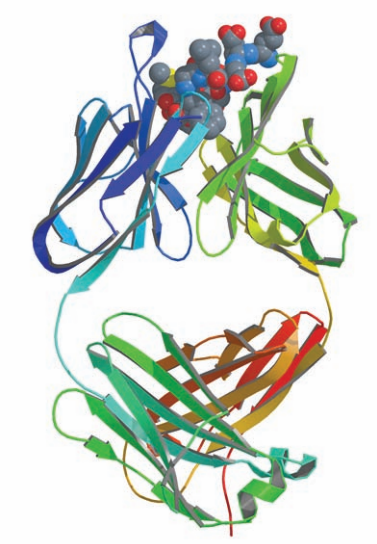
NCSRT

Works™ Case Study

Isolation of secreted proteins from mammalian cells with *SmartFlow*™ TFF



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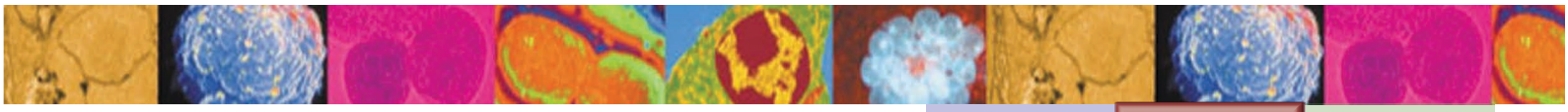

Isolation of secreted proteins from mammalian cell culture

Overview:

The *Isolation of secreted proteins from mammalian cell culture* procedure from NCSRT is intended for developing an optimized process for isolating secreted proteins such as monoclonal antibodies from mammalian cell culture. The process incorporates a lower shear environment to avoid cell lysis. The volume to membrane area ratio plays an important role developing an optimized process. The microfiltration (MF) membranes used for optimized mammalian cell culture processes are prone to plugging if the fluid flow at the membrane surface is not managed properly. Once the optimum flow rate is determined at a small scale, the ratio of the starting volume to membrane area must be kept constant during scale up. When using MF filters the TMP should be kept very low, less than 6 psi. This Case Study presents the results of a study that was performed at a major biopharmaceutical manufacturer on the isolation of a human monoclonal antibody from CHO cells using the process development methodology developed by NCSRT.

Case Study:

Process development scientists at a major biopharmaceutical company have looked at concentrating CHO cells while passing IgG through a MF as an alternative to centrifugation for the initial step in their downstream processing. Industrial scale cell clarification with traditional TFF formats have been subject to low flux rates and unacceptable product yields. Centrifugation combined with depth filtration is reported to provide acceptable yields but require significantly higher initial capital investment to implement.

Two different sets of studies were undertaken. First, a comparison of the filtration performance of the three basic tangential flow formats was performed in their cell culture system. Secondly, a series of optimization runs were performed to select the parameters for further scale-up and determine if the optimized process would deliver acceptable results. In the optimization studies, membranes chemistries, pore sizes, shear rates, and volume to membrane area ratios were examined within the selected platform.

CHO cell cultures were grown and stored at 4°C until these experiments were performed. Antibody passage was measured using HPLC with a protein A column.

The clarifications were performed using a constant 30 LM (L starting volume / m^2 membrane area) for the base line test of the competing TFF formats. The results are summaried in Table 1.

The *SmartFlow*™ TFF module was the only TFF format to provide acceptable passage of the target antibody. However, the evaluation test conditions demonstrated that under unoptimized process conditions permeate flux rates will decline very rapidly. Process optimization studies for the *SmartFlow* TFF filters were conduced at the 0.03 m^2 of membrane area scale. This format was selected because of its ease of use and the small quantities of starting CHO cell culture broth needed to perfrom the experiments. Scale up of a MF step such as a mammalian cell harvest differs from the scale up of an ultrafiltration (UF) step. The MF process is scaled up based upon the starting volume to membrane area ratio. Therefore, the scaled up process should take the exact same time as the development process. If a small scale process is running in less than the ideal time, the experiment should be repeated using a lower starting LM ratio.

Based on the few number of cell aggregates, a channel height of 0.25 mm was used for optimization studies. The membranes tested were the polysulfone (PS) membrane with a 0.45 µm or 0.2 um pore sizes and a regenerated cellulose (RC) membrane with a 0.45 and 0.2 µm pore sizes. In these tests, only the cell concentration step was optimized as diafiltration was not performed. Tests using diafiltration are needed to finalize an optimized procedure.

Two (2.0) liters of starting material were added to the retentate reservoir and the pump was adjusted so that the inlet pressure reached 4 psi. The solution was concentrated approximately 5X if the permeate flux was over 100 LMH. Samples were taken at the beginning, 2X, and end of the experiment. The results of these experiments are summarized in Table 2.

Although all the membranes were capable of finishing the filtration, the MPS 0.45 µm pore size was considerably faster than the other membranes in the test. For example, the 0.2 µm and 0.1µm MPS membrane evaluations were stopped at the 2X sample due to their lower permeate flow rates when compared to the MPS 0.45 µm membrane. Additionally, the MPS 0.2 µm and 0.1µm had lower antibody passage than the MPS 0.45 µm membrane. The RC membranes had high antibody passage, but the flux rate through these membranes was lower than the flux through the MPS 0.45 µm membranes. Therefore, the results of the initial screen showed the 0.45 µm MPS membrane to have the desired combination of high flux rates and high Mab passage. Based upon these initial data, additional tests were made using the MPS 0.45 µm

Table 1

Flux and % Passage by Platform Technology

Format	Membrane	Pore size	LMH	% Passage
SmartFlow™ TFF 0.68mm channel	MPS	0.45	900-10*	>95
Traditional Cassette[1] 0.5mm channel	PVDF	0.45	10	40
Hollow Fiber[1] 1.0 mm diameter	PS	0.22	10	65

* LMH range during the screening process

pore size membrane to optimize the filtration efficiency by examining the effects of increasing the shear rate and increasing the L/ m² (LM) ratio on the cell harvest results.

The starting volume to membrane area ratio was studied. The starting volume was increased to 4.0 L. This increased the volume to membrane area from 63 to 125 LM for this set of experiments. The result from this experiment is summarized in Figure 1. As expected, it was found that the filtration was slower at the higher starting ratio (open symbols in Figure 1). However, the overall flux remained over 200 LMH for the experiment. Therefore, scale up can proceed using the PS 0.45 µm membrane with a starting volume to membrane area of 125 LM.

The final variable studied was the shear rate. Increasing the shear rate tends to increase the permeate flux. However, if the shear is increased too high, the shear-sensitive CHO cells can be torn apart, negatively impacting the filtration. In this study, the shear rate was increased from the baseline of 7600 sec^{-1} to 12000 sec^{-1}. The recirculation rate was increased 55% to achieve the higher shear at the membrane surface. The increased recirculation rate necessary to increase the shear rate will produce an increase in the filter inlet pressure and thus increae the TMP. For reference, the 0.45 µm RC membrane was run under the same conditions. For these experiments, a 125 LM ratio was utilized. The high shear studies concentrated the CHO cells to 9X. Under high shear conditions, the flux rate of the 0.45 um MPS membrane increased throughout the course of the experiment, when compared to the flux rate at 7,600 sec^{-1} shear. The flux rate of the 0.45 um RC membrane decreased throughout the course of the experiment when compared to the flux rate of the same membrane at 7600 sec^{-1} shear (Figure 2).

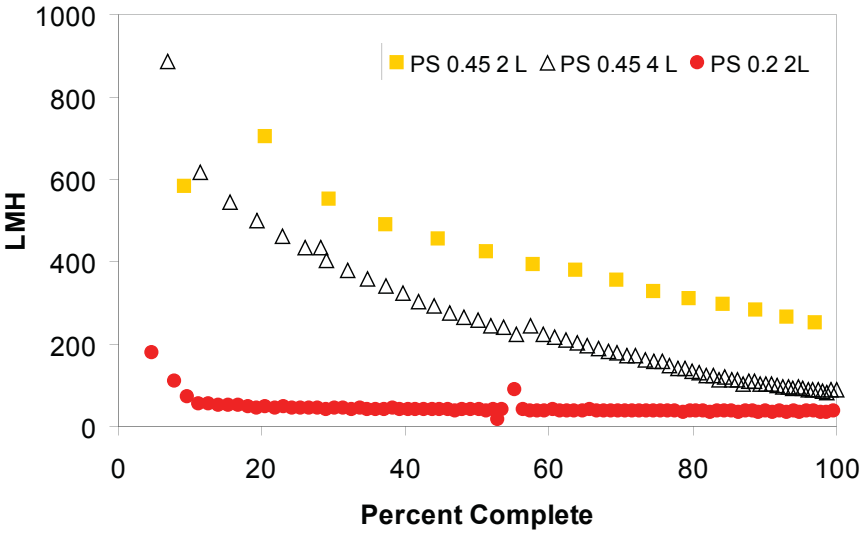

Figure 1 Performance of MPS membrane for CHO cell harvest. The closed symbols have a starting LM of 63. The open symbols have a LM of 125.

Samples were analyzed during the filtration to determine the fraction of the antibody that was passing through each membrane. The 0.45 MPS maintained complete protein passage throughout the different process conditions tested (at least 98% passage). In constrast, the 0.45 µm RC membrane exhibited a decrease in the protein passage when operated at the same increased shear and increased LM ratio (Figure 3). Due to its high permeate flux and high antibody passage, the 0.45 MPS membrane was selected for further scale up. The operating conditions chosen based upon these trials were a LM of 125 and a shear of 12,000 sec^{-1}.

The true advantage of the *SmartFlow*™ filter technology clearly presents itself when the optimized results presented and compared with the initial trial results in the same cell system as reported earlier (Table 3).

Table 2 - Summary of *SmartFlow* TFF harvest experiments with CHO cells producing antibodies

Membrane	*Pore size (µm)*	*Starting Volume (L)*	*Shear (sec^{-1})*	*Average LMH ($L/m^{-2}h^{-1}$)*	*Starting TMP (PSI)*	*Protein Passage at 2X (%)*	*Final Concentration*	*Protein Passage Final (%)*
MPS	0.45	2	7600	405	2.2	100	5.6 X	99
MPS	0.2	2	7600	43	1.8	69	2 X	69
MPS	0.1	2	7600	30	1.65	57	2 X	57
RC	0.45	2	7600	250	6.5	99	4.8 X	95
RC	0.2	2	7600	178	3.65	99	7 X	98
MPS	0.45	4	7600	213	2.25	99	9 X	97
RC	0.45	4	7600	305	8.6	99	6.9X	98
MPS	0.45	4	12000	278	3.2	100	9 X	99
RC	0.45	4	12000	128	6.55	99	10 X	90

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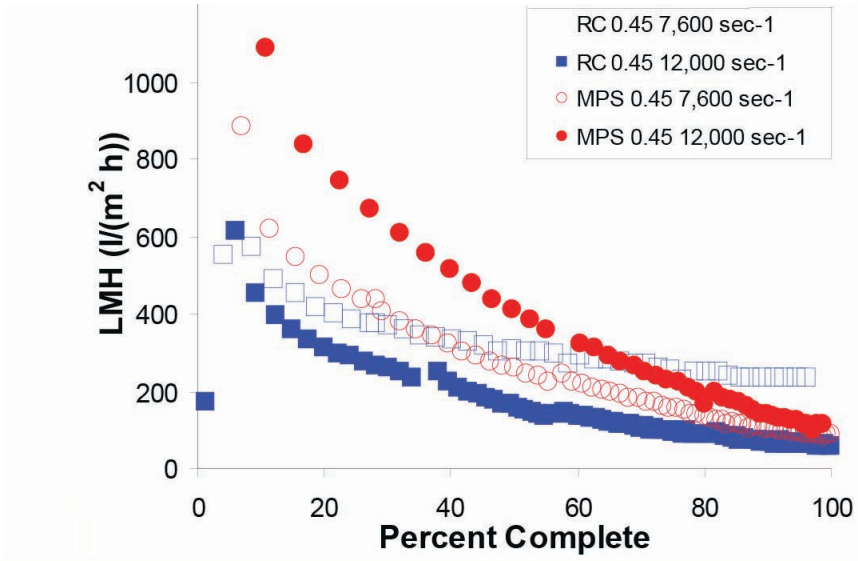
Figure 2



LMH under starting and high shear conditions. Open figures are low shear, filled figures are high shear data. Experiment was complete at 9X concentration.

Figure 3

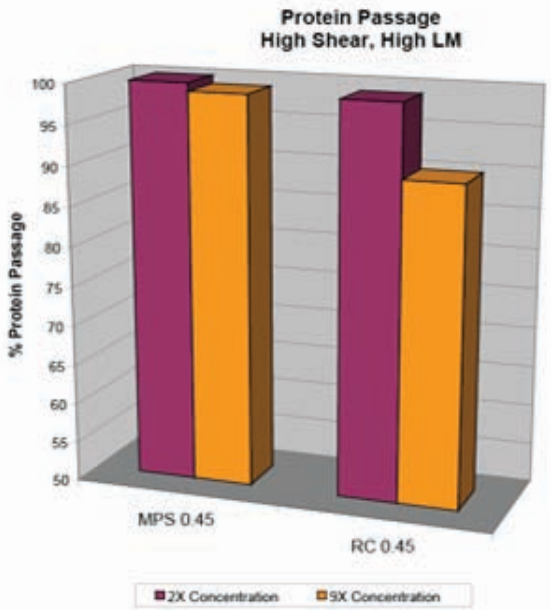


Table 3

Flux and passage under optimized *SmartFlow*™ parameters

Format	Membrane	LMH	% Passage
SmartFlow TFF	0.45 µm MPS	>200	>99
Cassette	0.45 µm PVDF	10	40
Hollow Fiber	0.2 µm PS	10	65

Conclusion:

SmartFlow TFF provides the optimum platform for the isolation of secreted proteins from mammalian cell culture in both membrane performance as measured by LMH and protein passage which drives the production process yield for the subsequent downstream processing procedures. NCSRT *SmartFlow* technology provides unparalleled value in downstream process for cell harvest applications in CHO cell culture systems.

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