

Technical Brief

Techniques for Demonstrating Cleaning Effectiveness of Ultrafiltration Membranes

A Case Study: Pellicon® 2 Cassette Processing of Therapeutic Proteins

Tangential flow filtration cartridges designed to isolate, purify and concentrate therapeutic biomolecules are typically reused for multiple process cycles. After each process run, the system is cleaned to remove foulants and contaminants from the membrane in preparation for the next cycle. To maintain optimal membrane performance, biotech and pharmaceutical manufacturers must establish effective, reproducible cleaning techniques to prevent microbiological contamination and ensure against cross contamination of batches. Effective and consistent membrane cleaning after each process cycle is the single most important factor in maintaining system performance.

Increasing regulatory requirements and proactive plant improvements are spurring the development of new methods to evaluate cleaning effectiveness leading to improved cleaning validation protocols. In this study, the cleaning procedures for Pellicon® 2 cassettes (Figure 1) are evaluated in the processing of human serum therapeutic proteins. Techniques are presented to demonstrate the effectiveness of cleaning procedures for Pellicon® 2 cassettes with Biomax® 10 kD polyethersulfone and Ultracel® 10 kD regenerated cellulose membranes.



Figure 1. Pellicon® family of cassettes ranging from 50 cm² to 2.5 m² of filter area.

Measuring Cleaning Effectiveness

An effective cleaning protocol removes residual protein, foulants, and other contaminants from the membrane surface and cassette feed channels, and restores the membrane so that performance returns to a predictable, consistent level. Key elements to monitor when assessing cleaning effectiveness include:

- Normalized Water Permeability
- Flush Water Residuals
- Process Reproducibility
- Physical Analysis of Contamination Through Destructive Techniques

Measurements of normalized water permeability (NWP) and process flux reproducibility directly indicate both the effectiveness and consistency of the cleaning procedures. Visual assessment and Fourier Transform

Infrared Spectroscopy (FTIR) analysis of the membrane surface are destructive tests that may be used during the development phase to evaluate the membrane and device after cleaning.

Selection of cleaning chemicals, flow and pressure conditions, cycle times and cleaning sequences are key elements in a cleaning protocol. Guidelines and process conditions are specific for the membrane type, device configuration, and process fluid, and are provided in Instruction Manuals.

For Pellicon® products refer to:

Pellicon® 2 Cassettes User Guide P35430

Pellicon® XL Cassettes User Guide P60085

Figure 2.
Generalized process schematic to measure Normalized Water Permeability (NWP).

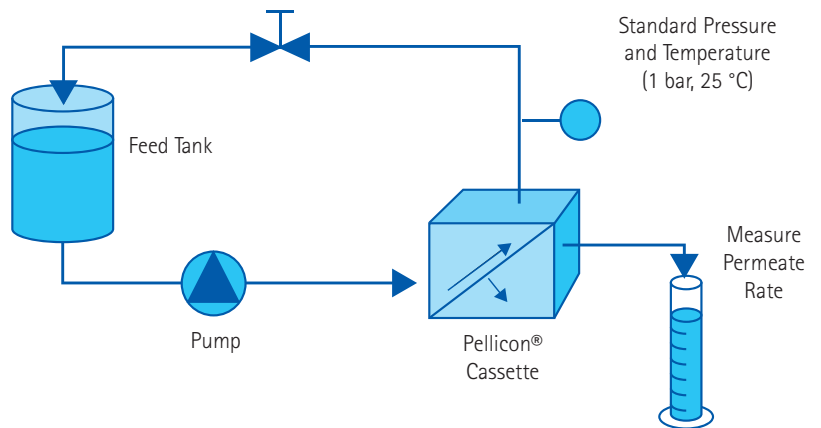
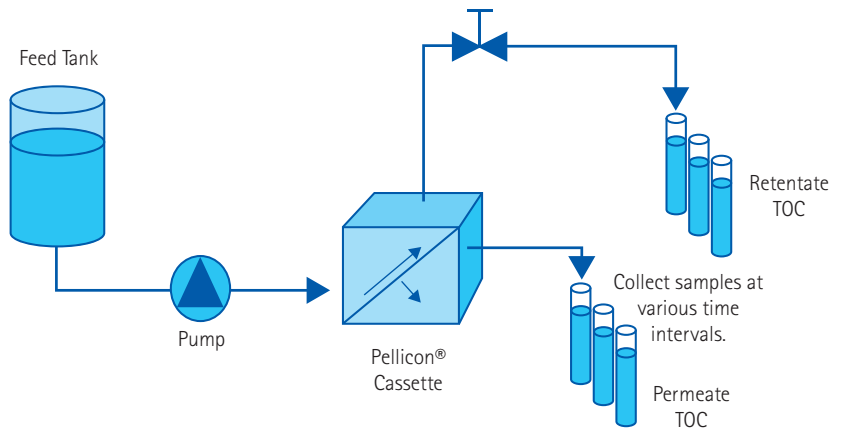


Figure 3.
Generalized schematic for collecting samples to measure TOC during the critical flush cycle.



Normalized Water Permeability (NWP)

Normalized water permeability (NWP) is an established method for determining the cleanliness of a cassette after cleaning. This method involves measuring the passage of clean water through the membrane under standard pressure and temperature conditions (Figure 2). The rate of clean water flux through the membrane is measured as liters per membrane area per hour (L/m²-h). Water flux divided by the transmembrane pressure is the normalized water permeability or NWP (L/m²-h-bar). The NWP values are compared to initial (pre-process) levels and may be analyzed for trends over time.

Fouled membranes typically have NWP values that are significantly less than 50% of the membrane's original NWP specification because of adsorption of materials such as proteins on the membrane surface. Cleaning cycles remove these foulants by oxidizing, emulsifying and removing the foulants from the membrane surface. After cleaning, if the membrane NWP is $\pm 20\%$ of the pre-run NWP value, process stream reproducibility will result. The acceptance criterion for cleaning efficacy is membrane and application specific, and may vary between plants.

The procedures for measuring NWP are:

- Fill tank with clean water
- Set standard conditions
- Record water permeability rates, pressure, and temperature

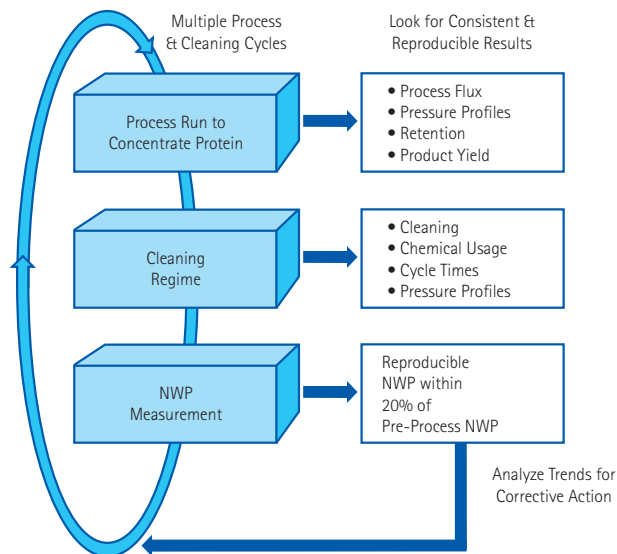
TOC as a Measurement of Flushing Effectiveness

In commercial (TFF) applications, the membrane cassette and system are flushed to displace storage and cleaning solutions, and to remove residual process materials. TOC measurements of the permeate and retentate flush streams provide a reliable and highly sensitive means of detecting organic contamination in a cassette (Figure 3). Because of its sensitivity, TOC is becoming the preferred method for identifying contamination from inadequate cleaning protocols, product carryover between batches, and cross-contamination of products exposed to the same process equipment. The flush cycle performed immediately prior to the process feed is termed the "critical flush" and is the most important cycle to identify trace contaminants using the TOC analysis. Typical TFF system critical flush volumes of 20 L/m² of membrane are used and should yield TOC values below a 1.0 ppm level.

The procedures are summarized as follows:

- Fill clean tank with water-for-injection (WFI)
- Divert permeate and retentate lines
- Set standard flush conditions
- Sample flush water over time
- Measure TOC

Figure 4. Key elements demonstrating cleaning effectiveness in a TFF system.



Process Reproducibility

The goal of a cleaning cycle is to ensure consistent process performance on a product stream. Reproducible process flux and predictable product yield are directly related to membrane cleaning. Long-term monitoring of the process and cleaning performance provides a means to proactively address potential operating problems and enhances overall process consistency.

Examination for Physical Evidence of Fouling

After many process cycles, the feed screen and membrane surface can be examined for residual protein by autopsying the cassette to determine cleaning effectiveness. The surface of the cleaned membrane may also be analyzed by high resolution FTIR spectrum for evidence of protein foulants and compared to the FTIR of an unused membrane sample. This procedure is a destructive technique that may be used to assist in validating a cleaning protocol.

Case Study

Experimental Technique

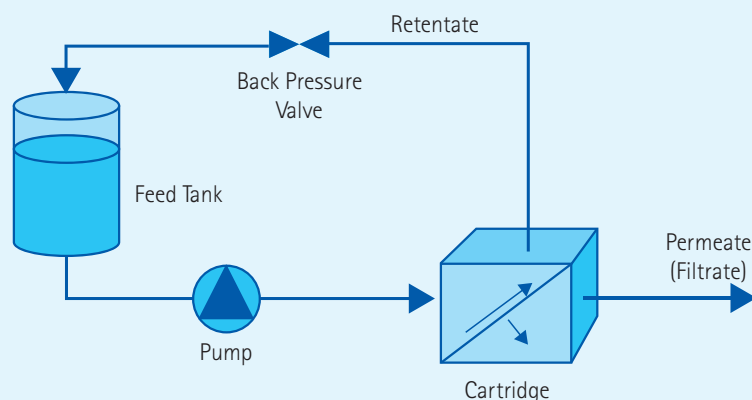
Pellicon® 2 cassettes were subjected to multiple processing and cleaning cycles on a human serum albumin (HSA) feed solution concentrating from 2% to 25% protein and in separate tests with a solution of 8% Fraction 1 protein. Biomax® 10 kD (polyethersulfone) and Ultrace® 10 kD (regenerated cellulose) membranes were chosen to represent two different membrane formulations. Membranes were exposed to identical process environments under production-scale conditions to compare performance and demonstrate applicability.

Multiple 2-hour concentration cycles established process reproducibility. Worst-case conditions were simulated during extended process cycles (up to 19.5 hours) at the maximum commercial protein concentration.

After processing, membrane cassettes were flushed with 1% NaCl at 10 °C and cleaned independently according to membrane type: Biomax® membranes were cleaned with a caustic/chlorine procedure (0.25N NaOH, 250 ppm sodium hypochlorite) and Ultrace® membranes were cleaned with a caustic-only (0.25N NaOH) procedure. The duration of both membrane cleaning cycles was 1 hour at 40 °C. Operating conditions were 2.0/0.5 bar with a crossflow of 6 Lpm/m². Following each cleaning cycle, WFI flush water TOC levels were tracked in the permeate and retentate lines, and NWP was measured at standard conditions. The key elements in operation of the pilot system were to:

- Match process-scale conditions
- Perform multiple process runs
- Compare flux performance
- Concentrate to maximum levels

Figure 5.
Generalized pilot schematic for HSA cleaning trials.



Case Study: Results

Consistent Return of Water Permeability

Membrane cleaning procedures effectively restored NWP following each process trial. When compared to "Pre-Run" NWP, the combined caustic and hypochlorite regime for the Biomax® membrane and the caustic-only cleaning for the Ultracel® membrane returned NWP values to near-initial levels. The lower NWP values of the Ultracel® membrane compared to Biomax® membrane is an inherent membrane trait related to membrane composition and is not indicative of process flux.

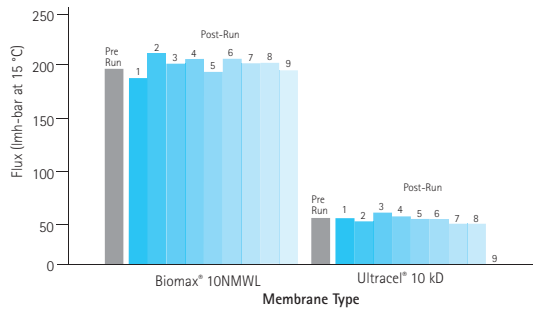


Figure 6.
Water Permeability
After Cleaning
Sequential Process Runs

Consistent Process Performance

Both the Biomax® and Ultracel® membranes demonstrated reproducible and predictable flux in all trials. Ultracel® membrane flux was stable at high protein concentrations during extended runs, demonstrating the low fouling nature of the regenerated cellulose membrane. Stable process pressures and flow conditions indicate absence of plugging and suitability of cassette design.

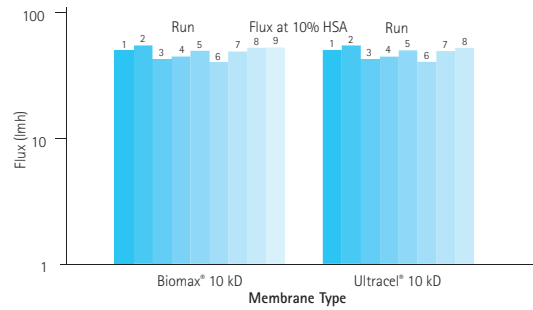


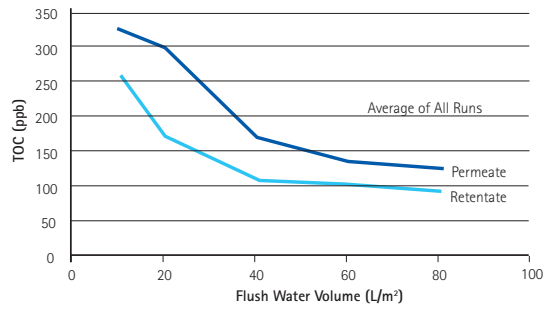
Figure 7.
Human Serum
Albumin Performance
Biomax® 10 and Ultracel®
kD Cassettes

No Protein Loss

Samples of composite permeate from each process run were analyzed for protein using the biuret method. No protein loss was detected in the permeate, indicating greater than 99.9% protein yield.

Run Number	Ultracel® 10 kD	Biomax® 10
1	0.00	0.01
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	0.00	0.00
6	0.00	0.00
7	-	0.00
8	-	0.00

Figure 8.
Flushing Efficiency of
Biomax® 10 Cassettes

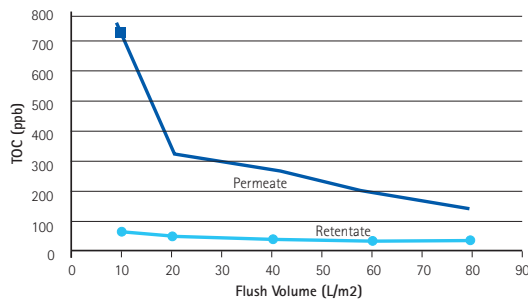


Case Study: Results

Low Protein Carryover After Flushing

Residual protein carryover between process runs is readily detected by TOC analysis of the flush waters. Cassettes of both membranes demonstrated efficient flushing to less than 500 parts per billion (ppb) TOC at flush volumes of 10 to 20 L/m² of membrane area.

Figure 9.
Flushing Efficiency of
Ultracel® 10 kD Cassettes



Case Study: Results

No Adsorptive Fouling on Ultracel® Membrane

After multiple process cycles, an autopsy revealed no visible foulants or protein material in the cleaned Ultracel® membrane cassette. Comparing the FTIR spectrum to that of a new membrane confirmed the absence of protein on the cleaned Ultracel® membrane surface.

Cleaning Results

- Both membrane types show return of NWP to original values
- Complete restoration of process flux
- Flushing studies show low residual protein
- FTIR shows no adsorption on Ultracel® membrane

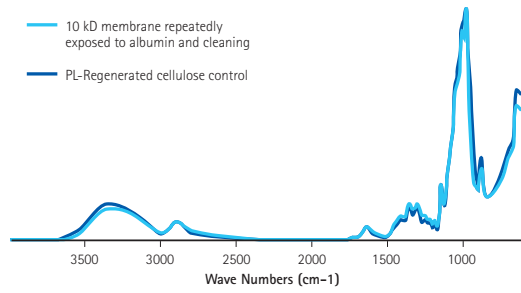


Figure 10. FTIR Analysis of the Ultracel® 10 kD Membrane Surface After Fraction 1 Processing

Case Study: Results

Residual TOC in a New Membrane

Pellicon® cassettes are shipped with storage preservative to prevent microorganism growth and to maintain membrane performance specifications. The TOC test allows for a simple technique to verify removal of the organic based preservative. To remove the preservative, new cassettes were clean-water flushed with permeate and retentate directed to drain. Measurement of permeate and retentate TOC residuals of a new cassette and successive flushes (indicated as 1-4 in Figure 6) demonstrated the removal of residual TOC to below 1.0 ppm after the first flush using 20 L/m² of WFI flush water. Repeated cleaning and flush cycles show some additional removal of TOC down to the 200 ppb level.

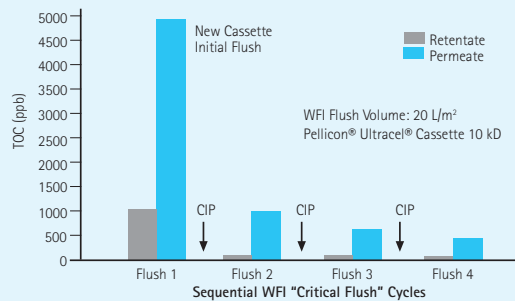
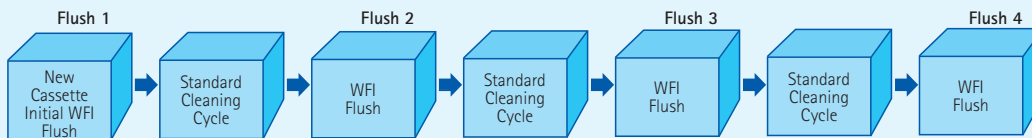


Figure 10. New Cassette Flushing Efficiency

Figure 11. Sequential WFI "Critical Flush" Cycles



Membrane Selection

Pellicon® 2 cassettes with either Biomax® 10 or Ultracel® 10 kD membranes demonstrate process consistency and reproducible cleanability. Both types are the latest generation of void-free, controlled pore membranes designed for high flux and excellent product retention. Membrane selection may be based on preference for a specific chemical cleaning procedure, membrane type, or other process attribute.

The techniques used for evaluating cassette cleanability may be extended to our other tangential flow products when there is a need to demonstrate process consistency and reproducible cleanability with membrane-specific cleaning procedures. Ask our Applications Specialist for additional guidance on cleaning optimization, enhancing yields and improving membrane performance.

Membrane Type	Biomax® 10 KD	Ultracel®
Membrane	Polyethersulfone	Regenerated cellulose
Molecular weight cut-off	5 kd – 1000 kd	1 kd – 1000 kd
Relative protein binding	Low to medium, use with > 0.1 mg/mL protein solutions	Ultra-low, far superior for use with dilute protein solutions
pH range	1 – 14	2 – 13
Chemical compatibility	250 ppm sodium hypochlorite	Use with anti-foams resists organic solvents
Cleaning	Easy to clean	Easy to clean
Common attributes		Excellent protein retention High process flux Long membrane life

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