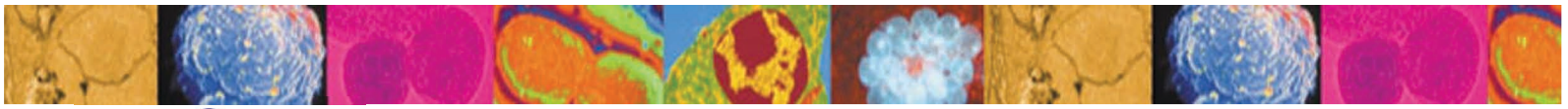

NCSRT

Works™ Optimization Procedure

Ultrafiltration, concentration, and diafiltration with *SmartFlow*™ TFF

The *SmartFlow*™ filter *WORKS*™ *Ultrafiltration, concentration, and diafiltration* optimization procedure from NCSRT is intended for concentrating and desalting a target molecule. This procedure is not for separating two molecules other than to separate simple salts and water from a target molecule.

This optimization procedure uses an ultrafiltration (UF) membrane to retain the desired protein in the retentate while small sugars and salts are able to pass through the membrane. The passage characteristics of proteins change with different buffers, temperatures, concentrations, and membranes. By examining the passage 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.

To learn how others have applied the *SmartFlow* filter technology to similar separations, please review the *WORKS Ultrafiltration, concentration, and diafiltration* Case Study.

Ultrafiltration, concentration, and diafiltration with *SmartFlow*™ TFF

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), TFM, and polyethersulfone (PES) membranes. The pore size of the initial membrane to test should be selected from Table 1.

Table 1. Recommended membranes for Ultrafiltration, concentration, and diafiltration

Target Molecule	Membrane polymer	Pore size
Protein < 5 kD	TFM®	1 kD
Protein 5-10 kD	TFM®	3.5 kD
Protein 10-30kD	RC	5 kD
Protein 30-100 kD	RC	10 kD
Protein > 100 kD	RC	50 kD
Complex Carbohydrate > 50kD	RC	30 kD
Protein 10-30kD	PES	5 kD
Protein 30-100 kD	PES	10 kD
Protein > 100 kD	PES	50 kD
Complex Carbohydrate > 50kD	PES	30 kD

3) Select the channel height for the module.
   a) For the concentration and diafiltration of protein, a channel height between 0.75 and 1.5 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 concentration to high solids is desired (above 20% protein), 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 proteins, the membrane capacity or LM ratio is not essential.
   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 ultrafiltration concentration and diafiltration processes ranges from 7,500 sec^{-1} to 20,000 sec^{-1} .
   b) The typical starting shear rate for a process development run is 10,000 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) The higher pump costs due to higher recirculation flow rates.
      ii) The higher pressure drops and TMPs which may decrease the passage of desired molecules.
      iii) An increase in the shear rate should be balanced by an increase in the flux rate 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 operate 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 2 to determine the module(s) part numbers for ordering.

Table 2: *SmartFlow*™ filter module part numbers

Module Size		Channel Height		Membrane polymer and pore size	
_____ -		____		____ -	_____
74	100 ft² Optisep® 11000	D	0.5 mm	FD-0001	TFM 1kD
72	50 ft² Optisep 11000	E	0.75 mm	5B-0005	RC 5 kD
71	10 ft² Optisep 11000	G	0.875 mm	5B-0010	RC 10 kD
41	10ft² Optisep 7000	H	1 mm	5B-0030	RC 30 kD
40	5 ft² Optisep 7000	J	1.5 mm	5B-0050	RC 5 kD
52	2 ft² Optisep 3000			FD-0003	TFM 3.5 kD
51	1 ft² Optisep 3000			2B-0010	PES 10 kD
				2B-0030	PES 30 kD
				2B-0050	PES 50 kD

Ultrafiltration, concentration, and diafiltration with *SmartFlow*™ TFF

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 3.
   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) With each sample 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) With each sample 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 alternatative 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.

Table 3 Typical TMP Pressures for *SmartFlow*™ filter modules

Membrane Pore Size	Transmembrane Pressure Starting Value PSIG (Bar)	Transmembrane Pressure Ranges 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)
300 kDa	10 (0.69)	15 to 45 (1 to 3)
500 kDa	7.5 (0.5)	10 to 30 (0.7 to 2)
Microfiltration Membranes		
0.1µ	2 (0.13)	4 to 15 (0.27 to 1.0)
0.2µ	2 (0.13)	4 to 15 (0.27 to 1.0)
0.45µ	2 (0.13)	4 to 10 (0.27 to 0.69)
0.8µ	1 (0.07)	1 to 6 (0.07 to 0.41)
1.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)
2.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)
3.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)

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. With each sample, 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 until 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 (such as a salt molecule) is 95%.

ii) Increasing the diafiltration factor will increase the yield of salts and sugars in the permeate. 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 of the salt and sugar in the permeate. 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 Anlaysis:

Sample Analysis:

1) Check the permeate protein passage. The permeate protein passage should be close to zero.
2) Calculate the membrane flux rate or LMH (L/m²/h) by dividing the measured permeate flow rate at each sample by the membrane area.
3) Record the data on the Membrane Test worksheet.

Process Optimization:

Repeat the procedure under different process conditions to ensure that the optimized conditions are reached.

1) The module used is an important optimization parameter. By changing the membrane chemistry or membrane type, optimized flux rates and protein retention may be found.
2) 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 protein retention. 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 protein, permit the desired media components to pass into the permeate and have a high permeate flux.

Conclusion:

This *SmartFlow*™ filter *WORKS*™ *Ultrafiltration, concentration, and diafiltration* optimization procedure provides guideline for optimizing the application of NCSRT's *SmartFlow* filters. To see how others have applied the technology to their separations operations, please refer to the *WORKS Ultrafiltration, concentration, and diafiltration* case study. To receive the complete application package, please request the *Ultrafiltration, concentration, and diafiltration WORK*book.

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

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