Increased Productivity Using Minimate™ Capsules to Replace Stirred Cell Systems

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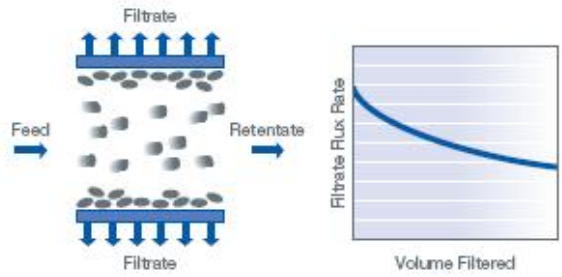
Introduction

Ultrafiltration Preserves Biological Activity and Time
Protein purification technology has progressed from methods as diverse as chemical precipitation for sample concentration or dialysis for buffer exchange towards pressure-driven purification cross flow systems utilizing ultrafiltration membranes. Ultrafiltration (UF) techniques rely on the use of polymeric membranes with highly defined pore sizes to separate molecules according to size. Simply put, UF procedures rely on the use of fluid pressure to drive the migration of the smaller molecules through a UF membrane with the simultaneous retention of larger molecules.

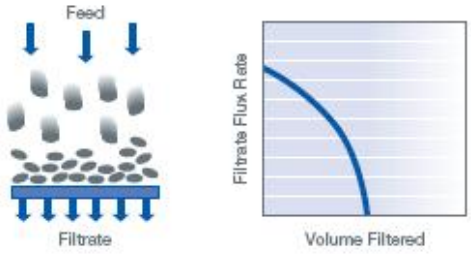
While chemical precipitation can be used to concentrate a protein sample, separation with ultrafiltration is based on mechanical rather than chemical interactions allowing a researcher to perform sample concentration without the addition of denaturing solvents or salts. Buffer exchange using dialysis technologies use large volumes of buffer and since the only force acting upon the solution is diffusion, the process can take several days. Pre-assembled and simple to use ultrafiltration devices can rapidly perform either concentration or buffer exchange procedures without the extensive handling required for many other techniques.

Optimization of Ultrafiltration Using Crossflow
Ultrafiltration can be performed in one of two operational modes: Direct Flow Filtration (DFF), or Tangential Flow Filtration (TFF, Figure 1). DFF works well for small volumes (up to 30 mL) using centrifugal devices, however, DFF technologies can fall prey to problems with membrane fouling (1). To reduce the formation of a gel layer, cross flow can be generated on the upstream side of the membrane using a floating stir bar configuration (stirred cell) or by creating a controlled laminar flow. While stirred cell operations tend to improve UF performance, they are still limited to achieving the optimal performance since the velocity and subsequent level of agitation is dependant on the sweep of the bar that varies along the radius of the sweep.

Figure 1
Graphic Comparison of DFF Versus TFF



(A) Sample solution flows through the feed channel and along (tangent to) the surface of the membrane as well as through the membrane. The crossflow prevents build up of molecules at the surface that can cause fouling. (B) The TFF process prevents the rapid decline in flux rate seen in direct flow filtration allowing a greater volume to be processed per unit area of membrane surface.



(A) The feed is directed into the membrane. Molecules larger than the pores accumulate at the membrane surface to form a gel, which fouls the surface, blocking the flow of liquid through the membrane. (B) As the volume filtered increases, fouling increases and the flux rate decreases rapidly.

Crossflow Performance Optimized Using the Minimate Capsule
In comparison with stirred cell technologies the TFF processing is reproducible, easy to control and amenable to monitoring (4). Overall, TFF operations allow a uniform and gentle recirculation of sample flow over the surface of a membrane effectively controlling membrane fouling. Use of the entire membrane surface results in improved flux rates significantly reducing processing times and increasing productivity. The Minimate capsule is pre-assembled, ready to use and can process volumes to 1 liter without the need for user intervention. In contrast, most stirred cell devices have a 350 mL or smaller processing capacity and must be opened repeatedly risking integrity failures and requiring valuable technician time. We demonstrate significant time savings with the Minimate capsule over stirred cell devices when performing a simple concentration protocol or for a more complex enzyme purification process.

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Materials and Methods

Minimate TFF Capsule
Minimate TFF capsules contain the Omega™ ultrafiltration membrane (polyethersulfone) integrally sealed into a self-contained device which is available with a wide range of molecular weight cut-offs. Using the Minimate capsule, sample batch sizes of up to 1 liter can be concentrated to volumes as low as 5 mL with little user intervention. The reusable/disposable capsule can perform single or sequential concentration/diafiltration steps using the same device in a closed loop connection. A variety of peristaltic pumps can be used for TFF processing.

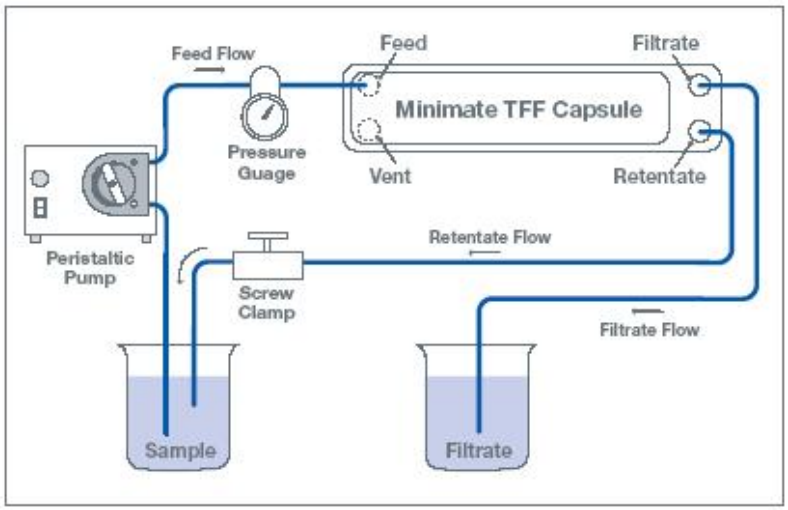
Minimate Capsule Setup

Stirred Cell Devices
Standard commercially available stirred cell devices were assembled, operated, and cleaned in accordance to manufacturers instructions. One of the primary differences in operation from TFF was the need for a pressure vessel or nitrogen tank to create the desired pressure. In addition, the stirred cell devices required the researcher to independently assemble, open, refill and disassemble the device during use if diafiltration was required.

Technical Data	Minimate	Stirred Cell
Effective Filtration Area	50 cm^2 (0.05 ft^2)	28.7 cm^2 (0.03 ft^2)
Recommended Cross Flow Rate	40-50 mL/min	Not applicable
Operating Temperature Range	5-50 °C (41-122 °F)	5-50 °C (41-122 °F)
Maximum Operating Pressure	4 bar (60 psi) 400 kPa	55 psi (N_2 or filtered air)

System Working Volume (feed/retentate)	5-10 mL	5-10 mL

Diagram 1
Minimate TFF Capsule System with Pump, Pressure Gauge, Retentate Screw Clamp, Reservoirs, and Tubing Connections



Simple Startup Using Minimate Capsules
The easy-to-use Minimate capsule can be connected to a peristaltic pump system to create crossflow in the retentate channel. A more detailed and complete protocol for the operation and fine-tuning of the Minimate device can be found in the Care and Use Guide and other listed publications (2, 3, and 5). A brief operation overview is presented:

Setup

1. Connect all required tubing to capsule, keeping lengths as short as possible to reduce system working volume. Install pressure gauge on the feed port of the capsule.
2. Calibrate the peristaltic pump for the required flow rates.
3. Slightly elevate the retentate side of the capsule to facilitate the removal of air.
4. Loosely attach screw clamp to the retentate tube.

Processing

1. Place solution to be filtered into feed reservoir and direct both retentate and filtrate lines to a separate container. Pump solution through capsule at a flow rate of 40 mL/min.
2. After a volume of fluid approximately equal to the system hold-up volume (10 mL) has been pumped through the retentate and filtrate lines, place retentate line back into feed reservoir and place the filtrate line in a separate container.
3. Continue processing until desired concentration is reached. If concentrating 500 mL of solution to 5X, stop filtration when there is 100 mL remaining in the feed reservoir.
4. Perform diafiltration (optional) by adding fresh buffer to the sample reservoir (2).
5. Use a 15 mL Syringe to collect the retentate if the volumes are less than 10 mL, slow the pump for the final concentration to avoid product foaming.

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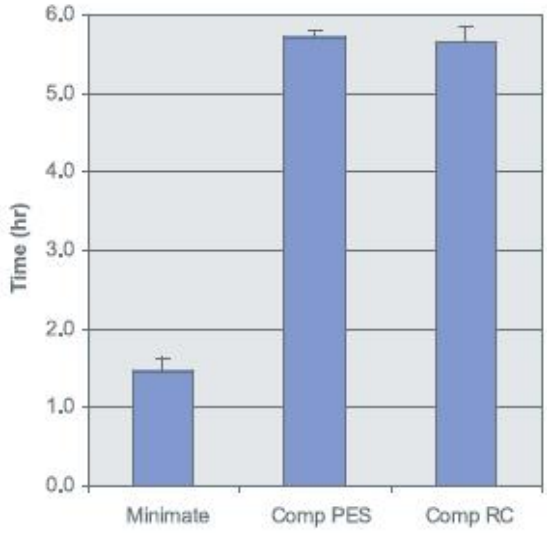
Results and Discussion

Increased Productivity Using the Minimate Device
Processing time is a function of overall device performance. The processing operation of five Minimate 10K Omega capsules was directly compared to similar processing conditions using comparable stirred cell configurations containing hi-flow PES or regenerated cellulose membranes. An experiment where 1 liter of a 2 mg/mL BSA was concentrated ten-fold (to 100 mL) was performed to demonstrate the difference in processing times between the configurations. Under these operational conditions, complete processing using the Minimate Capsule was achieved in just over one hour (Figure 2). In contrast, processing times for stirred cell devices took five times longer regardless of membrane configuration. In addition to the long processing times the stirred cell devices required user intervention in the form of repeated fill cycles.

Figure 2
Use of the Minimate Capsule Significantly Reduces Processing Times



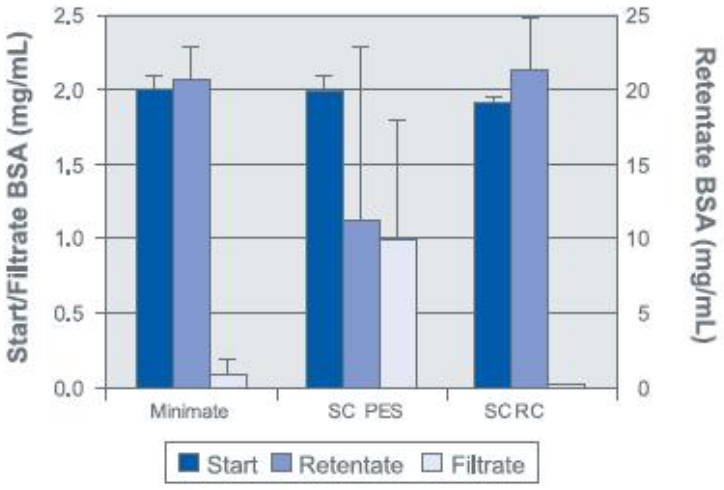
A 2 mg/mL BSA solution was concentrated ten-fold (1000 to 100 mL) in either a 350 mL stirred cell device or Minimate capsule. The Minimate contains a pre-assembled Omega 10K membrane. The crossflow, set at 50 mL/min with retentate loop backpressure applied to create an initial filtrate flow of about 15 mL/min. The stirred cell devices used polyethersulfone (PES) or regenerated cellulose (RC) disks and were pressurized with filtered air at 55 psi giving a starting filtrate flow of about 6 mL/min. Error bars indicate standard error for five independent runs.

Measuring the absorbance at 260 nm for both the filtrate and retentate fractions provided verification of protein concentration process. Using stirred cell devices it was observed that BSA leaked through the 10K PES membrane during processing indicating a failure in integrity. Subsequently, in order to insure a statistically significant comparison between configurations additional experiments were performed until a total of five successful stirred-cell runs were completed.

Handling and Device Integrity
Integrity failures during processing can not only result in lost time but also end up with the destruction of valuable sample. The Minimate device is manufactured as a sealed, self-contained capsule that requires no end-user assembly. Using TFF, a 1 liter protein sample can be processed in a single step reducing the risk of disturbing the sample. In contrast, the SC devices have a complex assembly where UF membrane disks are placed on a device landing and screwed into place, an action that can create leaks or bypass. Major leaks are easily identified by abnormally high filtrate flow rates. More critical however, a partial bypass can look like a normal run, but the loss of protein would not be detected until it was too late or even at the end of a run.

By monitoring the filtrate for protein bypass, we observed that the competitive PES membrane in stirred cell operation suffered significant integrity failures (Figure 3). The Minimate TFF and regenerated cellulose stirred cell devices were all integral, however, the competitive PES membranes failed in 5 out of 10 runs each at different stages in the processing.

Figure 3
The Pre-assembled Minimate Eliminates Integrity Failures



Sample concentration was performed as described in Figure 2. Aliquots of the starting material, final retentate, and final filtrate pools were analyzed for protein concentration at 280 nm. Average concentrations are plotted with error bars indicating standard error for (5 - Minimate), (10 - SC PES), and (5 - SC RC) runs respectively For simplicity in the graphical representation, the retentate values are

plotted on the Y2 axis to accommodate the ten-fold concentration that occurred in the processing.

Purification of Cyclooxygenase-2 (COX-2)
Cayman Chemical (Ann Arbor, MI) performed a comparison study to validate the use of the Minimate capsule over the stirred cell configuration for the purification of the Ovine COX-2 enzyme from tissue homogenate (Table 1). They were able to easily develop a new protocol that replaced the stirred-cell device in an enzyme production process. They found that the COX-2 specific activity derived from the TFF procedure matched that from the stirred cell device they had been using. Most critical however, was that the processing time was cut to nearly a third of the time for TFF ultimately allowing a second run to be performed during a shift.

Cayman COX-2 protocol using both the Pall Ultrasette™ and Minimate devices:

1. Homogenize sheep cotyledons in buffer.
2. Centrifuge homogenate at 10,000 x g for 20 min at 4 °C.
3. Ultracentrifuge supernatant at 100,000 x g for 1 hr at 4 °C.
4. Resuspend microsomal pellet in solubilization buffer containing 1% Tween 20.
5. Solubilize by stirring for 1 hr at 4 °C.
6. Ultracentrifuge at 100,000 x g for 1 hr at 4 °C.
7. Concentrate supernatant at 4 °C using a Pall Ultrasette with a 30K MWCO membrane. Concentrate 300 mL to 10 mL then dilute to 100 mL with DE-53 column buffer.
8. Load sample onto the DE-53 anion exchange column, wash, and elute enzyme with a pH gradient.
9. Concentrate eluent with a Minimate capsule or stirred cell device containing a 30K MWCO membrane. Concentrate 350 mL to 6 mL then wash membrane with 3 mL buffer.
10. Load concentrate onto a Sephadex G-200 sizing column for final processing.

Table 1
Competitive Testing with Cyclooxygenase-2

Independent Purification	Stirred Cell 1	Stirred Cell 1	Stirred Cell 1	Minimate 1	Minimate 2	Minimate 3
Initial Protein Conc. (mg/mL)	15.0	14.5	15.0	13.6	14.1	14.7
Initial Specific Activity (U/mg)	66.7	62.1	70.9	73.5	67.4	74.8
Concentration Factor	50	46	50	40	44	41
Final Specific Activity	220	196	191	200	217	206
Final Yield (SA/mg total protein)	0.048	0.045	0.043	0.042	0.044	0.040
Time to Process (hr)	8.0	8.0	8.0	2.5	2.5	2.5

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Conclusions

The simple-to-operate Minimate TFF system is the most rapid, reliable choice for buffer exchange or protein concentration for samples up to 1 liter. The self-contained TFF capsules can be cleaned and reused saving time in setup and operation (5). In contrast, stirred-cell systems require assembly, operate more slowly, require more intensive monitoring and have to be filled multiple times in order to process sample volumes up to 1 liter. In this paper we observed that the Minimate capsule was able to concentrate a 1 liter protein solution five times faster than a comparable stirred-cell system. This finding was further confirmed by an independent researcher for the purification of the Cyclooxygenase-2 enzyme resulting in a reduction in processing time over three-fold without the loss of protein activity.

While the Minimate TFF capsule is a valuable tool for small process volumes (< 1 liter), larger volumes can be processed by multiplexing several Minimate TFF capsules in a parallel configuration. In addition, the Minimate TFF capsule was designed with the same flow path length as larger TFF devices saving valuable optimization time when scaling beyond lab scale to volumes used in pilot and production plants.

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References

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2. L. Schwartz, 2003. *Diafiltration: A Fast, Efficient Method for Desalting or Buffer Exchange of Biological Samples*. Pall Scientific & Technical Report, PN 33289.
3. L. Schwartz, 2003. *Desalting and Buffer Exchange by Dialysis, Gel Filtration or Diafiltration*. Pall Scientific & Technical Report, PN 33290.
4. J. Jenco, T. Hu, L. Schwartz, and K. Seeley, *The partnership of the Minimate TFF Capsule with Liquid Chromatography Systems Facilitates Lab-Scale Purifications and Process Development Through In-Line Monitoring* Technical Report.
5. Minimate *Care and Use Manual*, electronic only; on CD within Minimate TFF product package.

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