Application Note

Plasmid DNA Purification: Process Intensification Using Ultrafiltration SepraPor® Hollow Fibers

Introduction

This cell and gene therapy (CGT) application demonstrates the improved efficiency of an ultrafiltration and diafiltration (UF/DF) process using SepraPor® hollow fiber filters. The inherent design of a hollow fiber membrane provides rheology and throughput advantages to overcome the design limitations of cassettes. In this study, 300 kDa SepraPor® hollow fiber filters were evaluated for plasmid DNA (pDNA) purification. Compared with historical processing data using cassettes, SepraPor® filters maintained product integrity and high yields while decreasing processing times and consumables used.

SepraPor® in Cell & Gene Therapies

Cell and gene therapies using viral vectors are frequently generated through transient expression routes based on pDNA. pDNA is a circular double helix DNA molecule used as a therapeutic transgene, allowing coding for the viral capsid or being the therapeutic itself. The typical manufacturing process of pDNA **(Figure 1)** is carried out by fermentation, clarification and tangential flow filtration (TFF) purification, and a final sterile filtration step.

Ultrafiltration/diafiltration (UF/DF) is a critical step in pDNA manufacturing for impurity removal and buffer exchange. In this particular pDNA purification application, filtration challenges stem from the high biological burden of the process solution and high concentration factor required, which increase membrane fouling. The membrane form factor of hollow fiber filters provides several advantages over cassettes when processing challenging solutions.

SepraPor® Hollow Fiber Filters for Demanding Applications

Cassette filters employ a tortuous flow path with restricted fluid entrance and exit openings for using the entirety of the membrane surface area. However, liquids in hollow fiber filters enter each lumen directly, maximizing the use of the membrane surface area, i.e. minimizing filtration inefficiencies due to filter geometry as in cassettes. Minimal restriction creates a smooth tract for more gentle separations and the most efficient and precise processing possible.

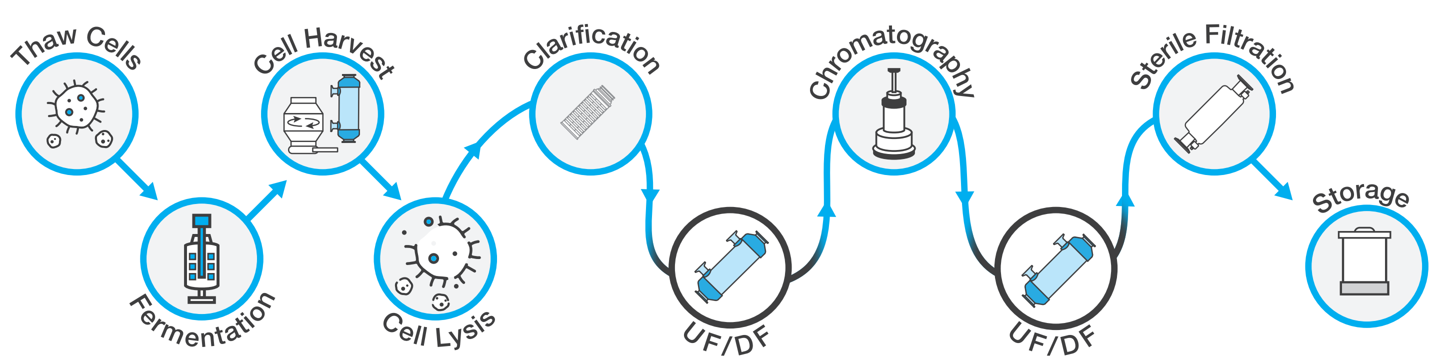

Figure 1: Different steps involved in the manufacture of pDNA involving TFF purification in 2 UF/DF steps.

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As shown in **Figure 2**, cassette filter geometry imposes a fluid path restriction that prevents higher feed flow rate values from being achieved and therefore limits high transmembrane pressures. Filtration, which ultimately is a pressure driven process, cannot be optimized as effectively. These TMP limitations can lead to process inefficiencies. In this respect, hollow fibers have no such limitations and are ideally suited for high performance separations of the most challenging solutions.

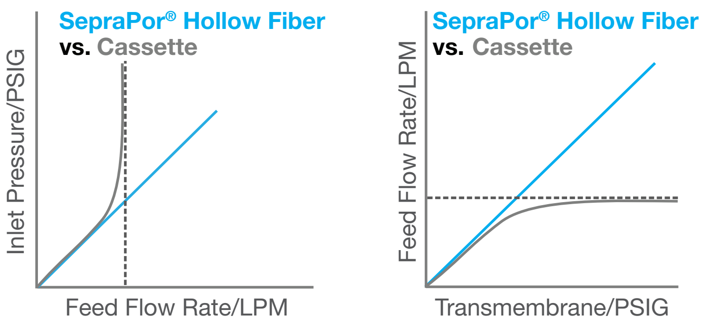

Figure 2: Transmembrane pressure (TMP) in cassettes is limited by the flow geometry. Increases in feed flow rate to increase TMP and separation only increase the inlet pressure, while the feed flow rate is only marginally improved.

With SepraPor® hollow fibers, the solution moves through a simple, linear flow path, maintaining smooth and gentle flow. Hollow fiber membranes minimize shear, so sensitive products are protected during processing.

Case Study: Purification of Plasmid DNA using SepraPor®

In this application, pDNA cell lysate was first concentrated 10x in an ultrafiltration step. Then a diafiltration buffer exchange was completed with Tris-EDTA (TE) buffer. This UF/DF process was historically carried out using stacked cassettes with a 1.5 m^2 total effective filtration area (EFA). SepraPor® hollow fibers were first evaluated in a small-scale (0.023 m^2) experiment, then scaled up to production runs (0.4, 0.8 m^2) using the filter part numbers identified in **Table 1**. Comparing this UF/DF process to historical processing conditions with cassettes highlights several processing advantages of UF SepraPor® hollow fiber filters for the TFF purification of pDNA.

Process parameters monitored during UF/DF runs include: inlet, outlet, permeate and transmembrane pressures, conductivity, concentration factor, diafiltration volumes, permeate and solution volumes, flux, and feed flow rate.

SepraPor® Product Number	Surface Area m^2	Concentration Factor	Feed Flow Rate L/min	Diafiltration Volumes	UF/DF Process Time
Cassette	1.50	10 X	6	20	> 8 hours
XFC300C024-7711	0.023	10 X	0.3	15	< 5 hours
XFC300C412-00CC	0.36	10 X	12	12	< 5 hours
XFC300C424-00CC	0.84	10 X	12	14	< 5 hours

Table 1: Comparison of 300 kDa Cassettes and SepraPor® Filters for UF/DF of pDNA. Highlighted in gray is the smallest surface area filter, which was used for bench scale small volume runs.

The first part of the process involved a 10x pDNA concentration. The recirculation rate and TMP remained constant. **Figure 3** shows the progressive flux decrease as the concentration and membrane loading increased.

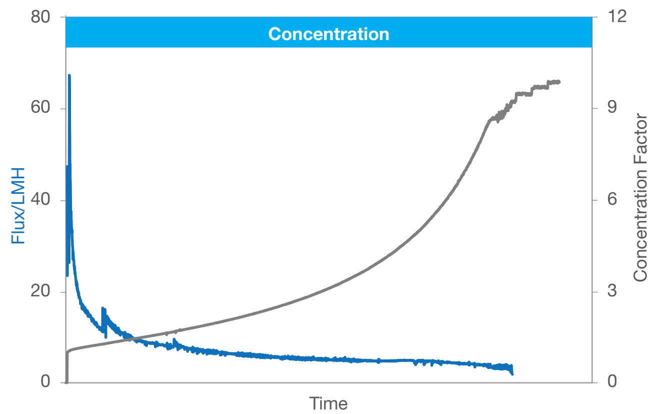

Figure 3: A pDNA concentration factor of 10x was obtained using the 300 kDa SepraPor® filter with 0.8 m² EFA.

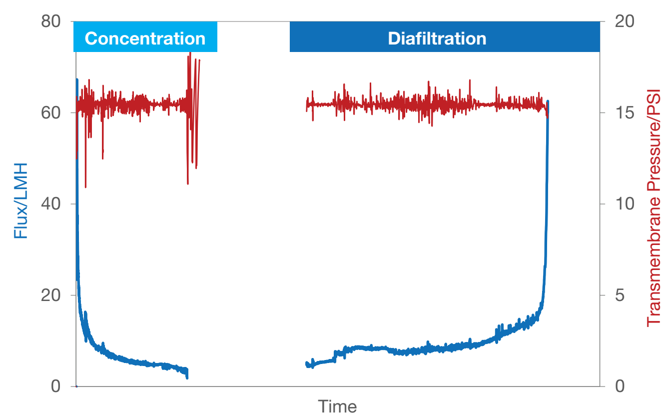

Figure 4: Concentration and diafiltration steps of a pDNA solution under constant TMP using SepraPor® 300 kDa hollow fiber filter with 0.8 m² EFA.

Permeate flux and TMP, shown in **Figure 4**, were monitored throughout the production-scale concentration and diafiltration steps. A transient recirculation period was utilized between the two steps to maintain solution stability overnight, but no pressure data was recorded.

In **Figure 5**, once 10x concentration was achieved, diafiltration using a TE buffer was carried out until the conductivity was lowered to a target setpoint (**Figure 5**). The full 20 diafiltration volume (DV) run was carried out to maintain equivalent process conditions between hollow fiber and cassettes, however, the target setpoint

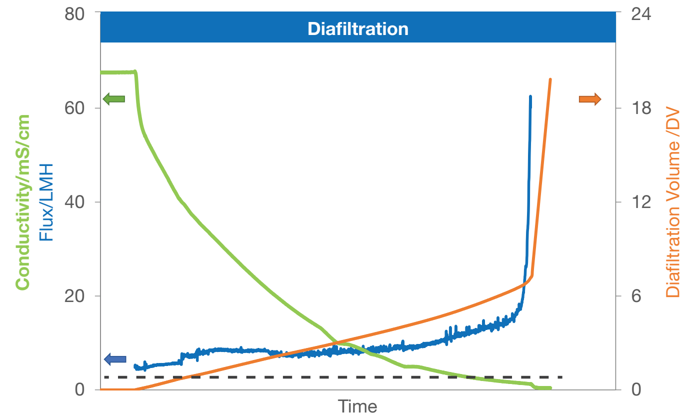

Figure 5: Using a 300 kDa SepraPor® filter with 0.8 m² EFA reached the target conductivity setpoint in 12-15 DV. Dotted line represents the time at which the conductivity target setpoint was achieved during diafiltration.

was achieved by the SepraPor® filter with significantly less recirculation. The SepraPor® filter increased efficiency of the diafiltration step, saving at least 25% of the used TE buffer and decreasing the necessary processing time.

Using SepraPor® 300 kDa molecular weight cut-off (MWCO) ultrafiltration hollow fiber filters shortened processing times by 40% compared with cassettes. Recovery yields and product purity was maintained above 98.5% even after regeneration and repeated use using NaOH as a cleaning agent.

Historically, in this application, the limiting factor in processing efficiency with cassettes was back pressure, therefore limiting the recirculation rate to 6 liters per minute, which may not be the case for other applications or types of solutions used. Hollow fibers did not exhibit this limitation. However, we did observe significant solution foaming above 12 liters per minute, which is solution dependent.

Conclusion

In conclusion, SepraPor® filters achieved the following performance improvements when compared to historical pDNA UF/DF processing with cassettes and therefore are better suited for process intensification.

- 40% reduction in processing time
- 50% reduction in effective filtration area
- 25% less buffer consumed

Choose SepraPor® for Your Demanding Applications

SepraPor® UF hollow fibers are developed with higher industry standards:

- Improved flow rate performance
- Scientifically rigorous characterization of MWCO, using multiple Mw markers
- Improved lot-to-lot consistency

With SepraPor® ultrafiltration membranes, we are committed to achieving a higher level of material characterization to help our customers develop processes that run at peak performance, consistently.

Well-characterized membranes with enhanced consistency can improve the performance of your purification process.

Learn How SepraPor® Can Help You

We welcome customer collaborations to continuously develop the best products and membranes in the industry. For additional information about SepraPor® ultrafiltration membranes, please visit www.meissner.com/seprapor.

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