95% for all runs.

For nylon filter treatment, control solution was filtered through the nylon filter, and the output was adjusted to 11 mg/mL mAb.

For the reference and control solutions (no processing), output from each chromatography columns and permeate from the nylon filter, 40 mL was prefiltered through a 0.2 μm microfilter (Minisart RC 25 mm, Sartorius) to remove potential precipitates and placed in a feed vessel. The feed solution was then filtered with a 0.0003 m^2Planova BioEX filter in constant pressure mode at 0.3 MPa. Filtration runs had a throughput of 130 L/m^2 or 1500 g/m^2, and protein recovery of [?]99% was confirmed by UV absorbance for all runs.

2.8. Plasma IgG with IgG aggregate spike experiments

Commercially available plasma IgG (50 mg/mL) was diluted in 20 mM sodium acetate, 100 mM NaCl, pH 6 (12 mS/cm) to prepare test solutions. Plasma IgG solution at 11 mg/mL was prepared as a reference solution for virus filtration. Plasma IgG solution prepared at 15 mg/mL in the same buffer and spiked with 0.5% IgG aggregate was used as the control and was processed by one of these types of column chromatography: AEX resin (mixed-mode AEX1 and normal AEX) or sulfate ligand resin (modified CEX1 and modified CEX2).

For column chromatography on 1 mL columns operated in flow-through mode, 20 mL of aggregate-spiked plasma IgG solution was loaded at a flow rate of 0.5 mL/min (residence time, 2 min), resulting in a plasma IgG loading capacity of 300 mg/mL-resin. Following a 7 mL wash with equilibration buffer (20 mM sodium acetate, 100 mM NaCl, pH 6; 12 mS/cm), the entire 27 mL output of 11 mg/mL plasma IgG was collected. Protein recovery was [?]90% for mixed-mode AEX1 and modified CEX1 and [?]95% for normal AEX and modified CEX2.

For the reference and control solutions (no processing) and the output from each chromatography column at 11 mg/mL, 27 mL was filtered with a 0.0003 m^2 Planova BioEX filter in constant pressure mode at 0.35 MPa. Filtration runs had a throughput of 90 L/m^2 or 900 g/m^2, and protein recovery of [?]99% was confirmed by UV absorbance for all runs.

2.9. Clogging model analysis

For purposes of modeling, pores in the filter are simplified to a collection of cylinders. While no filter has this simplified pore structure, we can expect to see differences in filtration behavior for the different clogging models that are representative of the processes. Considering that filtration occurs as the filtration solution passes through a collection of uniform cylindrical pores of some length and inner diameter, these models can be used to express the theoretical filter behavior due to changes in flow path resistance as a result of substances that cause clogging being retained in the filter based on shape. Equations expressing the relationship between filtration volume (throughput) and flow rate (flux) by constant pressure filtrations for the four clogging models (cake filtration, intermediate blocking, standard blocking and complete blocking) and the explanation of each model described in Sumiya[10] and Grace[11] are shown below.

2.9.1. Cake filtration model

In this clogging model, the substances causing clogging do not directly block the flow path through the pores but rather adhere to the pore surfaces, causing the formation of new flow paths that are different from those

of the original filter material.

$$J = \frac{J_0}{(kV + 1)} \quad (1)$$

$$\frac{J_0}{J} - 1 = kV \quad (2)$$

where J_0 is the initial flow rate (flux), J is the flow rate (flux) achieved at filtration volume (throughput), V, and k is the clogging factor specific to the solution being filtered.

2.9.2. Intermediate blocking model

In this clogging model, the substances causing clogging accumulate on already trapped substances and the entrances of the cylindrical pores.

$$J = J_0 \exp(-kV) \quad (3)$$

$$-ln\left(\frac{J}{J_0}\right) = kV$$

(4)

2.9.3. Standard blocking model

In this clogging model, the substances causing clogging are distributed evenly over the inner walls of the cylindrical pores, and the inner diameter of the pores gradually becomes smaller.

$$J = J_0\left(1 - \frac{kV}{2}\right)^2$$

(5)

$$2\left(1 - \left(\frac{J}{J_0}\right)^{\frac{1}{2}}\right) = kV$$

(6)

2.9.4. Complete blocking model

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

$$J = J_0(1 - kV)$$

(7)

$$1 - \frac{J}{J_0} = kV$$

(8)

Filtration behavior parameters (filtration volume and flow rate) were applied to the rearranged equations for each clogging model (Equations 2, 4, 6 and 8), and the left side of each equation was plotted against filtration volume, V, expressed as throughput (L/m^2), and k was obtained for each model as the slope. For the standard blocking and complete blocking models, V_{max} is determined by setting J = 0 in the above equations to obtain 2/k and 1/k, respectively. According to Grace,[11] k is proportional to the volume of solid particles removed by filtration. Additionally, the mAb and plasma IgG filterability data for the control were plotted to visually show the determination of k for each molecule according to Equations 2, 4, 6 and 8.

Differences between experimental values and modeling results were plotted and compared for all four models. Using k, theoretical filtration volume and flow rate were obtained using Equations 1, 3, 5 and 7, and the mean difference between the experimental and calculated values at each point of measurement was obtained by Equation 9 for each solution and model.

$$\overline{\sqrt{\Delta^2}} = \sum_{i=1}^{N} \sqrt{\left(J_{exp,i} - J_{cal,i}\right)^2} / N$$

(9)

Here, $J_{exp,i}$ and $J_{cal,i}$are the i^{th} filtration behavior measurement for experimental and calculated flow rate values and N is the number of experimental measurements for each solution. The smaller the mean difference

between experimental and calculated values obtained using Equation 9, the better the fit of the clogging model.

3. Results

3.1. Column chromatography and filtration for mAb with aggregate spike

The effect of column chromatography and nylon prefilter treatments on mAb filterability is shown in Figure 1A and 1B. Protein aggregate characterization by SEC analysis is shown in Figure 1C and a comparison of dimer and trimer or larger aggregate and HCP content are shown in Figure 1B. From the figures, we see that the purified mAb (reference solution) with 175 ppm HCP and 1.3% dimer content has a stable flow rate on Planova BioEX. The control, which is aggregate-spiked mAb (spiked at 1.0%), has markedly lower throughput from shortly after the start of filtration, showing that an increase in larger aggregate (trimer or larger species) content from 0.2% to 1.2% is the likely cause for the marked decrease in filterability. Further, aggregate-spiked mAb processed in flow-through mode with normal AEX has similarly high trimer or larger aggregate content and filtration behavior that is almost the same as the control, showing that normal AEX column chromatography has no impact on improving filterability. The moderate improvement by the nylon prefilter on filterability of the aggregate-spiked mAb can be attributed to the reduction of trimer or larger aggregates to 0.5%, which is less than half of the level in the control. The output from mixed-mode AEX1 and mixed-mode AEX2 shows markedly higher filterability, surprisingly higher than the reference. These chromatography processes effectively reduced HCP and aggregate, particularly reducing trimer or larger aggregates to below detectable levels. HCP was also decreased for both outputs, but the similar improvement in filterability increase observed for both despite mixed-mode AEX2 output having twice the HCP as mixed-mode AEX1 output suggests that the cause of decreased filterability for aggregate-spiked mAb is the trimer or larger aggregates more so than HCP. These results suggest that filterability was not greatly impacted by the HCP or mAb dimer content at these concentrations.

The molecular weight distribution profiles based on SEC analysis are shown in Figure 1C for the reference, control, mixed-mode AEX1 output and nylon prefilter output. The aggregate-spiked mAb (control) has increased dimer and especially more trimer or larger aggregate content, which is believed to have a great impact on filterability in the virus filtration step. For mixed-mode AEX1 output, which shows improved filterability at the virus filtration step, the molecular weight distribution profile clearly shows that this processing almost completely removed trimer or larger aggregates and reduced the dimer content. Filtering the solution with a nylon prefilter moderately improved filterability and it decreased the proportion of trimer or larger aggregates, but its effect on improving filterability is very small compared to mixed-mode AEX1.

3.2. Column chromatography and filtration for plasma IgG with aggregate spike

Figure 2 shows the effect of column chromatography on the filterability of polyclonal plasma IgG isolated from plasma derivatives with 0.5% aggregate spike. Aggregate content by SEC analysis is shown in Figure 2A. Plasma IgG spiked with 0.5% IgG aggregate (control) had increased trimer or larger aggregate content (from 0.3% for the reference solution to 0.5%) and 7.8% dimer content as shown in Figure 2A. The figure clearly shows that the 0.5% IgG aggregate spike causes a marked decrease in filterability as evidenced by achieving flux of 100 LMH at nearly 80 L/m^2 for the reference, while the flux of the control had become nearly zero and the run was ended by 12 L/m^2. Normal AEX processing of aggregate-spiked plasma IgG produced no aggregate removal and there was no improvement in filterability.

Mixed-mode AEX1 shows more than double the throughput of the control and high aggregate removal with dimer content decreasing from 7.8% to 5.8% and trimer or larger aggregate content decreasing from 0.5% to below detectable level. On the other hand, while trimer or larger aggregate content was also reduced to below detectable level by modified CEX1 and modified CEX2 processing, dimer was not reduced markedly from the control and remained at 7.3% and 7.7%, respectively. Interestingly, modified CEX output shows significantly higher filterability than the mixed-mode AEX1 output and even exceeds the flux of the reference in the early phase of filtration (Figure 2B). Modified CEX1 and modified CEX2 both show high filterability, but modified CEX2 output shows a greater flux decay than does modified CEX1 output.

3.3. Determination of clogging factor k for model analysis

For clogging model analysis, clogging factor k is determined by applying the obtained filtration data (filtration volume and flow rate) to Equations 2, 4, 6 and 8 for the cake filtration model, intermediate blocking model, standard blocking model and complete blocking model, respectively. Graphical results of applying the control (aggregate spike) filtration results of both mAb and plasma IgG to each clogging model and finding the line of best fit using the least squares method are shown in Figure 3. Based on the overlap between experimental and calculated data, the mAb is best fit to the standard blocking model (Figure 3C) and plasma IgG is best fit to the complete blocking model (Figure 3D).

3.4. Clogging model analysis for aggregate-spiked mAb

Evaluation of filtration behavior for the reference (no spike) and control (aggregate spiked) mAb solutions in Figure 1B with each of the models is shown in Figure 4A and 4B, respectively. For the reference (no aggregate spike), values calculated based on each of the models overlap with experimental values, indicating that pronounced clogging was not observed for this purified protein (Figure 4A). In contrast, the control (aggregate-spiked mAb) had distinct plots for each clogging model, and the standard blocking model plot overlapped the most with experimental values (Figure 4B).

The control, output from normal AEX and output from nylon prefilter (all three solutions having relatively high proportions of trimer or larger aggregate content) had markedly higher k than column output from mixed-mode AEX (low proportion of aggregate), and the higher the aggregate content, the larger the k (Figure 4C). Further, for all three solutions with trimer or larger aggregates, the k decreased in the order of cake filtration, intermediate blocking, standard blocking and complete blocking models. The differences between experimental and calculated values for each solution with all four clogging models in Figure 4D show that for solutions with high aggregate content and high k, the standard blocking model is the best fit for mAb.

3.5. Clogging model analysis for aggregate-spiked plasma IgG

Evaluation of the filtration behavior for the reference and control for plasma IgG solutions in Figure 2B with each of the four clogging models is shown in Figure 5A and 5B. While the calculated results for all four clogging models overlap with experimental results (Figure 5A), indicating no significant clogging for the reference (no aggregate spike), control (spiked with aggregate) had distinct plots for each clogging model, and the complete blocking model showed the smallest differences between experimental and calculated values (Figure 5B).

Plots of k and mean differences between experimental and calculated values for filtration behavior (Figure 5C and 5D) show that plasma IgG solutions with relatively high proportions of trimer or larger aggregate content (control and normal AEX output) showed the best fit with the complete blocking model, which is a different from that for mAb (standard blocking model). A deeply interesting point is that the output for mixed-mode AEX1, which removed the trimer or larger aggregates through column chromatography and had lower dimer content than the outputs for modified CEX1 and modified CEX2 (5.8% vs. 7.3% and 7.7%, respectively), had a markedly higher k than the output for both modified CEX resins and the reference, which had 0.3% trimer or larger aggregate content as shown in Figure 5C. Furthermore, the differences between experimental and calculated values for each solution in Figure 5D show that mixed-mode AEX1 output has the best fit with complete blocking model. These results suggest that there are components besides aggregates detected by SEC that impede the filterability of plasma IgG on the virus filter, and the components formed in the process of producing aggregates are reduced by processing with modified CEX but not by mixed-mode AEX1.

4. Discussion

Plots of filterability profiles for mAb (Figure 1) and plasma IgG (Figure 2) with aggregate spike and after processing with various chromatography columns clearly show that chromatography processing significantly affects filterability for both solutions, but with different results for various chromatography resins. It should

be noted that all runs were conducted with the same solution conditions for control and simplicity of the experimental design, but optimizing solution conditions for each different resin could potentially result in different outcomes. Differences in initial flux, even for constant pressure filtration at the same pressure, may be due to differences in viscosity resulting from the chromatography processes.

Based on analysis of filtration behavior and the addition of column chromatography on filterability, users can consider choosing chromatography resins that will improve the overall performance of their virus filtration process. For aggregate-spiked mAb processing, the output from mixed-mode AEX1 and mixed-mode AEX2 showed improved filterability while normal AEX did not. Based on manufacturer information on the resins, mixed-mode AEX1 has a primary amine and butyl base and mixed-mode AEX2 has a tertiary amine and phenyl group, and as such, these mixed-mode AEX resins do not rely on the strength and weakness of an anion exchange group and hydrophobic group. Similarly, for aggregate-spiked plasma IgG processing, filterability was improved over reference by two resins with a sulfate ligand, modified CEX1 with dextran sulfate and modified CEX2 with cellulose sulfate, indicating that plasma IgG filterability improvement is due to dextran sulfate being more effective for flow-through processing. However, while both mixed-mode AEX and modified CEX column chromatography removed trimer or larger aggregates from plasma IgG, there were differences in removal of dimers by these two methods. Despite greater removal by mixed-mode AEX, modified CEX showed markedly better improvement in filterability with flux at the start of the filtration exceeding that for the reference. This observation suggests that the decrease in filterability of plasma IgG, which is polyclonal, is not dependent solely on the aggregate content determined by SEC, unlike the pattern observed for mAb solutions.

From clogging model results based on filtration behavior, we see that mAb with aggregate was best fit to the standard blocking model (Figure 4) and plasma IgG with aggregate was best fit to the complete blocking model (Figure 5). Appropriate selection of the best-fit model for each molecule was shown as the lowest k for both solutions with aggregate spike (control) and solutions with moderate reduction of aggregate following chromatography processing. Based on selection of the standard blocking model for mAb, the pores of the filter are likely narrowed by molecules adhering to the walls of the pores. In contrast, plasma IgG likely obstructs the pores based on the selection of the complete blocking model.

Although the clogging models assume simplified uniform cylindrical pores, which may not be exactly representative of virus filters, based on the studies and analyses presented here, applying the clogging models to filtration behavior could be an insightful way to characterize filtration processes. Our findings indicate that, by selecting chromatography processes that are compatible with virus filtration and that improve the filterability of the feed stream, the capacity of production processes can be increased. These processes can be conducted at large scales of at least 1000 L/m^2, and even larger throughput can be expected, for example, as has already been put into practice (Lute et al., 2020).[12]

Optimizing filterability through consideration of aggregate removal is of great interest for downstream process development. As future work, correlation of clogging model results and analytical results of solution characteristics including aggregate content along with the use of visualization techniques will deepen our understanding of filtration mechanisms. Applying filtration data from higher throughput runs (more than 500 L/m^2) to clogging model analysis will provide guidance for scaling up.

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Nomenclature

J [L/(m^2 h)] flow rate (flux) achieved at filtration volume

J_0 [L/(m^2 h)] initial flow rate (flux)

$J_{exp,i}$ [L/(m^2 h)] i$^{\text{th}}$ filtration behavior measurement for experimental flow rate

$J_{cal,i}$ [L/(m^2 h)] i$^{\text{th}}$ filtration behavior measurement for calculated flow rate

k [-] clogging factor

N [-] number of experimental measurements for each solution

V [L] filtration volume (throughput)

References

[1] a) L. Besnard, V. Fabre, M. Fettig, E. Gousseinov, Y. Kawakami, N. Laroudie, C. Scanlan, P. Pattnaik, *Biotechnol. Adv,* **2016** ,*34* , 1, https://doi.org/10.1016/j.biotechadv.2015.11.005; b) T. A. Grein, R. Michalsky, P. Czermak, in *Animal Cell Biotechnology*(Ed: R. Pörtner), **2014,** *1104* , 459-491, Methods in Molecular Biology (Methods and Protocols), Humana Press, Totowa, NJ,. https://doi.org/10.1007/978-1-62703-733-4_26; c) G. Miesegaes, S. Lute, K. Brorson, *Biotechnol. Bioeng.***2010** , *106* , 238, https://doi.org/10.1002/bit.22662; d) D. Strauss, J. Goldstein, T. Hongo-Hirasaki, Y. Yokoyama, N. Hirotomi, T. Miyabayashi, D. Vacante, *Biotechnol. Prog.* **2017** ,*33* , 1294, https://doi.org/10.1002/btpr.2506.

[2] a) S. Manabe, Y. Kamata, H. Iijima, K. Kamide, *Polym. J. (Tokyo, Jpn.)***1987** , *19* , 391, https://doi.org/10.1295/polymj.19.391; b) S. Manabe, T. Tsurumi, G. Ishikawa, M. Satani, T. Yamashiki, Y. Hamamoto, K. Yamaguchi, S. Kobayashi, N. Yamamoto, *Membrane* **1989** ,*14* , 77, https://doi.org/10.5360/membrane.14.77.

[3] a) J. G. Barnard, D. Kahn, D. Cetlin, T. W. Randolph, J. F. Carpenter,*J. Pharm. Sci.* **2014** , *103* , 890, https://doi.org/10.1002/jps.23881; b) M. Bieberbach, P. Kosiol, A. Seay, M. Bennecke, B. Hansmann, S. Hepbildikler, V. Thom, *Biotechnol. Prog.* **2019** ,*35* , e2776, https://doi.org/10.1002/btpr.2776; c) Q. Chen, D. Chen, *PDA J. Pharm. Sci. Technol.* **2015** ,*69* , 142, https://doi.org/10.5731/pdajpst.2015.01038; d) P. Genest, H. Ruppach, C. Geyer, M. Asper, J. Parrella, B. Evans, A. Slocum, *BioProcess Int* **2013** , *11* , 54.

[4] a) A. Brown, C. Bechtel, J. Bill, H. Liu, J. Liu, D. McDonald, S. Pai, A. Radhamohan, R. Renslow, B. Thayer, S. Yohe, C. Dowd, *Biotechnol. Bioeng.* **2010** , *106* , 627, https://doi.org/10.1002/bit.22729; b) P. Genest, H. Ruppach, C. Geyer, M. Asper, J. Parrella, B. Evans, A. Slocum, *BioProcess Int***2013** , *11* , 54; c) S. Roederstein, V. Thom, *BioPharm Int.* **2013** , Special Supplement, A Renaissance in Biomanufacturing: The Art of Purification.

[5] Y. Yigzaw, P. Hinckley, A. Hewig, G. Vedantham, *Current Pharmaceutical Biotechnology* **2009** , *10* , 421.

[6] H. P. Grace, *AIChE J.* **1956** , *2* , 316, https://doi.org/10.1002/aic.690020308.

[7] F. Badmington, R. Wilkins, M. Payne, E. S. Honig, *Pharm. Technol.* **1995** , *19* , 64.

[8] G. Bolton, M. Siwak, T. Ireland, H. Lutz, *BioPharm Int***2004** , *17* , 38.

[9] a) C. C. Ho, A. L. Zydney, *J. Colloid Interface Sci.* **2000** ,*232* , 389, https://doi.org/10.1006/jcis.2000.7231; b) G. Bolton, D. LaCasse, R. Kuriyel, *J. Membr. Sci.* **2006** ,*277* , 75, https://doi.org/10.1002/btpr.2514; c) G. R. Bolton, A. J. Apostolidis, *Biotechnol. Prog.* **2017** ,*33* , 1323.

[10] M. Sumiya, *SPRING Pall News* **2013** , *117* , 10.

[11] H. P. Grace, *AIChE J.* **1956** , *2* , 316, https://doi.org/10.1002/aic.690020308.

[12] S. Lute, J. Kozaili, S. Johnson, K. Kobayashi, D. Strauss,*Biotechnol. Prog.* **2020** , *36* , e2962, https://doi.org/10.1002/btpr.2962.

Figure legends

Figure 1. Filterability for mAb preparations and size exclusion chromatography profiles. A) Throughput against filtration time for reference (mAb without aggregate spike), control (1.0% aggregate spike) and chromatography and nylon filter outputs. B) Permeate flux (L/(m^2 h)) against throughput and aggregate and HCP content of the preparations. C) Size exclusion chromatography profiles of reference, control, mixed-mode AEX1 flow-through fraction of aggregate-spiked mAb and nylon prefilter filtrate of aggregate-spiked mAb. For all filtrations, mAb at 11 mg/mL in 20 mM Tris-Acetate, pH 7 adjusted to 15 mS/cm with NaCl was filtered at 0.3 MPa. Reference (mAb at 11 mg/mL with no aggregate spike) and control (mAb at 15 mg/mL spiked at 1.0% mAb aggregate for application to chromatography and nylon filter and adjusted to 11 mg/mL) have no column chromatography treatment. mAb aggregate spike was 11.4% dimer and 33.3% trimer or larger species. n.d., not detected

Figure 2. Filterability plasma IgG preparations. A) Throughput against filtration time for reference (plasma IgG without aggregate spike), control (0.5% aggregate spike), chromatography outputs and aggregate content of the preparations. B) Permeate flux (L/m^2 h) against throughput. For all filtrations, plasma IgG at 11 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 6 was filtered at 0.35 MPa. Reference (plasma IgG at 11 mg/mL with no aggregate spike) and control (plasma IgG at 15 mg/mL spiked with 0.5% plasma IgG aggregate for application to chromatography and adjusted to 11 mg/mL) have no column chromatography treatment. Plasma IgG spike was 14.8% dimer and 57.1% trimer or larger species. n.d., not detected

Figure 3. Plots of calculated and experimental results for the control solution (aggregate spike) for each clogging model: A) cake filtration model using Equation 2, B) intermediate blocking model using Equation 4, C) standard blocking model using Equation 6 and D) complete blocking model using Equation 8.

Figure 4. Analysis of clogging models for aggregate-spiked mAb (1.0% aggregate spike) filtrations shown in Figure 1. Experimental values and calculated values for each model are shown for A) reference (no aggregate spike), B) control (1.0% aggregate spike). C) Clogging factor, k, for filtrations shown in Figure 1. D) Mean difference between experimental values and calculated values for each clogging model (calculated using Equation 9). Clogging models: cake filtration, intermediate, standard and complete. For all filtrations, mAb at 11 mg/mL in 20 mM Tris-Acetate, pH 7 adjusted to 15 mS/cm with NaCl was filtered at 0.3 MPa. Reference (no aggregate spike) and control (aggregate spike) have no column chromatography treatment.

Figure 5. Analysis of clogging models for aggregate-spiked plasma IgG (0.5% IgG aggregate spike) filtrations shown in Figure 2. Experimental values and calculated values for each model are shown for A) reference (no aggregate spike), B) control (0.5% aggregate spike). C) Clogging factor, k. D) Mean difference between experimental values and calculated values for each clogging model (calculated using Equation 9). Clogging models: cake filtration, intermediate blocking, standard blocking and complete blocking. For all filtrations, plasma IgG at 11 mg/mL in 20 mM sodium acetate, 100 mM NaCl, pH 6 was filtered at 0.35 MPa. Reference (no aggregate spike) and control (aggregate spike) have no column chromatography treatment.

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