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Advantages of Single-Use Crossflow Filtration in ADC Processes

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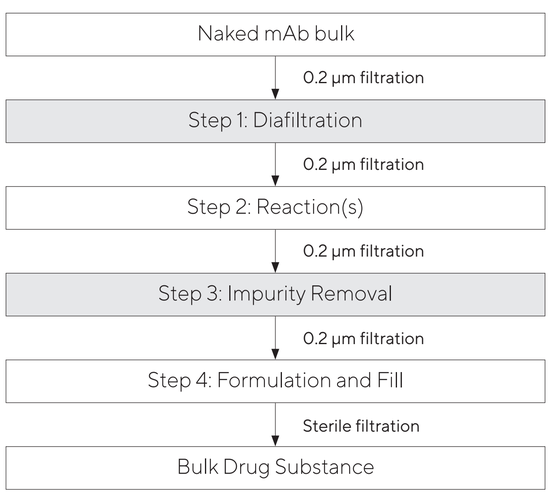
1. Introduction



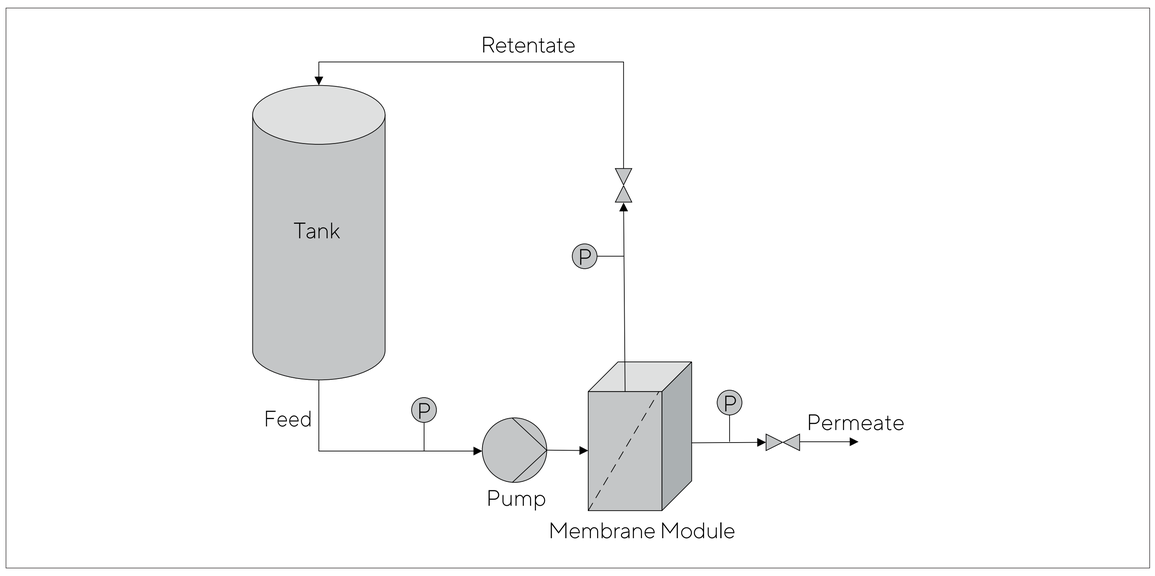
Antibody Drug Conjugates (ADCs) represent a significant area with clinical and economic growth for the bio-pharmaceutical market. ADCs combine the targeted specificity of a monoclonal antibody (mAb) with a cytotoxic small molecule. They are manufactured by attaching the monoclonal antibody to potent cytotoxic payload via a heterobifunctional linker. The typical process flow in manufacturing is indicated in the figure.

2. Crossflow Filtration in ADC

Crossflow filtration is an important unit operation used in ADC manufacturing processes and frequently serves the following processing objectives:

- The buffer exchange of the naked mAb formulation buffer to a basal buffer suitable for performing reactions
- Removal of reaction related impurities (e.g. solvent, small molecules, etc.)
- Buffer exchange of the ADC into its basal formulation buffer

The minimal equipment set-up of a single-use crossflow system in ADC application is indicated in the figure below. The set-up consists of a tank, a pump, a membrane module, pressure sensors, control valve, a feed, a retentate and permeate line.



Process Steps for Crossflow Filtration in an ADC Manufacturing Process

Naked mAb bulk

0.2 µm filtration

Step 1: Diafiltration
The first diafiltration step describes the exchange of the mAb formulation buffer to a basal buffer suitable for conjugating the small molecule to the naked mAb. A Hydrosart® (regenerated cellulose) membrane is recommended to reduce membrane fouling and nonspecific binding of the mAb. As a result, yield losses are minimized and processing time to complete the unit operation is reduced.

0.2 µm filtration

Step 2: Reaction(s)

0.2 µm filtration

Step 3: Impurity Removal
The crossflow membrane (typically 30 kD MWCO) is operated in diafiltration mode to remove reaction related impurities.

- Impurity Removal: These reaction related impurities can include reducing agent, excess linker, excess cytotoxic payload, solvents and unwanted buffer components. The mAb and | or ADC is too large to pass through the membrane pores and is therefore recirculated back into a recirculation tank via the retentate port. Reaction related impurities are small enough to pass through the membrane pores and exit through the permeate port.
- Diafiltration: As the reaction related impurities are being removed the buffer is replaced by the desired basal formulation buffer. The volume of the initial product is defined as the diafiltration volume. Usually, at least 10 diafiltration volumes (DV) are required to remove the reaction related impurities and exchange the buffer. The diafiltration takes place simultaneously to the impurity removal.

0.2 µm filtration

Step 4: Formulation and Fill

Sterile filtration

Bulk Drug Substance

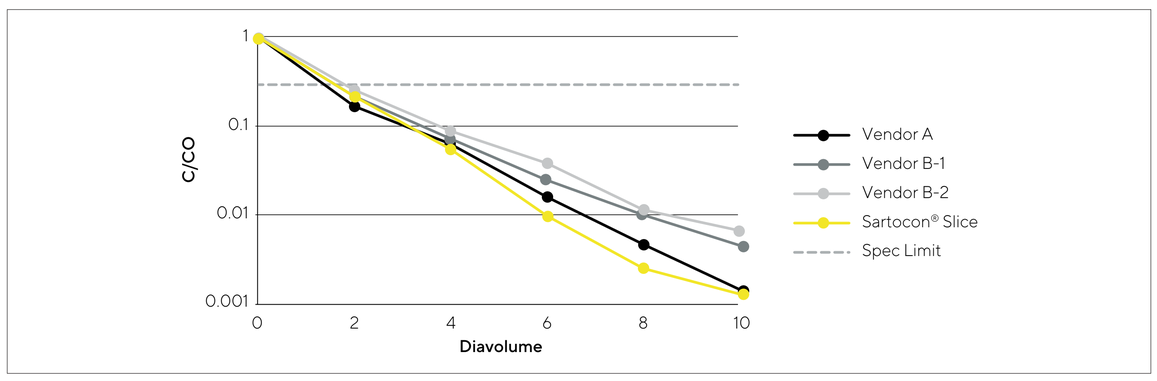
3. Case Study: Hydrosart® Cassettes remove reaction related impurity to lowest level with fastest processing time.

Experiment

A leading ADC company and collaborator of Sartorius evaluated different crossflow cassettes from multiple suppliers. The purpose of the evaluation was to determine which cassette(s) gave the lowest residual reaction related impurities after 10 DVs. Processing time and yield were also evaluated. In total, four different membranes from three separate suppliers were included in the evaluation. From Sartorius Stedim a Sartocon® Slice 200 Hydrosart®, eco channel 30 kd membrane was chosen. The process parameters for all tested crossflow membranes were identical. The membranes were all loaded with approximately 125 g of ADC/m² membrane, the feed rate was 6 L/min/m² and the transmembrane pressure (TMP) was controlled at 1 bar | 15 psi.

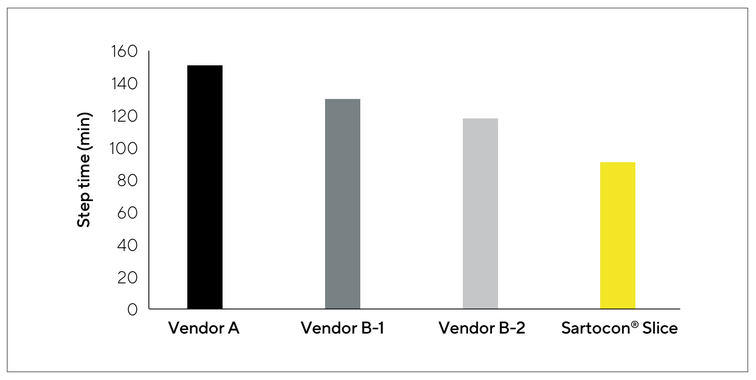
Results

Small Molecule Clearance



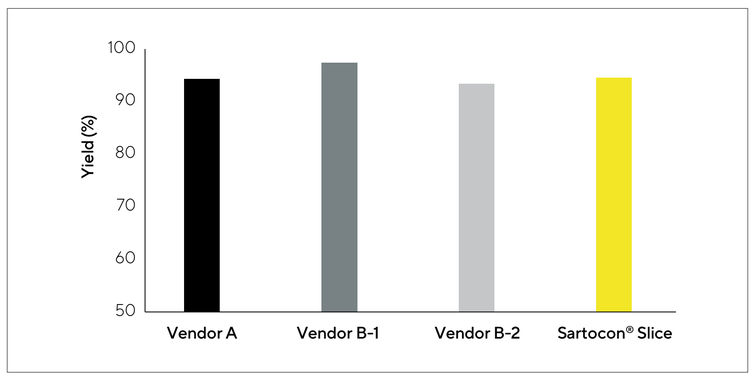
Hydrosart® and Vendor A membrane achieved lowest residual small molecule after 10 (DV).

Step Time



Hydrosart® processing time was significantly faster compared to the other crossflow membranes used in this study.

Step Yield



Step yields are comparable within the normal variation of the assay when comparing the different membranes that were evaluated.

4. Single-Use Crossflow Filtration in Manufacturing Scale

Due to the hydrophobicity of an ADC and the toxicity of the payload, a regenerated cellulose, closed loop system is recommended for ADC crossflow filtration. Hydrosart® is the only stabilized regenerated cellulose membrane on the market that is available as a pre-assembled, pre-sterilized by gamma irradiation and pre-flushed closed loop. Single-use technologies offer big advantages to manufacturers of ADCs as the crossflow flow paths are delivered pre-assembled and pre-sterilized. With this, operator error is reduced and pre and post use cleaning and intensive cleaning validation can be eliminated; thereby minimizing waste containing cytotoxins or cleaning agents and errors during assembly.

To reduce risk of exposure, fully closed, self contained crossflow systems and consumables are recommended. Also a high level of automation is recommended to minimize risk of exposure to operators.