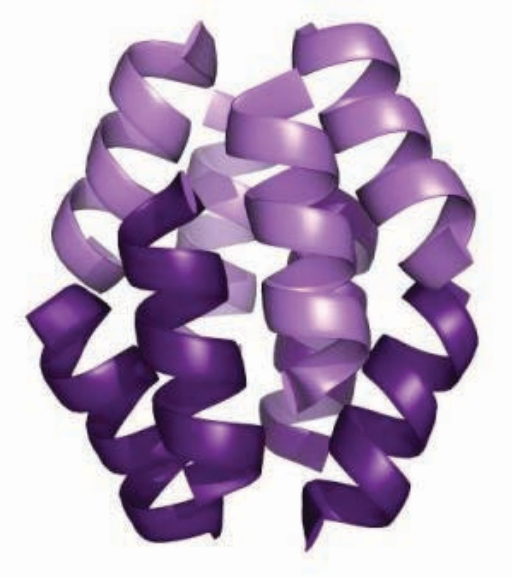
NCSRT

Works™ Case Study

Simultaneous isolation and concentration with *SmartFlow*™ TFF



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Simultaneous isolation and concentration *SmartFlow*™ TFF

Background:
The *Simultaneous isolation and concentration* procedure from NCSRT is intended for developing an optimized process for isolating biomolecules such as proteins from fermentors, bioreactors, and extraction processes. The process incorporates a harvest step to remove the target product from the starting medium using a membrane that freely passes the target molecule. The second process step is the concentration of the target molecule in an filtration step that retains it. The two steps are linked together in a closed loop system. The permeate from the harvest step provides the input for the concentration step. The permeate from the concentration step is feed back to the harvest step to serve as the diafiltration buffer for the process. This greatly reduces expenses for tanks and associated hardware, chemical makeup and disposal costs for the diafiltration buffer, and total process time.

This Case Study presents the results of a study that was performed on the harvest of a human monoclonal antibody from CHO cells. The test compared the yield from the *SmartFlow*™ TFF filter modules developed by NCSRT to the yield from the current centrifugation and depth filtration operations.

Case Study: Isolating Mab from a CHO cell culture

Process development scientists at a major biopharmaceutical company evaluated harvesting CHO cells and passing their Mab through a microporous polymeric membrane as an alternative to their current centrifugation and depth filtration process for the initial step in their downstream processing. Historically, 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 requires significantly higher initial capital investment to implement.

A test of the filtration performance of the NCSRT *SmartFlow* tangential flow filtration (SF-TFF) platform was performed with the cell line. The protocol was developed to test the SF-TFF in a two stage simultaneous process, using a 0.45 µm MF membrane for the CHO cell harvest and a 10kD UF membrane for the concentration step (Figure 1).

The current centrifugation/depth filtration process produces a yield of 90%. The objective of the test was to determine if the ST-TFF simultaneous process could meet or exceed the yield of the current process.

Methods:

One and one half (1.5) 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 diafiltered 5X. Samples were taken at the beginning, 1X, 2X, 3X, 4X, and end of the experiment. Quantification of antibody passage was determined by a proprietary customer assay. The antibody passage levels are summarized in Table 1 under the % Removed from Harvest column.

The harvest membrane evaluated was a 0.45 µm modified polysulfone (MPS) membrane at challenge level of 25 LM (L of media per square meter of membrane). The recirculation flow rate was set to 14 LPM (liters per minute) to achieve a shear rate of 8000 sec^{-1} at the membrane surface. Based on customer experience with the CHO cell culture being tested, there was not a concern of aggregation or cell clumping, so a 0.5 mm channel height was selected for the *SmartFlow* filter module. This resulted in an initial permeate flow of 60 mL/min.

For consistency, the customer used the same 10 kD regenerated cellulose (RC) ultrafiltration membrane for the Mab retention and concentration loop they use in existing intermediate downstream processes. The customer recognized that they would suffer final product yield losses due to the design of the traditional cassette system they used. For this study, performance of the harvest step is determined by the Mab passage into the Loop 2 retentate tank. Final total recovery would be reduced due to the unoptimized flow dynamics of the Loop 2 cassette. The total system recovery was measured also. The inefficiency of the second step was determined to be the cause of the product loss.

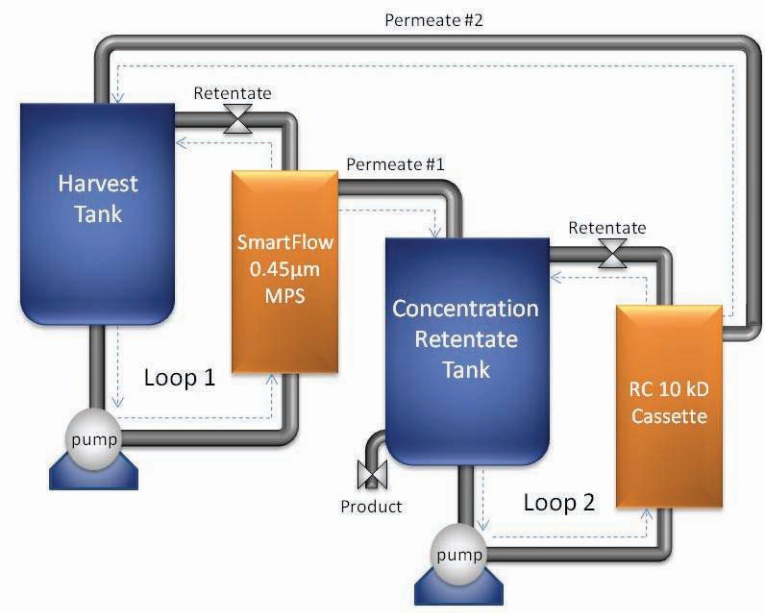

Figure 1 – Simultaneous Processing for Mab harvest.

Simultaneous isolation and concentration *SmartFlow*™ TFF

Results:

The use of the 0.45 µm MPS membrane in the *SmartFlow*™ filtration module produced a total recovery of the Mab in the Concentration Retentate tank of 96% (Table 1). The average LMH of the initial trial ranged from instantaneous levels of 53 to 12, with an average LMH of 20 (Figure 2).

Total system recovery was reduced to 89.5% due to Mab retained in the Loop 2 traditional TFF cassette. Total system recovery can in improved to>95% by optimizing Loop 2. Both Loop 2 and the total system yield will be optimized in subsequent experiments.

The close adherence in the observed Mab passage to the theoretical levels was observed. The correlation was computed as TFF efficiency (Removed/ Theoretical) at each step of the continuous diafiltration process. (Table 1)

Conclusion:

The *SmartFlow* TFF module provided improved yield of the target antibody compared to the current system of centrifugation and depth filtration (96% vs. 90%).

Additional benefits of reduced buffer volumes and their associated costs are attainable when implementing the *Simultaneous isolation and concentration* process using *SmartFlow* filter modules. Additional yield can be achieved by performing a systematic evaluation of the different membrane polymers, pore sizes, and process conditions using the NCSRT *Simultaneous isolation and concentration* membrane selection procedure in both loops of the system. *SmartFlow* TFF provides a preferred alternative platform to centrifugation/depth filtration for the isolation of Mab from mammalian cell culture in membrane performance as measured by protein passage.

Figure 2 Permeate Flux of Mab Harvest *SmartFlow* TFF with 0.45 um MPS

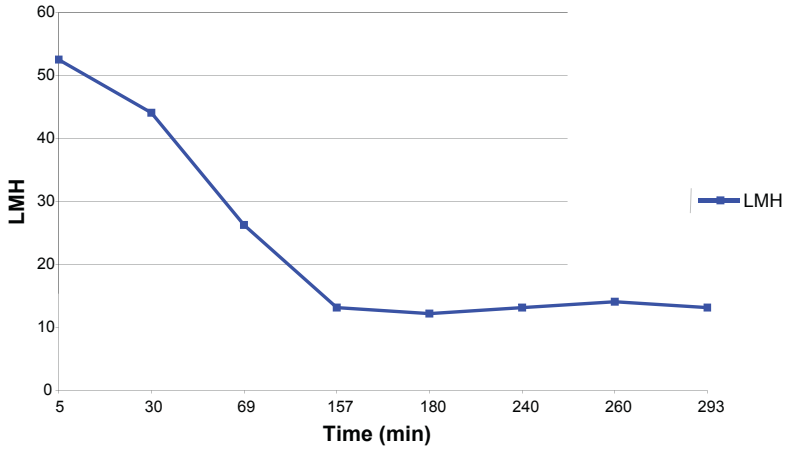

Table 1 Comparison of Actual to theoretical passage

Simultaneous Diafiltration	% Removed from Harvest Step	Theorectical Removal	Efficiency
1X	63%	63%	100%
2X	81%	86%	94%
3X	89%	95%	94%
4X	93%	98%	95%
5X	96%	99%	97%

NCSRT *SmartFlow* technology provides unparalleled value in downstream process for cell harvest applications in CHO cell culture systems.

To implement this type of process for small proteins or biomolecules (8-30 K) follow the *Works*™*Simultaneous isolation and concentration* protocol. To develop an optimized system for virtually any fermentation, cuilture, or extraction process product, refer to the *Works Simultaneous isolation and concentration* Optimization Procedure.

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