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Tangential Flow Filtration Evaluation of mRNA Using Hydrosart® Cassettes

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Abstract

mRNA manufacturing processes are unique and require carefully designed procedures to ensure they are produced and purified reliably. These include gentle filtration methods compatible with the sensitivity of mRNA to maximize recovery. Sartorius tangential flow filtration (TFF) cassettes provide high-performance separation in various upstream and downstream bioprocessing unit operations.

In this application note, we tested the performance of four TFF cassettes in the purification of a model 4000 nucleotide mRNA. All four Hydrosart® membranes showed effective performance in buffer exchange and concentration of mRNA drug substance.

mRNA recovery was higher than 89% for all tested membranes, and the product was not degraded in either narrow or wide-channel cassettes. Permeate fluxes were comparable, and the 300 kDa E-channel demonstrated the highest average permeate flow through the process, leading to the shortest process time.

Introduction

mRNA is an emerging biotherapeutic with some unique features that require particular attention during process set-up. mRNA (and other long RNA molecules) are produced during an in vitro transcription (IVT) reaction, in which a polymerase will generate the RNA from a DNA template. There are two typical options for purifying the mRNA: proceeding directly to an affinity chromatography column such as the CIMmultus™ OligodT (Sartorius) or first employing tangential flow filtration (TFF) before a potential chromatography step. Employing TFF before chromatography enables the separation of some small molecules such as nucleotides, representing an initial purification step.

mRNA molecules can range from 500 nt to over 10,000 nt,[1] making them at least 3 – 10 times larger than a monoclonal antibody (mAb). Moreover, while they may appear linear, mRNA molecules often possess complex folding. As such, traditional purification processes are often unsuitable for RNA purification because inappropriate pore size, molecular weight cut-off (MWCO), or shear stress can cause loss or degradation of the molecule.

TFF is a common technique used for concentration and diafiltration (buffer exchange). It is typically employed several times during downstream processes (DSPs).[2] As such, TFF steps must be suitable for mRNA purification, maintaining the integrity of the molecule and providing sufficient recovery.

In this application note, we tested the performance of four TFF cassettes in an mRNA purification process (Figure 1).

Materials

mRNA
The process material used for TFF testing was 4 kb mRNA with a polyA tail. mRNA was produced in an IVT reaction and purified on the CIMmultus™ OligodT column as previously described.[3]

Sample Preparation
mRNA was first diluted to a final concentration of 0.8 – 0.9 mg/mL in 50 mM Tris, 0.4 M NaCl, pH 7.2 buffer, mimicking the elution conditions of a CIMmultus™ C4 HLD mRNA purification step. We reused the mRNA after each TFF for another TFF run. Approximately 100 mg of mRNA was used for each TFF cassette test.

TFF System and Consumables
A Sartoflow® Smart was used for all TFF experiments. This system can directly use the Sartocon® Slice 50 TFF membrane, which generates low shear stress thanks to its four piston membrane pumps and low recirculation volume (20 mL).

Hydrosart® membranes are stabilized cellulose-based membranes optimized for biopharmaceutical process applications. They are stable across a broad pH range and extremely hydrophilic, making them non-protein binding and virtually non-fouling. They are available in various MWCOs, with two types of spacers: (1) the ECO screen for low viscosity feed stream, requiring a lower flow rate than (2) the E-screen, designed for more viscous products.

TFF Cassettes Used
- Sartocon Slice 50 Hydrosart 100 kDa ECO 50 cm²
- Sartocon Slice 50 Hydrosart 300 kDa ECO 50 cm²
- Sartocon Slice 50 Hydrosart 100 kDa E-screen 50 cm²
- Sartocon Slice 50 Hydrosart 1300 kDa E-screen 50 cm²

After clean water flux (CWF) was performed, the membrane was conditioned with the initial sample buffer (50 mM Tris, 0.4 M NaCl, pH 7.2) for 5 min with a closed permeate port. After conditioning, the buffer was removed from the system. The sample was introduced into the recirculation tank and circulated for an additional 5 min with a closed permeate port.

Figure 1: *mRNA Purification Flow Chart*

Methods

TFF Procedure

TFF System Preparation
Sartoflow® Smart system with a 1 L recirculation tank was used to perform TFF. The sanitization procedure was performed with 0.1 M NaOH for 30 – 60 min prior to use on all tubings and cassettes. After use, the system and membrane were sanitized again with 0.1M NaOH for 30 – 60 min.

Clean Water Flux (CWF)
To determine cleaning efficiency, CWF was performed before and after the use of each TFF cassette. Standard CWF parameters (TMP: 1.25 bar) were used, and permeate flux was measured three times. CWF was calculated as an average of three distinct measurements.

TFF Consumables

Transmembrane Pressure (TMP) Scouting
Optimal TMP was determined with TMP scouting, in which a series of TMP set-points were tested at different differential pressures (DP: P1 – P2). During TMP scouting, the volume (and, therefore, mRNA concentration) in the recirculation tank was kept constant by introducing the initial sample buffer through an external peristaltic pump into the recirculation tank. Optimal TMP and DP were determined for each Sartocon® Slice 50 cassette and continued with concentration and diafiltration at optimal conditions.

Concentration
During concentration, the optimal diafiltration point (the point which provides the fastest buffer exchange) was determined. Retentate volume, permeate volume, and permeate flux were measured and recorded every few minutes, and diafiltration time was calculated. If the optimal diafiltration point was not reached before the lowest recirculation volume (40 mL), the sample was concentrated up to 40 mL (concentration factor approximately 2.5).

Diafiltration
After concentration, we continued with the diafiltration of the sample into ddH_2O. Five diafiltration volumes were chosen for buffer exchange (this removes >99% of impurities). After diafiltration, the buffer-exchanged sample was recirculated for 5 min with a closed permeate port to increase sample recovery.

Sample Recovery

The sample was collected in clean, RNase-free containers. To further increase sample recovery (due to the dead volume of tubings and cassette), 20 mL of ddH_2O was introduced into the recirculation tank and recirculated for 5 min with a closed permeate port. Flushing was repeated three times.

Analysis

mRNA recovery was measured with on the PATfix® mRNA platform using CIMac™ PrimaS HPLC columns. Sample integrity was assessed by agarose gel electrophoresis using a Bioanalyzer electropherogram.

Results and Discussion

TMP scouting allowed us to select the appropriate pressure conditions for the experiment. Constant DP and flux (LMH) were used to evaluate the best TMP. We tested DP from 0.5 to 2 bar range across the four MWCO and screen combinations (Table 1).

Table 1: *Tested TFF Conditions*

Pressure Type [bar]	100 kDa ECO	100 kDa E-screen	300 kDa ECO	300 kDa E-screen
Feed (P1)	1.5	1.5	1.5	2
Retentate (P2)	0.5	0.5	0.5	0
Permeate Pressure (P3)	0	0	0	0
Differential Pressure (DP)	1	1	1	2
Transmembrane Pressure (TMP)	1	1	1	1

The TFF experimental run was performed with a concentration phase followed by a diafiltration phase (buffer exchange) (Figure 2-4).

During the concentration phase, there were large differences in feed flux, with E-Screen cassettes (particularly 300 kDa) being much higher than the ECO screens (Figure 2). Good stability is observed over the entire concentration for all cassettes.

In the permeate flux, differences were smaller (Figure 3). There were small differences early in the concentration, which stabilized later.

During the diafiltration, the differences in the feed flux were similar to during concentration, with the 300 kDa E-screen showing the highest flux (Figure 4). There was also good stability over the diafiltration, with a slight increase at the end.

There was a large increase of permeate flux in the second part of the diafiltration (Figure 5). Differences observed between the filter cassettes, with the 300 kDa E-screen performing best.

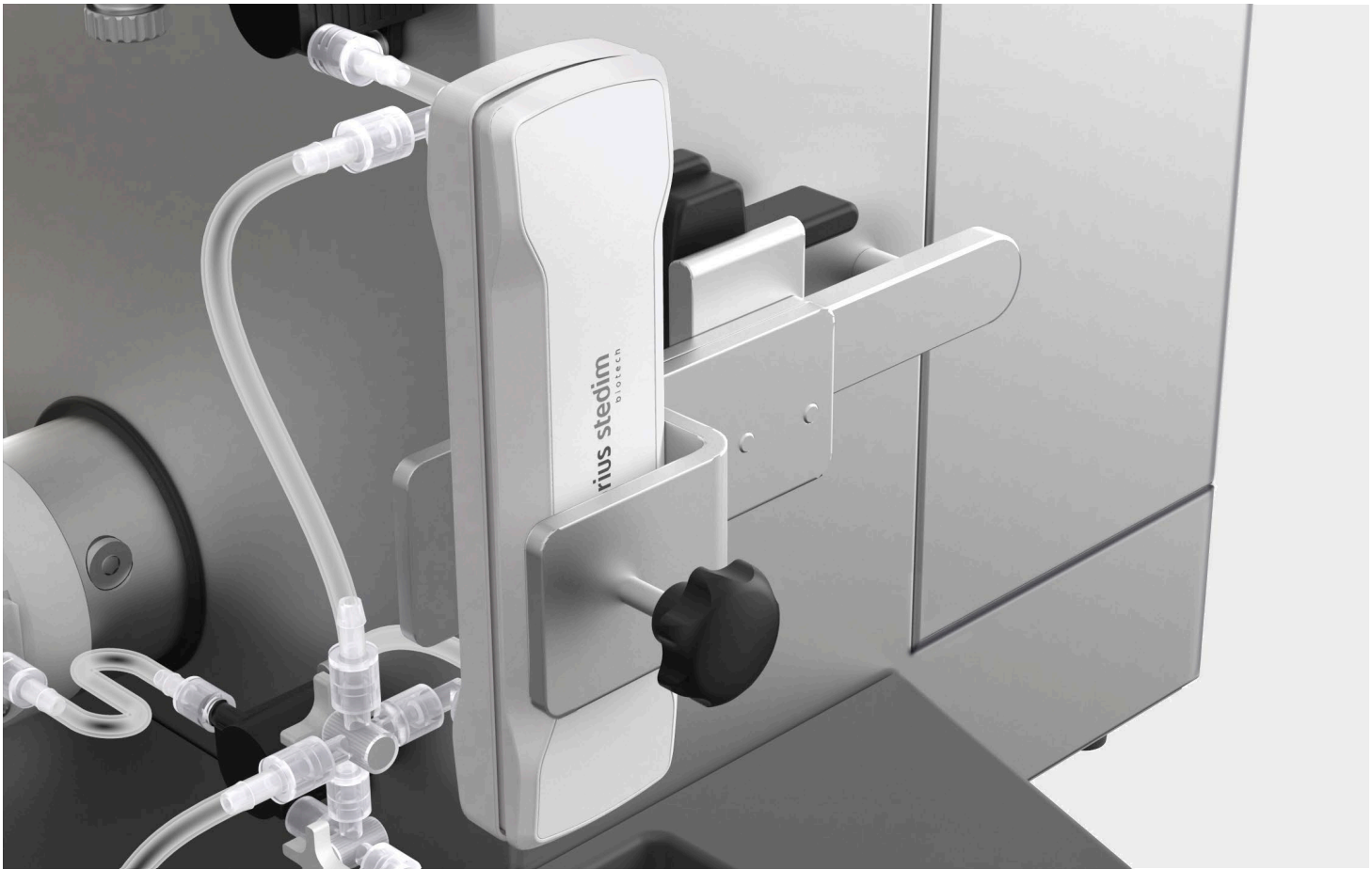


Figure 2: *Concentration Phase (Feed Flux) Across Four Filter Cassettes*

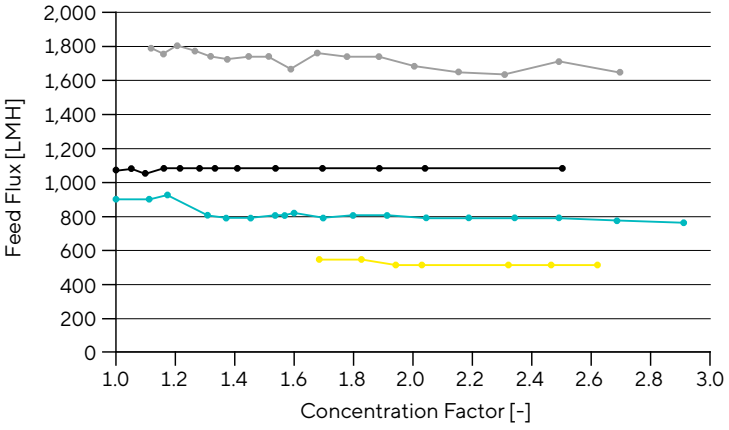


Figure 3: *Concentration Phase (Permeate Flux) Across Four Filter Cassettes*

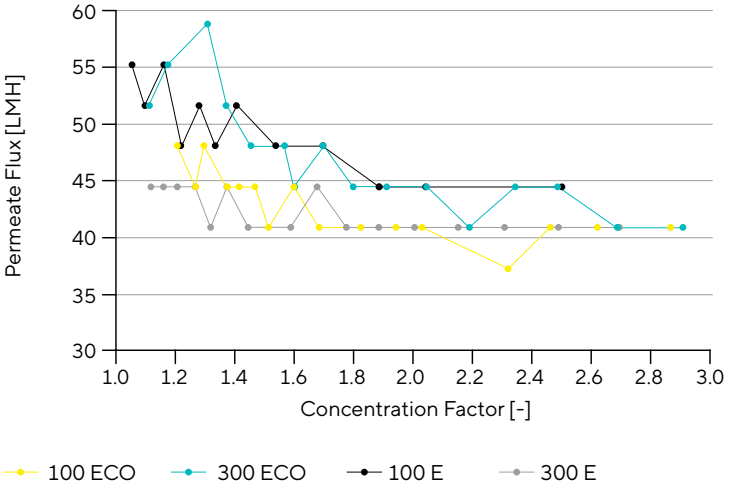


Figure 4: *Diafiltration Phase (Permeate Flux) Across Four Sartocon® Slice 50 TFF Cassetes*

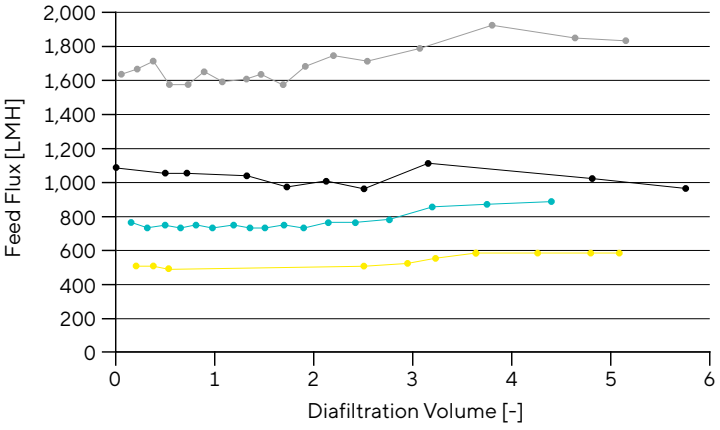
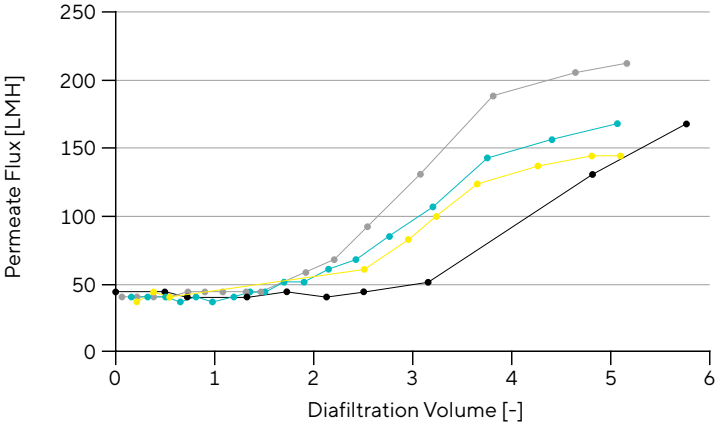


Figure 5: *Diafiltration Phase (Permeate Flux) Across Four Sartocon® Slice 50 TFF Cassetes*



mRNA Integrity Testing

To verify that there is no major loss or degradation of the biomolecule, we ran agarose gel electrophoresis and generated Bioanalyzer electropherograms for all cassette sizes. The worst-case scenario is the 100 kDa membrane with ECO-screen (i.e., small channels) (Figure 6).

The best-case scenario is the 300 kDa membrane with the E-screen (Figure 7). No significant changes in mRNA integrity were observed after TFF in both retentate and all three flushes. mRNA size is as expected, and a slight degradation of smaller molecular sizes was noted but also expected

Figure 6: *mRNA Integrity After TFF on 100 kDa ECO Hydrosart® Cassette. A) Agarose Gel Electrophoresis; B) Bioanalyzer*

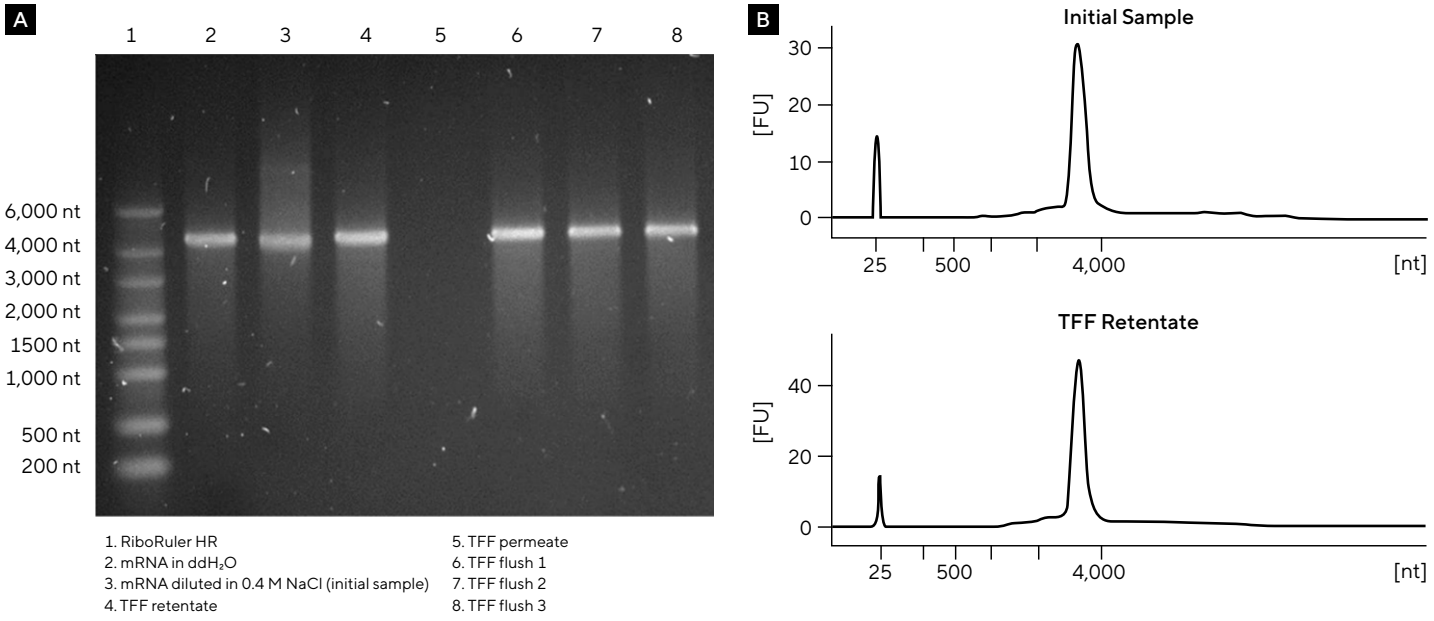
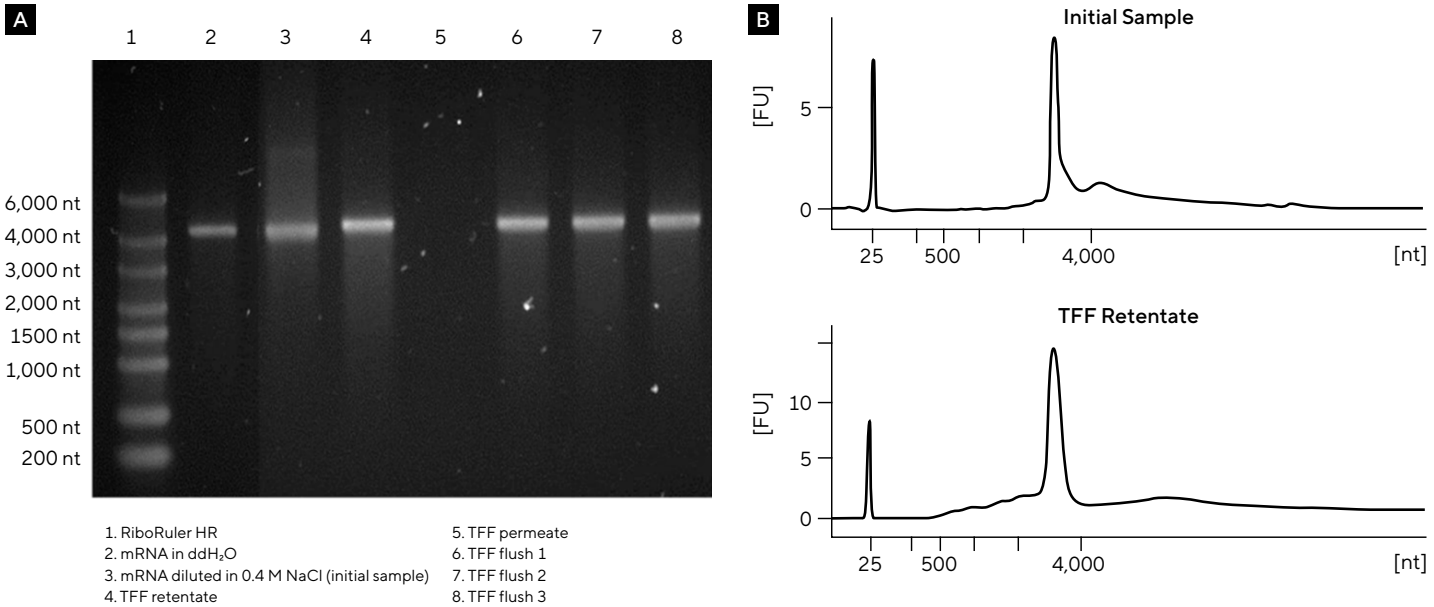
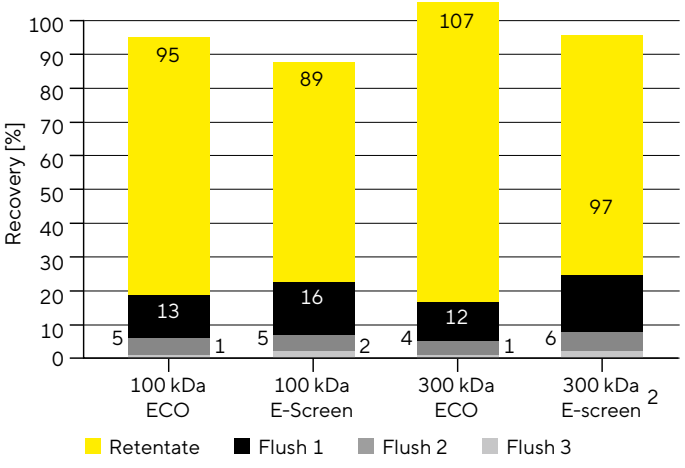


Figure 7: *mRNA Integrity After TFF on 300 kDa E-Screen Hydrosart® Cassette. A) Agarose Gel Electrophoresis; B) Bioanalyzer*



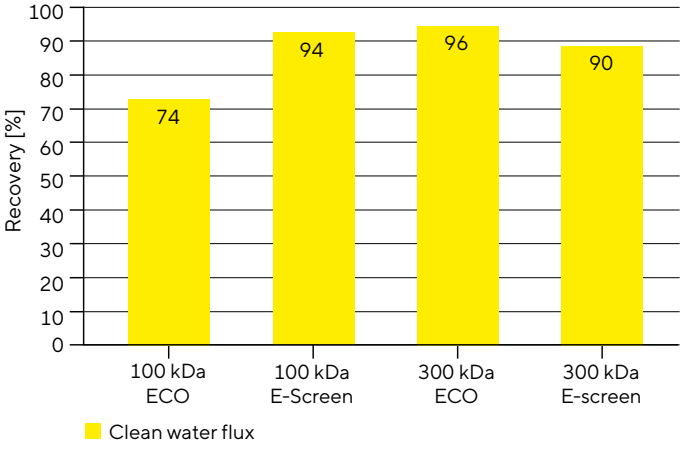
We also evaluated the recovery performance for the 4 cassette types. mRNA recovery was measured by HPLC using CIMac™ PrimaS HPLC column. Overall, we observed good recovery (average 97%) with the 300 kDa performing better. Flushes were necessary to recover around 20% of the product (Figure 8).

Figure 8: *Retentate Recovery Across Sartocon® Slice Hydrosart® TFF Cassettes*



Clean water flux recovery was 89% on average (Figure 9), meaning that the cassette can be reused successfully.

Figure 9: *Clean Water Flux Recovery Across Sartocon® Slice Hydrosart® TFF Cassettes*



Conclusion

TFF represents a valuable pre-chromatography purification step during mRNA processing. This application note showcases the performance of four TFF cassettes during ultrafiltration and dialfiltration of mRNA on the Sartoflow® Smart system.

The results show that all four tested TFF cassettes are suitable for ultrafiltration and diafiltration of mRNA, e.g., for buffer exchange from a high-salt matrix into water. Our findings are summarized in Table 2. To make a recommendation, we employ the following selection criteria:

- High recovery
- High speed, low feed flow
- High concentration
- High permeate flux
- mRNA stability

Table 2: *Cassettes Test Results Overview*

Cassettes	Recovery	Flux	Concentration	mRNA Stability
100kDa ECO	◖	◖	◖	◖
100kDa E	○	◖	◖	◖
300kDa ECO	●	◖	●	●
300kDa E	●	●	●	●

Note. ● best average, ◖ intermediate average, ○ lowest average,

The 300kDa Hydrosart® E-screen had the fastest process time and enabled high mRNA recovery (96%) with no mRNA detected in the permeate (which indicates no product loss). The 300 kDa Hydrosart® ECO cassette is also suitable, showing even higher recovery (~100%) at a low feed flow. The cassette was also the second fastest after the 300 kDa E-Screen.

All cassettes showed stable permeate flux during concentration, indicating that high concentrations should be reachable without issues. The large increase of the permeate flux during diafiltration with water may require particular attention when choosing the TFF system. Finally, the same sample of mRNA was used four times without noticeable degradation.

Based on these criteria, we recommend the 300 kDa Hydrosart® E-screen cassette for mRNA processing on the Sartoflow® Smart.

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