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Preparation, Separation,
Filtration & Testing Products

Application Note

Pellicon® Capsules for Ultrafiltration/ Diafiltration in the Antibody Drug Conjugate Manufacturing Process

Tangential flow filtration (TFF) is essential in the manufacturing of antibody drug conjugates (ADCs). Ultrafiltration (UF) is used to concentrate the protein product and diafiltration (DF) to exchange buffer and/ or remove process-related impurities from the ADC solution, including residual linker, organic solvent, and/or free drug. Clearance of organic solvent is required when processing the ADC solution directly after conjugation, for which the TFF filter must be solvent-compatible. ADC manufacturing also requires a containment strategy during post-conjugation TFF steps to protect operators from health risks associated with exposure to the highly potent ADC molecule and free drug, all while preventing contamination of the product and the environment. As a result, single-use TFF devices are a natural choice for UF/DF operations in ADC manufacturing.

Pellicon® Capsule for ADCs

Our proven and reliable Pellicon® Capsule with Ultracel® membrane fulfills the single-use TFF device requirements for the ADC manufacturing process (**Figure 1**). Engineered with operator safety in mind, the Pellicon® Capsule features an innovative, holderless and torqueless design for easy connection to a single-use TFF system. Its self-contained format enables use of a closed, disposable flow path for post-use containment. In addition, the capsules are resistant to organic solvents commonly used in the ADC manufacturing process and are supplied gamma sterilized with preservative-free reverse osmosis water for reduced device preparation time. Thus, operating the Pellicon® Capsule not only increases operator safety, but also saves time for improved batch turnaround and flexibility in the ADC manufacturing plant.

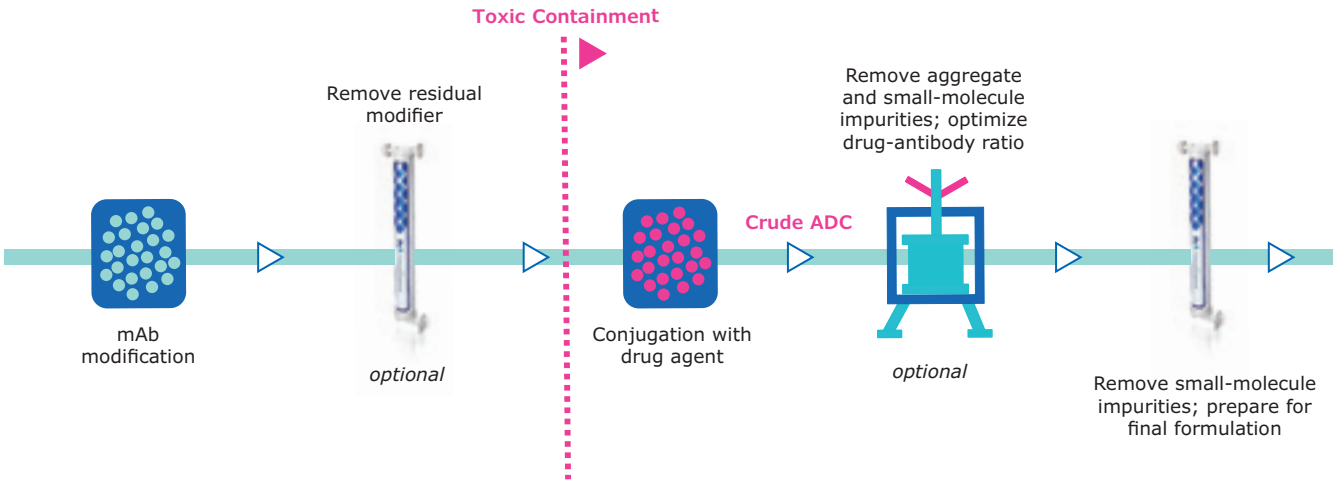

Figure 1. Example of an ADC manufacturing process with Pellicon® Capsule.

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Pellicon® Capsule Performance and Scalability

Pellicon® Capsules exhibit comparable UF/DF performance and linear scalability to Pellicon® cassettes for easy integration into existing ADC processes. To assess performance comparability, permeate flux and pressure drop of Pellicon® Capsules, sizes 0.1 m^2, 0.5 m^2 and 1.5 m^2, and benchmark Pellicon® 3 cassette, size 0.11 m^2, with 30 kDa Ultracel® membrane were evaluated over a range of feed flow rates using 40 g/L bovine gamma globulin (BgG). **Figure 2** shows that all three device sizes of Pellicon® Capsule provide comparable antibody flux and pressure drop to the Pellicon® 3 cassette when operated at the same feed flow rate.

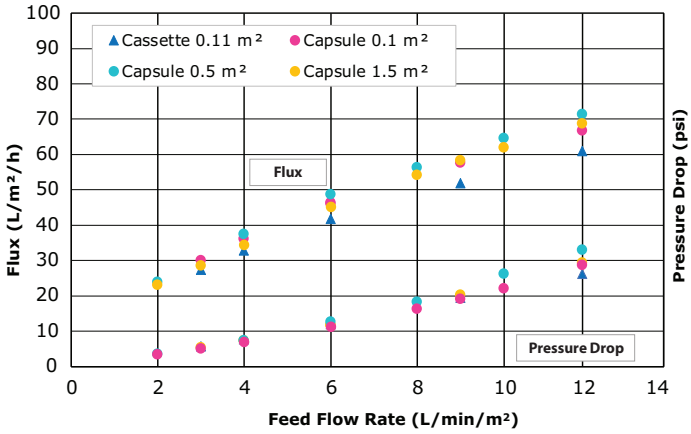

Figure 2. Effect of feed flow rate on permeate flux and pressure drop for Pellicon® Capsule and Pellicon® cassette; 40 g/L BgG, 15-20 psi retentate.

To evaluate scalability, a solution of 10 g/L BgG was recirculated and the permeate flux of all devices was monitored over a transmembrane pressure (TMP) range. **Figure 3** shows close antibody flux data distribution for both capsules and the cassette, demonstrating linear scalability within the Pellicon® Capsule family as well as to Pellicon® cassettes.

This performance comparability and linear scalability between both filter formats simplifies conversion from cassettes to capsules, all while the capsules offer enhanced ease-of-use, reduced device preparation time, and post-use containment for the filtration of ADCs.

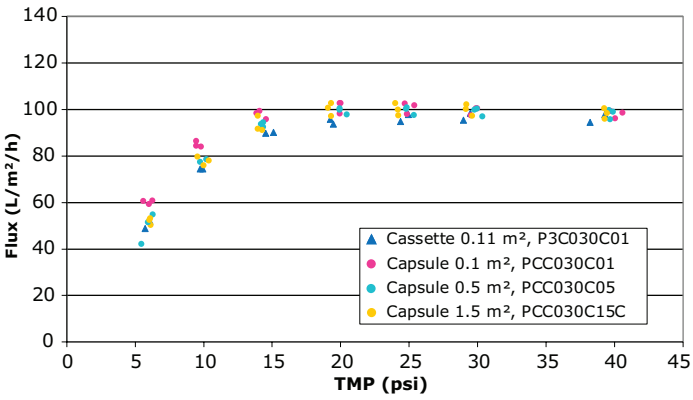

Figure 3. Effect of TMP on permeate flux for Pellicon® Capsules and Pellicon® cassette; 10 g/L BgG.

Organic Solvent Clearance and Compatibility

The ADC conjugation step occurs in the presence of an organic solvent, such as dimethyl sulfoxide (DMSO) or dimethylacetamide (DMAc). To process the crude ADC solution, the TFF filter must offer organic solvent compatibility with efficient clearance. Pellicon® Capsules have the same solvent-resistant, Ultracel® membrane of Pellicon® 3 cassettes, ensuring comparable solvent removal efficiency.

Figures 4 and **5** show the clearance of 20% DMAc and 20% DMSO, respectively, by diafiltration using capsule and cassette formats. Both filters performed with efficient solvent clearance and track a theoretical process in which no retention of the solvent by the membrane is assumed, calculated using a sieving coefficient (S) of 1. The decrease in DMSO removal rate after ~8 diavolumes may be due to the presence of dead legs in the system and charge interactions. Comparison of pre- and post-diafiltration parameters, including air diffusion, pressure drop, membrane permeability, and protein retention, indicates stability of the capsule to DMSO and DMAc under the tested conditions (**Figures 6** and **7**).

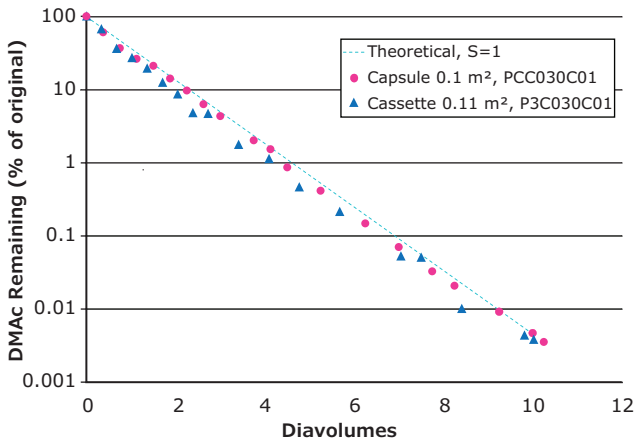

Figure 4. Diafiltration of 20% DMAc from aqueous solution.

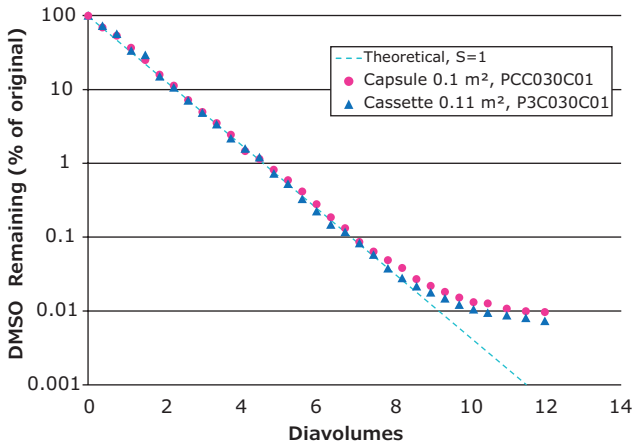

Figure 5. Diafiltration of 20% DMSO from aqueous solution.

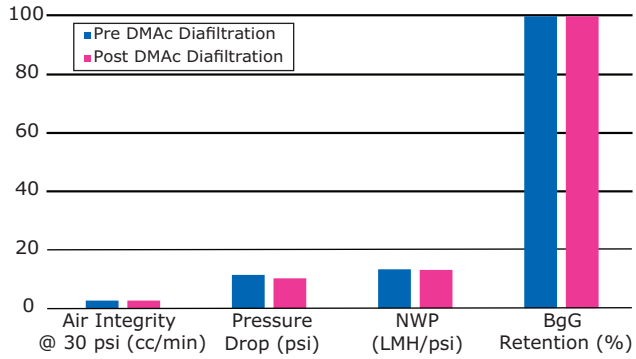

Figure 6. Compatibility of Pellicon® Capsule with DMAc.

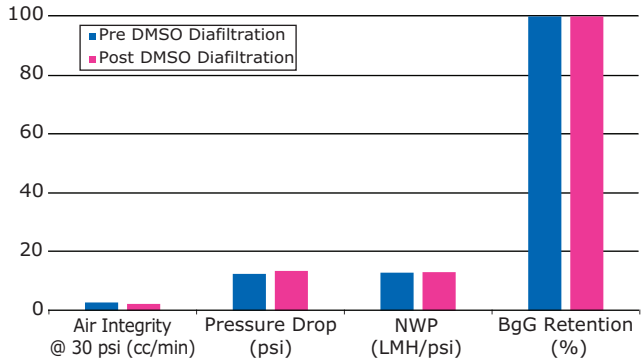

Figure 7. Compatibility of Pellicon® Capsule with DMSO.

Best Practices for ADC Processing with Pellicon® Capsule

The comparability between Pellicon® Capsules and Pellicon® cassettes simplifies conversion between both formats: similar membrane area and pump rate can be used. Further, because of its simpler, holderless format, the capsule can be integrated into a downstream process without major investments in new equipment.

When using Pellicon® Capsule for ADC feedstocks, the operating guidelines below should generally result in good process performance. These guidelines may be modified as needed based on findings from your process development work.[1]

1. Install Pellicon® Capsule(s) with 30 kDa Ultracel® membrane into the single-use TFF system (**Figure 8**) for the filtration of modified mAb or ADC with molecular weight of ~150 kDa. Installation of the capsule does not require a compression holder or single-use liners/insert plates to contain the feed solution.[2]
2. Confirm Pellicon® Capsule integrity, if desired.
3. Condition the TFF system and membrane by flushing with 20 L/m^2 of feed buffer (no sanitization is needed). After conditioning, prevent air from entering the capsule to avoid foam generation and protein damage from air/liquid interface.
4. Add feed solution to the feed tank. If feed volume exceeds tank capacity, then continue addition in constant-volume fed-batch mode (feed flow rate into tank equal to permeate flow rate).
5. Concentrate until the target concentration for diafiltration is achieved using a feed flow rate of 5 L/min/m^2, 10-20 psi TMP (optimal TMP determined by running TMP excursions), and room temperature. For ADCs, typical target concentrations for diafiltration are within 25 to 30 g/L.
6. Conduct diafiltration in constant-volume recirculation mode (buffer flow rate equal to permeate flow rate); this method typically gives the most efficient buffer exchange.
7. Conduct final concentration. Over-concentrate as needed to compensate for any dilution expected during recovery and formulation steps.
8. Recover the product from the Pellicon® Capsule and TFF system in a manner that gives desired yield, quality, and concentration. A reasonable recovery

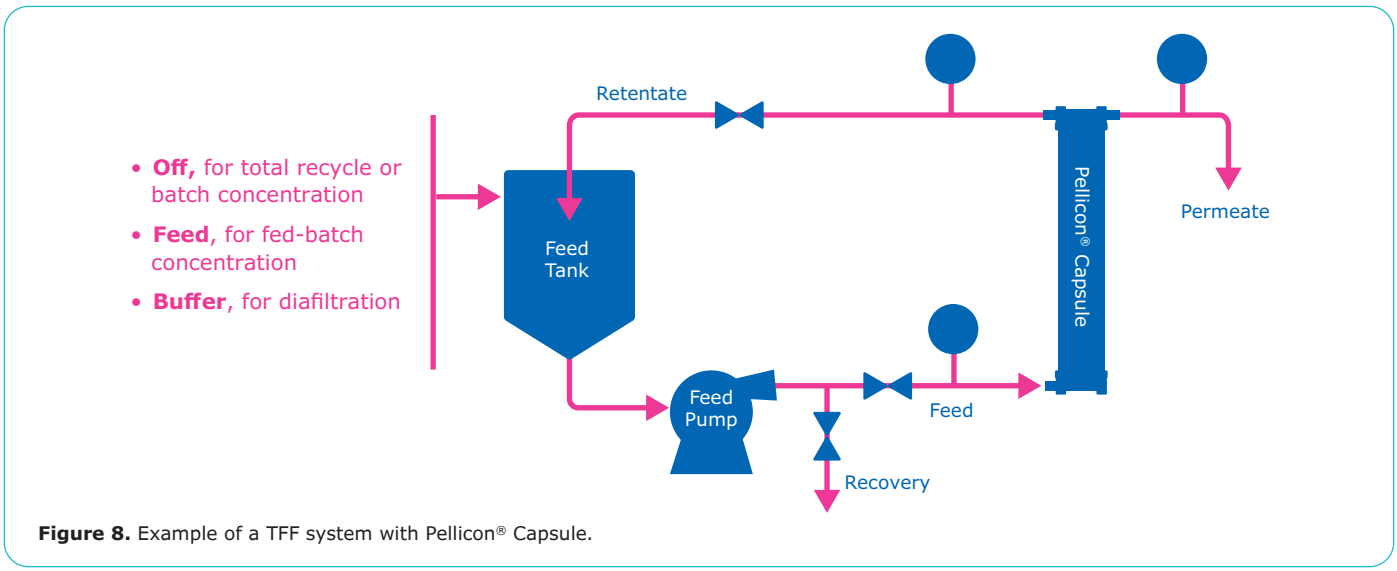

Figure 8. Example of a TFF system with Pellicon® Capsule.

approach is to depolarize the membrane, drain the feed tank to a collection container, and then conduct buffer displacement (from the highest point of the system, down through the capsule) or buffer recirculation with one minimum working volume.[3,4]

9. Remove the capsule and remainder of wetted flow path together from the single-use TFF system to keep the flow path closed, reducing risk of operator exposure to the process fluid.
10. To scale up, increase area proportionally with feed volume, while maintaining the same normalized feed flow rate, TMP, and temperature.

ADC Mimic Case Study

The applicability of Pellicon® Capsule in the processing of ADCs was showcased in a study reported by Czapkowski et al.[5] A column-purified, non-toxic ADC mimic (~150 kDa) was used to evaluate and compare performance parameters of Pellicon® Capsule and Pellicon® 3 cassette with 30 kDa Ultracel® membrane. The ADC mimic solution was spiked with DMSO and diafiltered at a feed flow rate of 5 L/min/m², TMP of 15 psi, and room temperature. The results indicated efficient clearance of DMSO from the ADC mimic feed solution by both capsule and cassette formats (**Figure 9**). In addition, permeate fluxes were stable throughout the diafiltration step for both filters, yields were high and comparable, and aggregate levels were also similar (**Table 1**).

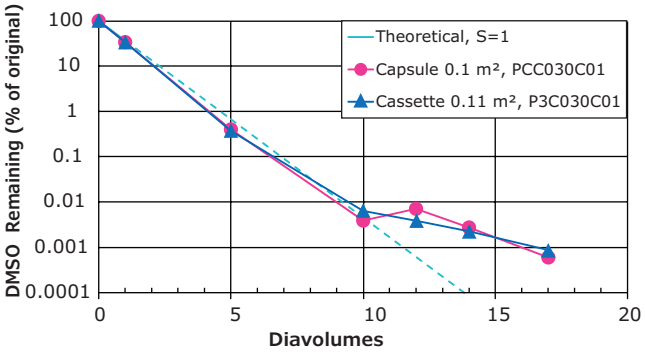

Figure 9. Clearance of 6.8% DMSO by diafiltration of 26-28 g/L ADC mimic.[6]

Table 1. Performance summary for the diafiltration of ADC mimic.[5]

Performance Parameter	Pellicon® Capsule	Pellicon® 3 Cassette
DMSO Clearance	4 log at 10 diavolumes	4 log at 10 diavolumes
Permeate Flux	68-92 L/m²/h	73-84 L/m²/h
ADC Mimic Yield	91-95% retentate 95-98% w/ rinse	91-96% retentate 93-98% w/ rinse
Aggregates (feed 0.875%)	0.93-1.16% retentate	0.91-1.16% retentate

Summary

The Pellicon® Capsule with Ultracel® membrane is ideal for single-use processing of ADCs. It enhances operator safety and offers organic solvent compatibility, all while providing high UF/DF performance comparable to that of Pellicon® cassettes. The Pellicon® Capsule is self-contained, gamma sterilized, and preservative-free. With these user-friendly features, the capsule is quickly and safely installed, used and removed, increasing plant productivity and reducing operator exposure to cytotoxic chemicals and aggressive solvents. Guidance on the use of the Pellicon® Capsule for UF/DF of ADCs is provided to assist with your process needs.

References

1. A hands-on guide to ultrafiltration/diafiltration optimization using Pellicon® Cassettes. Lit. No. AN2700EN00.
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3. Lutz, H. Ultrafiltration for Bioprocessing. Woodhead Publishing (Elsevier), Waltham, MA, USA, 2015.
4. Recovery optimization of process scale ultrafiltration/diafiltration systems. Lit. No. TB5882EN00.
5. Czapkowski, B. et al. Trial of high efficiency TFF capsule prototype for ADC purification. ADC Review, 2017.

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