Effectiveness of host cell protein removal during depth filtration – Effect of filter chemistry

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Abstract
Recent advancements in upstream processing (USP) of monoclonal antibodies (mAbs) have increased the load on downstream processing (DSP). The increased cell density of the product stream leaving the bioreactor means a higher concentration of contaminants associated with the production of mAbs. One such example is host cell proteins (HCPs), impurities that can create challenges for the operation of protein A affinity chromatography and hydrophobic interaction chromatography used for mAb purification. Recent studies have shown that depth filters can bind many of these small impurities, decreasing the load on subsequent filters and chromatography columns. This study aimed to examine the binding characteristics of two commercial depth filters to understand the relationship between a depth filter's chemical composition and its ability to remove HCPs. Two depth filters with comparable pore sizes, each made from different filter media, were tested using a series of model proteins. The model proteins were chosen to match the range of typical HCPs based on their isoelectric points (pI), molecular weights, and hydrophobicities. These model proteins were. The X0SP filter, which has polyacrylic fibers and a synthetic silica filter aid, showed a higher binding capacity for α-chymotrypsin, conalbumin, and myoglobin than the X0HC filter, which has cellulose fibers and diatomaceous earth as the filter aid. The X0SP filter is particularly well suited for removing positively charged HCPs with binding capacities of more than 600 g/m^2 at low conductivity. These results provide important insights into the performance characteristics and proper selection of depth filters for the purification of monoclonal antibodies.

Introduction

Monoclonal antibodies (mAbs) are a class of revolutionary pharmaceuticals used to treat a wide range of diseases, including cancer, multiple sclerosis, and rheumatoid arthritis.[1] By 2017, two-thirds of all mAbs in the market were produced inside Chinese Hamster Ovary (CHO) cells.[1]

One issue that arises when synthesizing mAbs is that, in addition to the antibody of interest, the product stream leaving the bioreactor will also contain residual host cell proteins (HCPs) generated by the CHO cells.[2] Due to their synthesis using non-human expression systems, the administration of these HCPs to humans has the potential to provoke an immune response, reducing both the safety and efficacy of the biopharmaceutical.[3] As such, the viability of mAbs is heavily dependent on the purification process designed to remove these HCPs, as

well as nucleic acids (DNA and RNA), cell membrane fragments, and any bacteria and viruses that might contaminate the antibody product.

The purification process of these contaminants is known as downstream processing (DSP). Many downstream processing steps, such as protein A affinity chromatography and hydrophobic interaction chromatography, can separate HCPs from the mAb solution.[4] However, these chromatographic operations are quite expensive, and resin fouling caused by HCP accumulation can shorten the lifetime of the columns, leading to frequent cleaning and eventual replacement.[5] Maximizing the number of HCPs removed in the initial clarification process could decrease the load on subsequent operations, thereby decreasing the overall cost of the DSP.[4]

We believe that depth filters are the key to reducing the number of HCPs in mAb solutions before they are processed by other unit operations. Depth filtration is commonly used as the first step in the clarification of the cell culture fluid from the bioreactor, effectively removing the host cells and large cell debris via size exclusion.[6] However, Nejatishahidein et al.[7] have shown that depth filters can also remove smaller impurities, such as HCPs, through intramolecular interactions with the depth filter media. Several studies have shown that depth filters are able to adsorb/bind HCPs due to a combination of electrostatic and hydrophobic interactions.[8]

The protein binding characteristics of a depth filter depend on the chemical composition of the adsorbent. Depth filters consist of several components, including polymeric fibers that provide structural integrity, a high surface area adsorbent (often referred to as a filter aid) that is able to filter out impurities, and a binder that holds the different components together. Conventional depth filters typically contain diatomaceous earth, a naturally derived silica-based porous material with a large surface area for HCP binding. One drawback of diatomaceous earth is that it can contribute to leachable components, which may impact the filter's performance and that of subsequent unit operations in the DSP.[9] Filters containing all-synthetic silica, as opposed to naturally-derived materials, have been shown to contribute significantly smaller amounts of leachable components, making them a more consistent and robust option.[9] Synthetic depth filters have also demonstrated improved HCP removal under some experimental conditions[9], although the generality of this result has not yet been established.

Nguyen et al.[9] compared the number of HCPs (in parts per million or ppm) that remained after filtration through a B1HC filter (containing diatomaceous earth) and an X0SP filter (containing synthetic silica). Although they conducted experiments with other filters, such as the D0SP and the X0HC, most of their experiments were run through either a B1HC filter or an X0SP filter. The problem with comparing these two filters is that they have different pore size ranges. Furthermore, this study did not standardize the pH of the feed solutions when running the experiments.

The objective of this study was to garner new insights regarding the relationship between a depth filter's composition (diatomaceous earth or synthetic silica) and its capacity for removing HCPs. The X0SP was compared with the X0HC to compare the effectiveness of filters containing diatomaceous earth and synthetic silica in adsorbing HCPs.

Materials and Methods

A series of model proteins were used based on their isoelectric points (pI), molecular weights (MW), and hydrophobicities (GRAVY number), as discussed by Nejatishahidein et al.[7] Table one shows the protein selection and their relevant values. All model proteins were obtained from Sigma-Aldrich as lyophilized powders, which were then dissolved in 150 mM (1x) phosphate buffered saline solution (PBS, AM9625, Thermo Fisher Scientific, Waltham, MA) with the pH adjusted to 7.4. All protein solutions were filtered through 0.2 μm polyethersulfone (PES) syringe filters (VWR 28145-501) immediately before running them through the depth filters to remove any large aggregates or undissolved protein.

TABLE 1: Physical properties of model proteins examined in this study

Protein	Catalog number	Source	MW (KDa)	pI	GRAVY
Albumin	A2153	Bovine serum	67	4.8	0.064
Ovalbumin	A5503	Chicken egg white	45	4.9	−0.663
α-Chymotrypsin	C4129	Bovine pancreas	25	8.8	−0.436
Conalbumin	C7786	Chicken egg white	77	6.6	−0.475
β-Lactoglobulin	L2506	Bovine milk	17.5	5.3	−0.006
Myoglobin	M0630	Equine skeletal muscle	16.9	7	−0.396
Ribonuclease A	R4875	Bovine pancreas	13.7	9.6	−0.162

Experiments were performed using either the Millistak+® Pod Depth Filter X0HC, which contains diatomaceous earth and cellulose, or the Millistak+® HC Pro Pod Grade X0SP, which contains polyacrylic fibers and synthetic silica (Millipore Sigma, Burlington, MA). Filters were initially flushed with 150 L/m^2 DI water for 30 minutes and then flushed with an additional 50 L/m^2 of 150 mM PBS. This extensive flushing was needed to remove any leachables. The model protein solution was then run through the filter at a constant filtrate flux of 150 L/m^2/hr (LMH), which was maintained by a Masterflex L/S Peristaltic roller pump (Gelsenkirchen, Germany) on the permeate exit line. All experiments were performed at room temperature (20-24°C).

Permeate samples were collected throughout the experiment. Feed and filtrate sample concentrations were measured using a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Waltham, MA) based on the UV absorbance at a predetermined wavelength for each protein.

Data and Results

Figure 1 shows data for the protein concentration in the permeate samples, normalized by the protein concentration in the feed solution, obtained during the depth filtration of 0.1 g/L feed solutions containing either α-chymotrypsin, myoglobin, or conalbumin, in 1x PBS at a filtrate flux of 150 LMH. Both myoglobin and conalbumin are nearly neutral proteins at pH 7.4, whereas α-chymotrypsin is positively charged. The filled symbols show results for the X0HC filter, while the open symbols show results for the X0SP filter. The data are plotted as a function of the volumetric throughput, defined as the cumulative filtrate volume divided by the membrane area. Larger volumetric throughput corresponds to a longer filtration time, with a volumetric throughput of 1500 L/m^2 corresponding to a 10-hr. filtration experiment.

The initial permeate samples have little-to-no proteins since all of the proteins in the feed solution are bound by the filter. As the protein binding sites within the filter become saturated, the protein concentration in the permeate solution increases, eventually approaching a value nearly equal to that in the feed solution. The X0SP filters show significantly higher binding capacities (delayed breakthrough curves) than the X0HC filters for all three proteins, with myoglobin having the most significant difference.

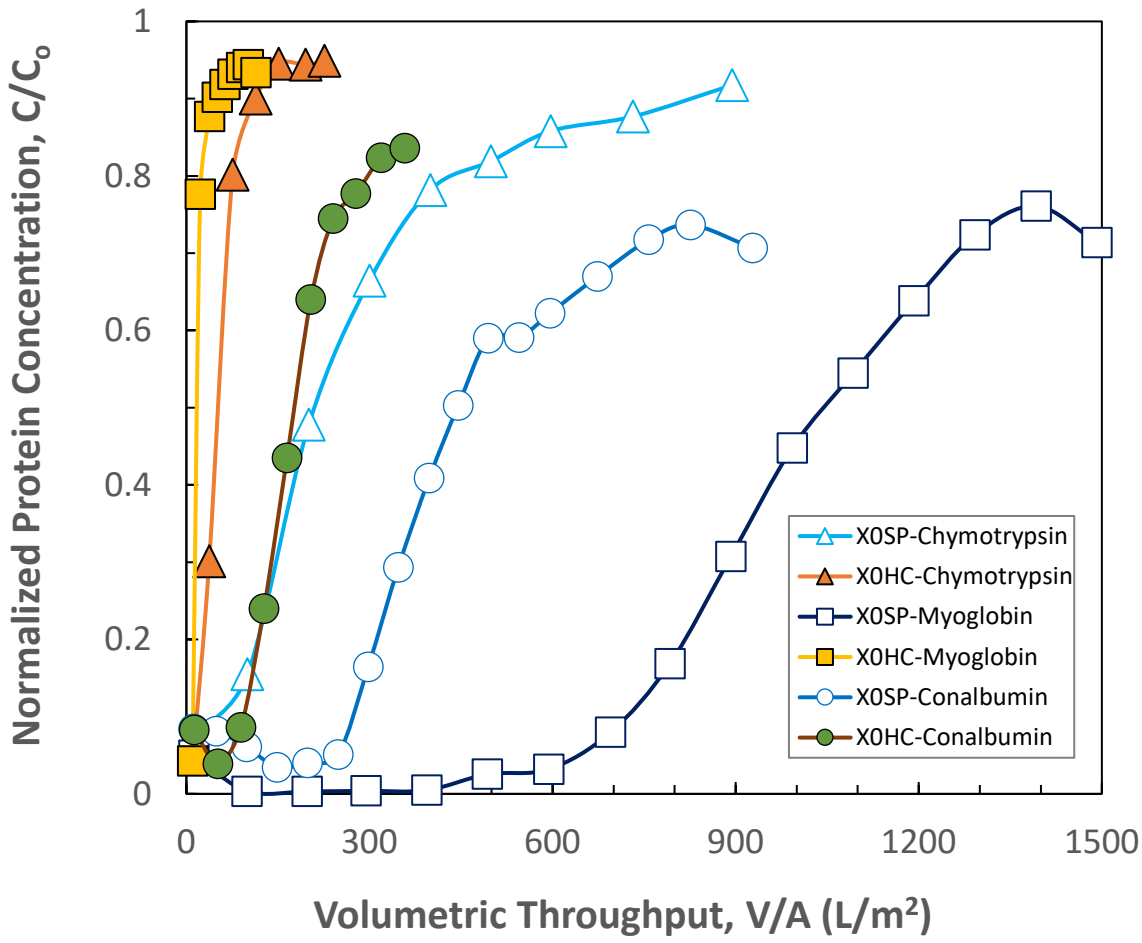

FIGURE 1: Normalized protein concentrations in permeate samples as a function of volumetric throughput during filtration of 0.1 g/L solutions of the model proteins in 1x PBS through the X0SP and X0HC depth filters

Figure 2 shows corresponding data for the filtration of the same set of model proteins but with 0.2 g/L concentrations. Again, the highest binding capacities were obtained with the X0SP filter, with the myoglobin requiring more than 900 L/m^2 of filtration to saturate the binding sites within the filter (i.e., to achieve a normalized permeate concentration of one). Interestingly, in the 0.2 g/L solutions, the binding capacity for chymotrypsin on the X0SP filter was greater than that for conalbumin. This behavior is precisely the opposite of what was observed in the more dilute 0.1 g/L solutions. This likely reflects a difference in binding affinities for these proteins, with the conalbumin saturating the binding sites at a lower concentration than the chymotrypsin.

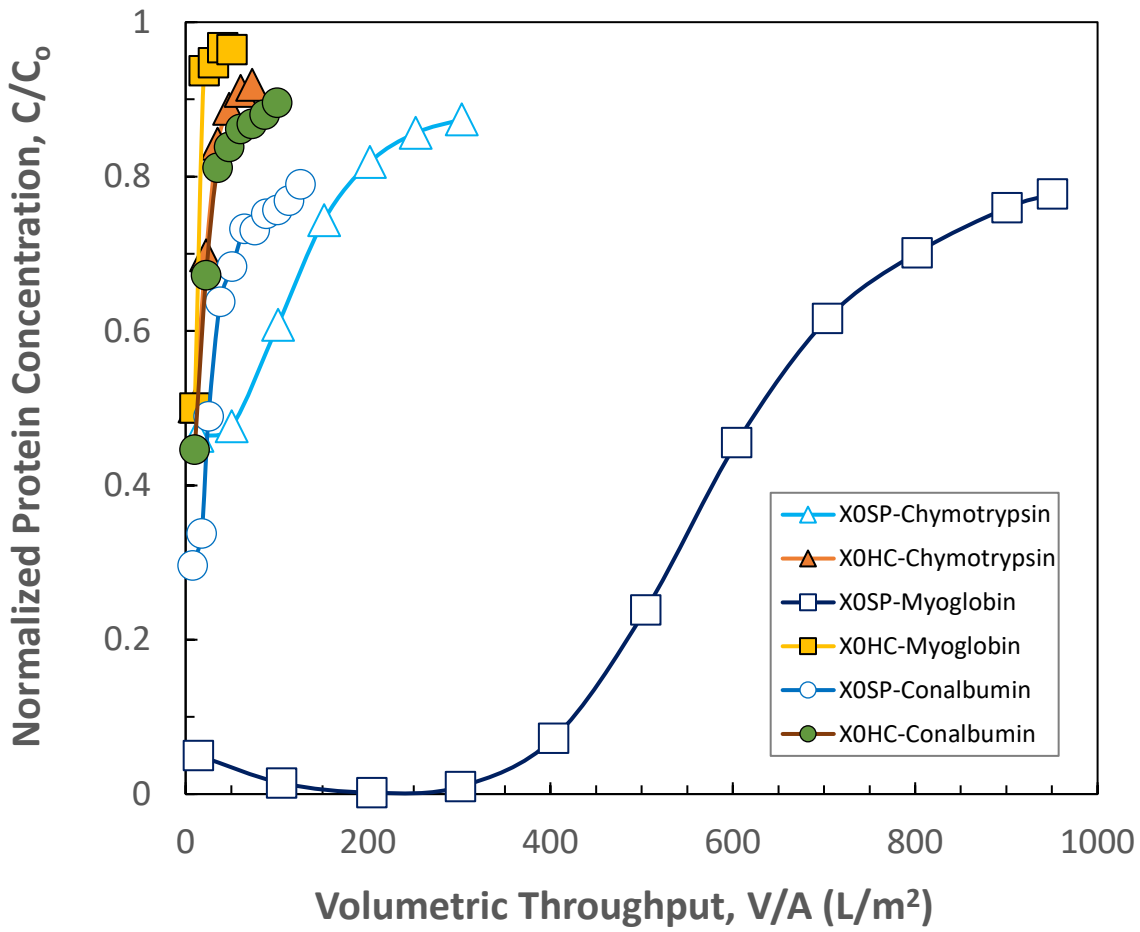

FIGURE 2: Normalized protein concentrations in permeate samples as a function of volumetric throughput during filtration of 0.2 g/L solutions of the model proteins in 1x PBS through the X0SP and X0HC depth filters

Figure 3 shows the breakthrough curves for the model proteins at a concentration of 0.5 g/L. The normalized protein concentrations increase much faster with increasing volumetric throughput for the 0.5 g/L solutions since the higher protein concentration in the feed leads to more rapid saturation of the binding sites within the depth filters. However, the general trends in the data for the different model proteins are similar to that seen with the 0.2 g/L solutions, with myoglobin having the greatest capacity on the X0SP filter but the lowest capacity on the X0HC filter.

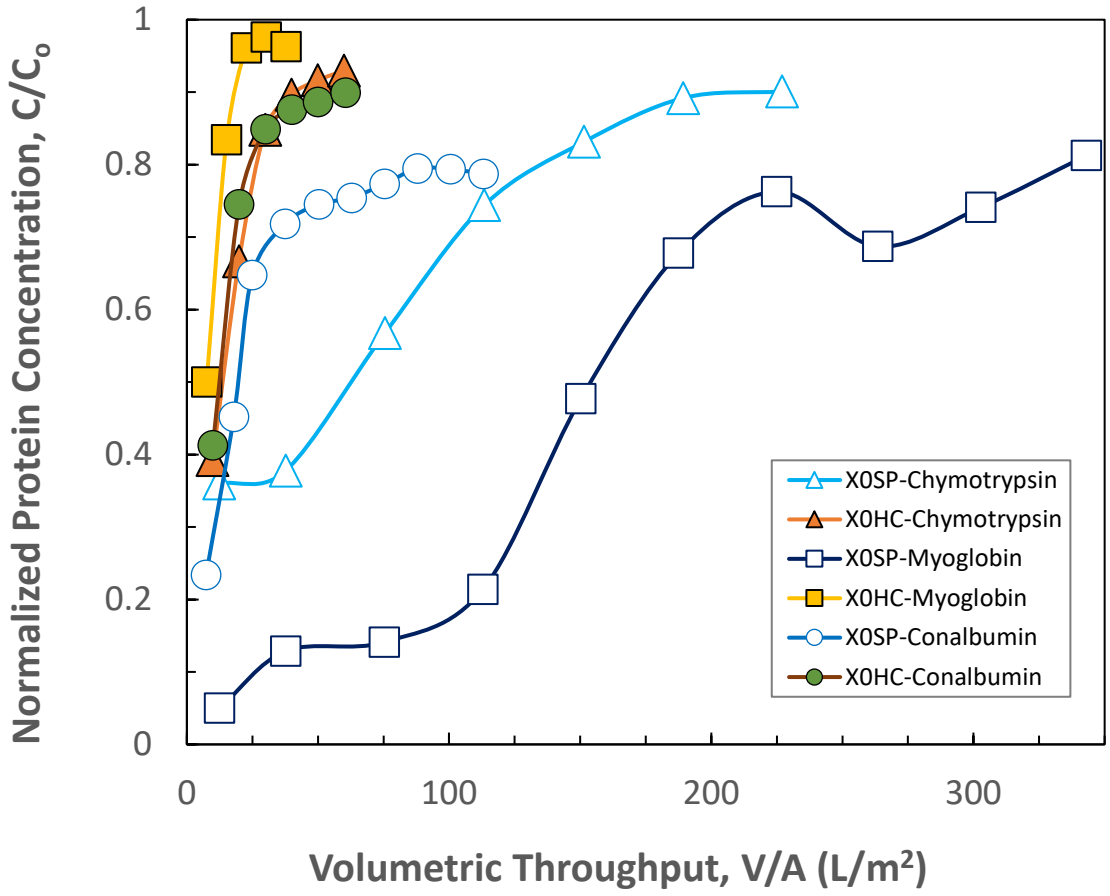

FIGURE 3: Normalized protein concentrations in permeate samples as a function of volumetric throughput during filtration of 0.5 g/L solutions of the model proteins in 1x PBS through the X0SP and X0HC depth filters

Conclusions

This study is the first to directly compare the binding capacities of model HCPs through a filter containing diatomaceous earth with one containing synthetic silica while controlling for the filter pore size, solution pH, and buffer strength. The X0SP filter showed much higher binding capacities for α-chymotrypsin, myoglobin, and conalbumin, indicating that replacing the traditional diatomaceous earth depth filters with synthetic silica depth filters may be key in decreasing the number of HCPs in the product stream after initial clarification by depth filtration.

The greatest binding capacity on the X0SP (synthetic silica) filter was seen with myoglobin, which is a small neutral protein that is relatively hydrophilic. This suggests that electrostatic interactions are relatively unimportant in protein binding to the X0SP depth filter, at least at the relatively high conductivity (150 mM PBS) examined in these experiments. In contrast, the X0HC filter showed very low binding of myoglobin, suggesting that the underlying binding mechanisms for the X0HC and X0SP depth filters may be very different.

Future experiments need to be performed with more proteins to be able to compare the two filters across a broader range of protein molecular weights, isoelectric points, and hydrophobicities. Such data could potentially be used to develop correlations between the binding capacity and the properties of both the proteins and the depth filters. This could enable biomanufacturers to select specific depth filters that are able to remove problematic host cell proteins based on their known biophysical properties. This would significantly improve the development of downstream processes that can effectively handle the increased product titer from modern bioreactors used to produce monoclonal antibody products.

References

(1) Dhara, V. G.; Naik, H. M.; Majewska, N. I.; Betenbaugh, M. J. Recombinant Antibody Production in CHO and NS0 Cells: Differences and Similarities. *BioDrugs* **2018**, *32* (6), 571–584. https://doi.org/10.1007/s40259-018-0319-9.

(2) Bracewell, D. G.; Francis, R.; Smales, C. M. The Future of Host Cell Protein (HCP) Identification during Process Development and Manufacturing Linked to a Risk-Based Management for Their Control. *Biotechnol. Bioeng.* **2015**, *112* (9), 1727–1737. https://doi.org/10.1002/bit.25628.

(3) Wang, X.; Hunter, A. K.; Mozier, N. M. Host Cell Proteins in Biologics Development: Identification, Quantitation and Risk Assessment. *Biotechnol. Bioeng.* **2009**, *103* (3), 446–458. https://doi.org/10.1002/bit.22304.

(4) Kornecki, M.; Mestmäcker, F.; Zobel-Roos, S.; Heikaus de Figueiredo, L.; Schlüter, H.; Strube, J. Host Cell Proteins in Biologics Manufacturing: The Good, the Bad, and the Ugly. *Antibodies* **2017**, *6* (3), 13. https://doi.org/10.3390/antib6030013.

(5) Zhang, J.; Siva, S.; Caple, R.; Ghose, S.; Gronke, R. Maximizing the Functional Lifetime of Protein A Resins. *Biotechnol. Prog.* **2017**, *33* (3), 708–715. https://doi.org/10.1002/btpr.2448.

(6) Nejatishahidein, N.; Zydney, A. L. Depth Filtration in Bioprocessing — New Opportunities for an Old Technology. *Curr. Opin. Chem. Eng.* **2021**, *34*, 100746. https://doi.org/10.1016/j.coche.2021.100746.

(7) Nejatishahidein, N.; Borujeni, E. E.; Roush, D. J.; Zydney, A. L. Effectiveness of Host Cell Protein Removal Using Depth Filtration with a Filter Containing Diatomaceous Earth. *Biotechnol. Prog.* **2020**, *36* (6), e3028. https://doi.org/10.1002/btpr.3028.

(8) Yigzaw, Y.; Piper, R.; Tran, M.; Shukla, A. A. Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal during Monoclonal Antibody Purification. *Biotechnol. Prog.* **2006**, *22* (1), 288–296. https://doi.org/10.1021/bp050274w.

(9) Nguyen, H. C.; Langland, A. L.; Amara, J. P.; Dullen, M.; Kahn, D. S.; Costanzo, J. A. Improved HCP Reduction Using a New, All-Synthetic Depth Filtration Media Within an Antibody Purification Process. *Biotechnol. J.* **2019**, *14* (1), 1700771. https://doi.org/10.1002/biot.201700771.