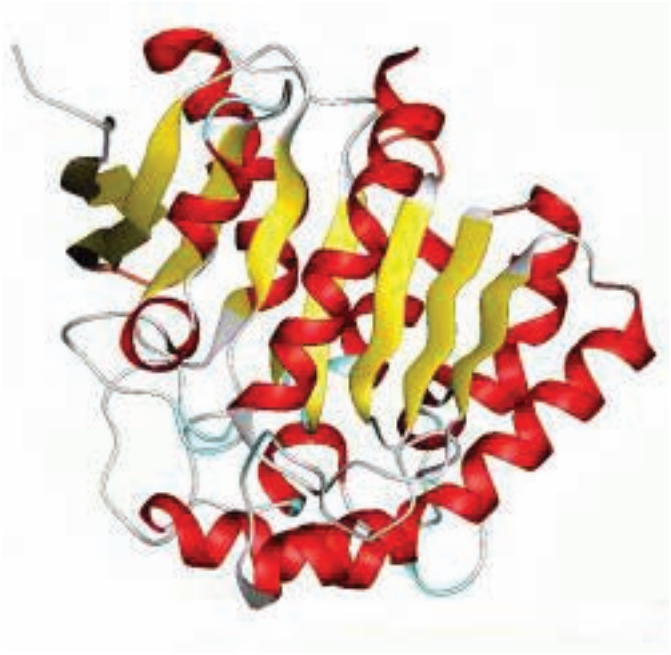
NCSRT

Works™ Case Study

Ultrafiltration, concentration and diafiltration with *SmartFlow*™ TFF



NCSRT, Inc.
1000 Goodworth Dr.
Apex, NC 27539
USA

Phone: +1 919 387 8460
Fax: +1 919 387 8540
email: info@ncsrt.com
Web: http://www.ncsrt.com

Ultrafiltration, concentration, and diafiltration

Overview:

The *Ultrafiltration, concentration, and diafiltration* process Optimization procedure from NCSRT is intended as a generalized protocol for developing an optimized process for virtually any TFF operation. Excellent examples include intermediate TFF steps such as preparing a product for loading on to a chromatography column, desalting and concentrating a product off an ion exchange, affinity, or HIC column, and preparing a product for final formulation.

The *Ultrafiltration, concentration, and diafiltration* processes use ultrafiltration membranes that retain the target molecule and allow lower molecular weight molecules and buffer salts to pass freely through the membrane. The process can often be run at high flux rates by using the most open UF membrane that retains the target molecule. The retention characteristics of a specific molecule will change with different buffers, temperatures, concentrations, and membrane polymer. By examining the retention characteristics of the different UF membranes available in the appropriate process conditions, a well defined and executed process development study can identify the most efficient membrane and process conditions to achieve the required separation performance.

An optimized TFF process is developed starting with the initial selection of the membrane polymer, pore size, and the operational parameters to evaluate. Within classes of molecules, like monoclonal antibodies, the performance of a *SmartFlow*™ TFF process may be very similar from one molecule to the next. This provides a basis for recommending the initial membrane and process conditions to one who is not experienced with the SmartFlow filtration technology. In addition to this procedure for developing a generalized intermediate process, NCSRT offers a library of application specific Optimization Procedures. These guides provide specific recommendations with respect to membrane type, pore size, and starting operational parameters such as shear and TMP for:

Concentration and diafiltration of viral antigens
Concentration and diafiltration of whole bacterial cells
E. Coli lysate filtration by simultaneous process
Isolation of bacterial phage
Isolation of proteins from cell lysate
Isolation of secreted proteins from mammalian cell culture
Separation of secreted proteins from whole bacterial cells
Purification of secreted viral antigens from cell culture
Isolation and concentration of small molecules

The following case study details an initial process evaluation that resulted in exceeding the customers target goals for the process improvement.

Case Study:

Process development scientists at a biopharmaceutical company examined isolating a monoclonal antibody produced in a CHO cell culture with the NCSRT OPTISEP® filter module. The objective of program was to develop a process that improved the efficiency of an intermediate concentration and diafiltration step in their downstream purification process. The step was designed to take a clarified cell culture supernatant and condition it to a specific pH and conductivity range in preparation for a chromatographic separation. The current process utilized a 30K PES membrane that delivers a flux rate of 31 LMH. Under these conditions, the runs took 3.5 hours to complete.

Additional problems with the process included precipitation of the product and clogging of the filters causing them to be replaced after each production run.

The results using the current customer processes are presented in Table 1.

The customer specified an acceptable range of concentration and diafiltration factors to achieve the desired end points in pH and conductivity.

NCSRT *SmartFlow* evaluation:
Based on the customer desire to maintain the current membrane polymer in the initial evaluations, the Applications and Process Development staff selected a PES 50K ultrafiltration membrane for the first process development tests. This membrane has been proven to successfully concentrate monoclonal antibodies and achieve greater than 90% product recovery in Mab applications.

The PUROSEP™ LT-2Q tangential flow filtration system (PN 0005-00-001) and the OPTISEP 800 filter holder (PN 10-900-2100) were used for the performing the process development studies. For the initial trial, 2.68 L of starting material was utilized. The starting material contained 0.8 g/L product in a clarified media. An LM ratio (liters of media to square meter of membrane) of 40 was evaluated using 0.064 m^2 PES 50 kD membrane in two OPTISEP 800 filter plates (PN 10-1XX-XXXX) for the studies. Because the starting material was clarified prior to the concentration and diafiltration steps, 0.25mm channel height OPTISEP 800 filter plates were selected for the tests. The low channel height reduces the pump requirements to achieve a targeted shear rate. The shear rate is critical in keeping the membrane surface free from the fouling in difficult separations. With precipates fouling the existing membranes reported as a problem, the shear was perceived to be more critical in this particular separation.

The recirculation rate was set to achieve a shear of 8500 sec^{-1} and the TMP was set to an initial value of 25 psi. During the course of concentrating the clarified media the TMP rose to a high of 32 psi. The recirculation rate was increased form 3.3 lpm to 3.7 lpm

Table 1 Current customer process

	Concentration	Diafiltration	LMH	Time	Recovery
Current Process	8X	5X	31	3:30	>90%

to maintain the permeate flow rate at an acceptable level during the run.(Figure 1) This increased the shear at the membrane surface to increase to 9500 sec^{-1}. The customer reported that the LMH remained steady, ranging from 52-50, throughout the run. Temperature was not controlled.

The results of the NCSRT *SmartFlow*™ filter experiment are summarized in Table 2. The increased efficiency of the *SmartFlow* filter enabled the end points of pH and conductivity to be reached using a 4X concentration and 4X diafiltration. The time to perform the process was reduced by 58 % from 3.5 hours to 1.5 hours. As with high value products such as monoclonal antibodies, process yield can drive the economics of one system over another. The increase in yield from 90% to 96% was viewed as significant by the customer when scaling up to commercial operations. No precipitation of the antibody was observed during diafiltration.

Conclusion:

Using the NCSRT *SmartFlow* the customer was able to achieve their process goals of improved efficiency of the concentration and diafiltration step. The end point was determined by attaining required pH and conductivity in the permeate stream. The efficiency gains observed were:

- increase in product yield to 96%
- reduction of the processing time from the current 3.5 hours, and
- elimination of the precipitation of the product.

The yield increase resulted in a 6.6% increase in Mab recovered during the run. These results would translate into an increase of 12 kg of product recovered per year based on a 20K reactor and 12 campaigns per year for the product (Table 3). Process time was reduced 58% while obtaining the product targets of pH and conductivity. The increased yield and sustained LHM during the entire run demonstrated that the product precipitation problem was eliminated and that the filters were not clogging.

These results are based on a single test result. The customer can further optimize their process for this and other antibodies within their product line by executing a systematic study of different membrane polymers such as regenerated cellulose, polysulfone, and other manufacturers' polyethersulfone membranes. Alternative pore sizes should also be evaluated to gain further efficiency in concentration and diafiltration. Product recovery may be increased even further by developing an optimized process. The NCSRT *Ultrafiltration, concentration, and diafiltration* Optimization Procedure provides a systematic approach to process development for operations such as the one described in this study.

NCSRT has also developed the *SmartFlow* Scale Up component sheet to assist you in determining the correct *SmartFlow* filter modules to meet your process needs and determine the appropriate PUROSEP™ filtration system to run you process development and pilot plant studies.

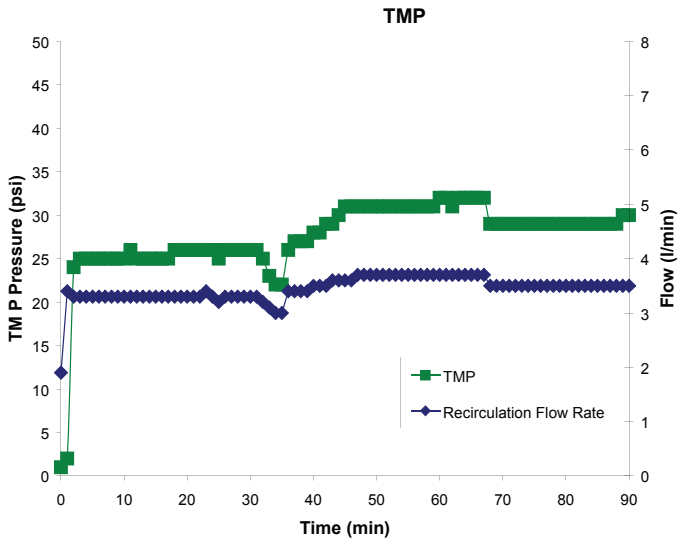


Table 3 *SmartFlow* TFF yield improvements

	SmartFlow filter Process	Current process
Batch size	20,000 L	20,000 L
Mab Concentration	0.8 g/l	0.8 g/l
Yield	96%	90%
Product recovered per batch	15.4 kg	14.4 kg
Batches per year	12	12
Total Product	185 kg	173 kg
Increased Product Yield	12 kg	

Table 2 *SmartFlow* TFF process

	Concentration	Diafiltration	LMH	Time	Recovery
SmartFlow Process	4X	4X	52	1:30	96%
Current Process	8X	5X	31	3:30	>90%