NCSRT

Works™ Optimization Procedure

Concentration and diafiltration of viral antigens with *SmartFlow*™ TFF

The *SmartFlow*™ filter *Concentration and diafiltration of viral antigens* *WORKS*™ Optimization Procedure from NCSRT is intended for concentrating viral antigens from both clarified and non-clarified sources. The subsequent diafiltration of the concentrated viral antigens is to remove low molecular weight media proteins and cellular metabolites.

This optimization procedure uses an ultrafiltration (UF) membrane to retain the desired viral antigen in the retentate while low molecular weight media proteins, cellular metabolites, and salts are able to pass through the membrane. The retention characteristics of proteins change with different buffers, temperatures, concentrations, and membranes. By examining the retnetion characteristics of the different 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 performance.

This optimization procedure starts with selecting a membrane module most likely to work with respect to polymer and pore size based upon thousands of NCSRT trials. Once this module is selected, ranges in which to begin optimizing parameters such as membrane capacity, recirculation rate, and pressure are presented.

Because of the variability in the products and processes using NCSRT's *SmartFlow* technology, we do not make specific process recommendations on parameters of temperature, pH, buffers, or other variables that may affect the separation process and the target product activity.

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Each parameter of the TFF process: product, membrane type, shear, pore size, temperature, concentration factor, pH, anti-foam, etc. may impact the phage passage through the membrane. This is why a systematic experimental plan must be developed and executed to optimize a concentration and diafiltration process.

Module and System Selection:

1) Select the *SmartFlow*™ filter module to evaluate. The selection requires specifying a combination of membrane type, channel height, and membrane area for a given module that will be tested.
   a) NCSRT has filtered thousands of solutions and therefore can provide several membrane chemistries and pore sizes that will likely work in the majority of cases. In general the pore size should be 5 to 10 times the size of the molecule to be passed through the membrane and one half to one third the size of a molecule to be retained.
   b) The combination of the channel height and the fluid velocity through the flow channel created by the recirculation pump produce a shear at the membrane surface. It is this shear that governs the separation performance and efficiency. Care must be taken in selecting and maintaining the shear at the membrane surface.
   c) The membrane area also affects the pump size required to achieve the necessary shear rates for a given separation.
2) Select the first membrane to test.
   a) Recommended membrane chemistries concentration and diafiltration of proteins are the regenerated cellulose (RC) 100 kD, polysulfone (PS) 100 kD, and modified polysulfone (MPS) 500 kD membranes.
3) Select the channel height for the module.
   a) For the concentration and diafiltration of the viral antigen, a channel height between 0.75 and 0.875 mm is recommended
   b) In most cases a channel height of 0.75 mm is recommended because it will require the lowest recirculation (and thus the smallest pump) and produce the highest flux rate.
   c) In cases where a concentration to high solids is desired, a channel height of 0.875 mm or above will be necessary.
4) Select the membrane area.
   a) The membrane area depends upon the batch size to be processed. For filtration process development trials, usually the smallest size membrane and thus the smallest batch size is desired.
   b) For ultrafiltration membranes filtering already clarified viral antigens, the membrane capacity or LM ratio is will determine the total process time.
   c) The minimum batch size is the system hold up volume times the concentration factor. For a continuous diafiltration, the minimum batch size is simply the hold up volume.
   d) A typical good starting point for solutions to be concentrated to 10X concentration is to use a starting capacity of about 100 LM. This ratio will usually result in a 1 to 3 hour process, and the ratio can be adjusted based upon the desired production process time.
   e) The membrane area needed is the batch size divided by the LM ratio.
5) Determine the shear rate.
   a) The typical shear rate for the concentration and diafiltration of viral antigens by continuous diafiltration ranges from 6,000 sec^{-1} to 15,000 sec^{-1}.
   b) The typical starting shear rate for a process development run is 9,000 sec^{-1}.
   c) The benefit of increasing the shear rate is an increased permeate rate.

d) The disadvantages of increasing the shear rate are:
   i) Higher pump costs due to higher recirculation flow rates.
   ii) Higher pressure drops and TMPs which may decrease the passage of desired molecules.

e) An increase in the shear rate should be balanced by an increase in the flux rate or phage passage for the process to retain the same overall efficiency. The energy costs of running the pump at a higher shear rate must be offset by savings on membranes to make increasing the shear rate efficient.

6) Calculate the flow rate needed to operated the selected module at the selected shear rate using the *WORKS*™ Scale-UP LPM GPM spreadsheet. Ensure that a pump is available that can produce this flow rate at the needed pressure. If a suitable size pump in not available, consider either running a smaller trial or calling NCSRT to determine if a suitable size pump is available.

7) Use Table 1 to determine the module(s) part numbers for ordering.

Table 1: *SmartFlow*™ filter module part numbers

Module Size			Channel Height		Membrane polymer and	
_______ -			___		____-_______	
74	100 ft²	Optisep®11000	D	0.5 mm	5B-0100	RC 100 kD
72	50 ft²	Optisep 11000	E	0.75 mm	1B-0100	PS 100 kD
71	10 ft²	Optisep 11000	G	0.875 mm	1N-B100	MPS 500 kD
41	10ft²	Optisep 7000	H	1 mm		
40	5 ft²	Optisep 7000	J	1.5 mm		
52	2 ft²	Optisep 3000				
51	1 ft²	Optisep 3000				

Filter Operation:

1) After loading the filter modules and making all the connections, the first step is to perform a water and/or buffer rinse of the system directing the permeate to the waste.

2) After the rinse, direct the permeate line back to the retentate tank so no concentration occurs prior to establishing the desired shear rate and performing the transmembrane pressure (TMP) optimization procedure.
IMPORTANT: Do not permit the permeate line to come in contact with the retentate fluid. This can contaminate the permeate pool in later samples.

3) Slowly increase the flow rate recirculation pump to the calculated rate from step 6 above.

4) Optimize the TMP.

a) TMP is optimized by setting the back pressure in the retentate loop downstream of the filter module using a back pressure valve. While increasing the back pressure, maintain a constant retentate recirculation rate. It is normal to increase the pump speed in order to maintain the desired recirculation rate as the backpressure is increased.

b) Increase the TMP to the lowest operational value for the current membrane. This value can be found in Table 2.

c) Measure the permeate flow rate and the passage of the target molecule by taking a permeate sample. This permeate flow rate is the base rate. Record these values.

d) Increase the TMP by 3 PSIG (0.2 bar) and measure the permeate flow rate. Record these values. Compare the permeate flow rate to the base rate or the previous permeate flow rate reading. If the rate has increased from the previous measurement go to step e, otherwise go to step g.

e) Wait three minutes and measure the permeate flow rate again. If the permeate rate has remained above the rate of the reading go to step f.

f) Repeat steps d and e until the permeate rate no longer increases with increasing pressure or does not hold that increase for three minutes.

g) Lower the TMP to the pressure that was measured before the permeate flow rate stopped increasing. This is the optimal TMP.

5) Remove the permeate line from the retentate tank and place back in the permeate vessel. Do not allow the permeate lines to contact the permeate fluid pool in the reservoir.

a) Take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously. Record the data on the Membrane Test Worksheet.

i) Record the permeate flow rate using a graduated cylinder, scale, or flow meter.

ii) It is critical to collect the data to be able to properly analyze the experimental results and develop an optimized procedure.

b) Concentrate the solution to be to the desired concentration factor.

i) Take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously at each concentration factor processed.

Note: for concentrations to a high concentration factor, not every concentration factor needs to be recorded

ii) Record the permeate flow rate using a graduated cylinder, scale, or flow meter.

6) After the final concentration samples are taken, record the volume of liquid remaining in the system at this time. There are two alternative methods for determining the end of concentration system volume.

a) The system volume can be determined by subtracting the volume of the permeate and the volume of all of the samples taken from the starting volume.

$$System\ Volume = Starting\ Volume - Permeate\ Volume - Retentate\ Sample\ Volume$$

b) If the system hold up volume is known and the volume in the retentate reservoir is known, adding these two values will produce the current system volume.

7) Diafiltration- The following describes the procedure for diafiltering the product 3X.

a) Start to monitor the permeate volume with a graduated cylinder or scale.

b) To start the diafiltration, add 5 to 15% of the retentate volume calculated in step 6 to the retentate tank. Optional - Remove the permeate line from the first permeate collection tank to a second permeate collection tank. By doing this, the effect of the concentration can be isolated from the effect of the diafiltration.

c) When the permeate volume has increased by the volume added in step b, take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously. Record the permeate flow rate using a graduated cylinder, scale, or flow meter.

d) Continue to add buffer at a rate equal to the permeate rate in aliquots equal to between 5 and 15% of the retentate volume calculated in step 6. Continue until 3 times the total volume of system recorded in step 6 has been added to the system.

e) Take samples from the permeate hose and retentate tank when each diafiltration factor is reached (i.e. take a sample when the permeate volume is equal to a multiple of the retentate volume such as 1X, 2X, etc.).

f) For other diafiltration factors, continue the process to the amount of diafiltration buffer equals number of desired diafiltration factor times the system volume recorded in step 6.

i) The theoretical recovery from a 3X diafiltration for a molecule with a 100% passage is 95%.

ii) Increasing the diafiltration factor will increase the yield especially when the target molecule has low passage. However, the cost of increasing the diafiltration volume is that the process time will be greater and a larger supply of buffer will be needed.

iii) Decreasing the diafiltration factor will decrease the yield. However, for molecules with high passage and low value, the small decrease in the yield may be worth the faster processing time and the saving on buffer.

Data Analysis:

Sample Analysis:

1) Check the permeate samples for cells.
2) Calculate the membrane flux rate or LMH ($L/m^2/h$) by dividing the measured permeate flow rate at each sample by the membrane area.

$$LMH = Permeate\ Flow\ Rate\ (mL/min) * \frac{1L}{1000mL} * \frac{60\ min}{1hr} \div Membrane\ Area\ (m^2)$$

3) Record the data on the Membrane Test Worksheet.

Process Optimization:

The procedure should be repeated under different process conditions to ensure that the optimized conditions are reached.

1. An important parameter for cell harvest is the membrane capacity or LM ratio.
2. Increasing the LM ratio decreases membrane performance, which increases processing time and decreases membrane costs. If membrane performance suffers greatly, then saving a little bit on membrane will not offset the costs in higher processing time.
3. Decreasing the LM ratio increase the membrane performance and increases membrane costs. Increasing membrane performance may decrease the processing time at a small incremental membrane cost, therefore decreasing total cost.
4. To find the optimal LM ratio:
   a) If the current trial was too fast, increase the LM ratio by starting with a larger volume of starting material.
   b) If the current trial was too slow, decrease the LM ratio by starting with a smaller volume of starting material.

5) The module used is an important optimization parameter. By changing the membrane chemistry or membrane type, optimized flux rates and passage may be found.
6) Using the same membrane, the shear rate can be optimized by increasing and decreasing the shear rate and measuring the effects on the membrane flux rate and passage. If an increase in the shear rate results in a relatively large increase in the flux rate, then the savings in membrane cost will offset the increased energy consumption.

After analysis of the data, select the best performing membrane. The best performing membrane will retain the cells, permit the desired media component to pass into the permeate, and have a high permeate flux.

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Table 2 Typical TMP Pressures for *SmartFlow*™ filter modules

Membrane Pore Size	Transmembrane Pressure Starting Value PSIG (Bar)	Transmembrane Pressure Ranges PSIG (Bar)	Cell Harvest Inlet PSIG (Bar) Starting Value	Cell Harvest Outlet PSIG (Bar)
Ultrafiltration Membranes				
1 kDa	75 (5)	90 to 150 (6 to 10)		
5 kDa	45 (3)	60 to 90		
10 kDa	30 (2)	45 to 90 (3 to 6)		
30 kDa	15 (1)	30 to 75 (2 to 5)		
100 kDa	15 (1)	20 to 60 (1.37 to 4)	20 (1.37)	12 (0.83)
300 kDa	10 (0.69)	15 to 45 (1 to 3)	20 (1.37)	10 (0.69)
500 kDa	7.5 (0.5)	10 to 30 (0.7 to 2)	7.5 (0.5)	0
Microfiltration Membranes				
0.1μ	2 (0.13)	4 to 15 (0.27 to 1.0)	4 (0.275)	0
0.2μ	2 (0.13)	4 to 15 (0.27 to 1.0)	4 (0.275)	0
0.45μ	2 (0.13)	4 to 10 (0.27 to 0.69)	4 (0.275)	0
0.8μ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
1.0μ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
2.0μ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
3.0μ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0

Conclusion:

This *SmartFlow*™ filter *WORKS*™ *Concentration and diafiltration of viral antigens* Optimization Procedure provides guideline for optimizing the application of NCSRT's *SmartFlow*™ filters. To receive the complete application package, please request the *Concentration and diafiltration of viral antigens* *WORK*book.

NCSRT's *SmartFlow* filter technology….It *WORKS*.

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