Scale-up of a highly concentrated 200 g/L bulk mAb product

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Introduction

High-concentration antibody drug formulations of over 100 g/L are becoming more common to facilitate subcutaneous administration, which offers benefits to patients. In this study, we ran a complete monoclonal antibody (mAb) purification process at 50 L, achieving a final concentration of 240 g/L. We scaled up to 500 L using our ReadyToProcess™ single-use solutions, achieving a final concentration of 223 g/L while maintaining key quality attributes.

We performed a 3-step chromatography purification process using ReadyToProcess chromatography columns, on ÄKTA pure™ 150 and ÄKTA pilot™ 600 at 50 L scale, while the 500 L scale ran on an ÄKTA ready™ system. The filtration steps below were investigated to a greater extent and optimized (orange steps in Fig 1) before scale-up.

- Depth filtration
- Viral filtration
- Tangential flow filtration (TFF) final formulation
- Single-pass TFF (SPTFF) final concentration to 200 g/L or more
- Final 0.2 µm filtration on Supor™ Prime sterilizing grade filter

Materials and methods

The monoclonal antibody was produced in a fed batch Chinese hamster ovary (CHO) cell cultures in Xcellerex™ XDR-50 and XDR-500 bioreactors. The cultures had a mAb titer of 5.1 and 4.6 g/mL and the maximum cell density was 51 and 48 million cells/mL, respectively.

Mid- and downstream purification was performed according to the process outlined in Figure 1 (500 L scale shown). Optimizations in orange are presented in this poster.

- Depth filtration on 1 m^2 Stax™ modules was performed using coarse PDK7 and fine PDCX depth filters (ratio 2:1). Filtration was done at 50 and 100 LMH. A comparison was done to the Stax mAx platform (PDP8+PDE2) recommendation for mAbs.
- The Mustang™ Q XT flowthrough eluate was virus filtered, at constant pressure in UNICORN™, on a filter train consisting of Pegasus™ Protect prefilter and Pegasus Prime virus filter connected to an ÄKTA pilot 600 at 50 L scale and an ÄKTA ready chromatography system for the 500 L scale.
- We performed the final formulation TFF step on a 30 kDa Delta membrane, 0.5 m^2 on ÄKTA flux™ 6 (50 L scale) and 2.5 m^2 on an ÄKTA readyflux™ 3/8 in. flow kit (500 L scale). The ultrafiltration and diafiltration ran at a transmembrane pressure (TMP) of 1.5 bar and a recirculation feed flux of 360 LMH.
- The final concentration was performed on a 4-in-series SPTFF module consisting of seven 30 kDa Delta membranes (93 cm^2 for the 50 L scale and 0.1 m^2 for the 500 L scale), using a Quattroflow 150S pump. Controlling the retentate flowthrough a valve prevented unstable performance.
- The final sterile filtration was performed at a maximum determined load of 390 L/m^2 when filtering at a flux of 400 LMH.

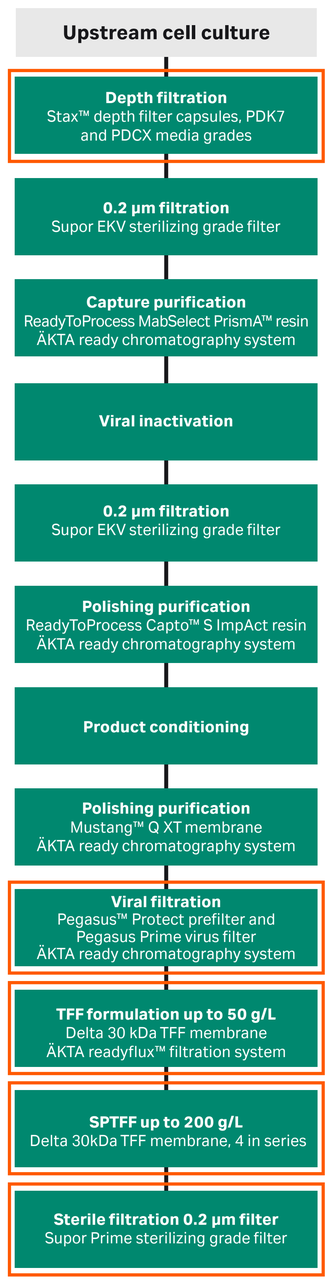

Fig 1. Midstream and downstream purification process.

References

1. cytivalifesciences.com/insights/production-of-a-highly-concentrated-monoclonal-antibody
2. cytivalifesciences.com/insights/scaling-up-production-to-500-l

Results

The results for the steps more thoroughly investigated according to Figure 1 are presented below.

Depth filtration on the tighter option PDK7 and PDCX Stax modules gave a clarified feed with a low turbidity of 9 to 10 FNU (Fig 2) compared to our Stax mAx recommendation for mAbs using PDP8 and PDE2 (expected turbidity in the range of 12 to 16 FNU). The differential pressures (Fig 3) were also quite low during the depth filtration. The total step yield was ~ 90.0% for both scales.

A lower turbidity in the clarified feed may offer a higher protection for the capture column with an increased lifetime.

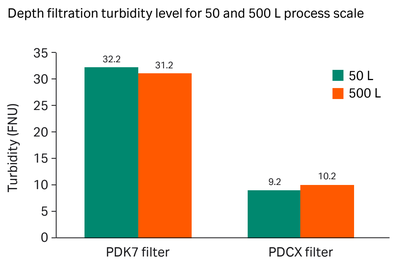

Fig 2. Depth filtration turbidity.

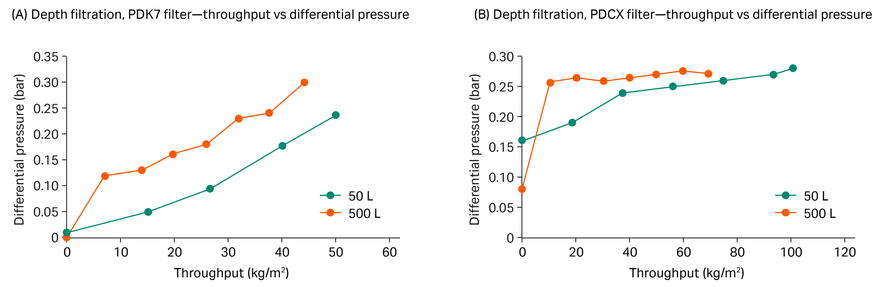

Fig 3. Depth filtration throughput vs differential pressure for primary clarification (A) and secondary clarification (B).

Virus filtration ran at a steady delta filter pressure over the filter train for both process scales (Fig 4). Only a slight decrease in flow occurred over time for the 500 L batch.

Here we have demonstrated that an ÄKTA ready™ system is suitable for running viral filtration and the yield was 97.8% and 99.9%, respectively for the 50 and 500 L scale.

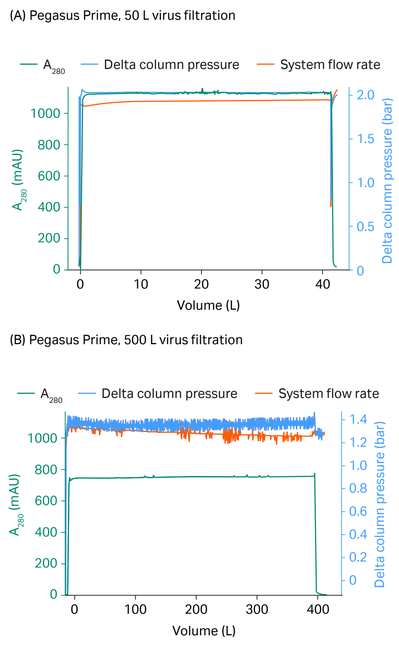

Fig 4. Viral filtration chromatogram showing UV, flow and delta filter pressure, 50 L (A) and 500 L (B).

TFF formulation of the virus filtered product was performed with initial UF of the mAb to 50 g/L followed by DF for 7 turn-over volumes in less than 2.5 h. The TMP and permeate flux is shown in Figure 5 below. The total step yield became 97.6% and 100%, respectively for the 50 and 500 L scale after product recovery. The 500 L scale TFF step was performed in 2 sublots (2).

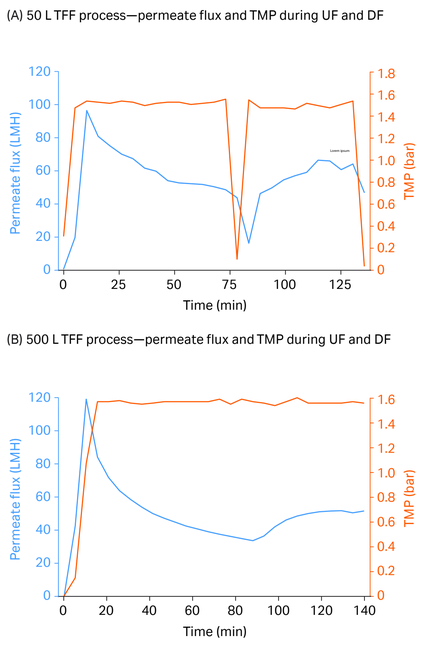

Fig 5. TFF final formulation TMP (orange) and permeate flux (blue), 50 L (A) and 500 L (B).

SPTFF concentration was finally concentrated to 240 and 223 g/L on an SPTFF unit for the two process scales. The overall concentration factor became 5.4 and 5.1, respectively, and the process ran at stable feed and retentate pressures as seen in Figure 6. Three hold-up volumes were used in the end for product recovery. The total step yield was 96.8% and 97.3% for the 50 and 500 L scale.

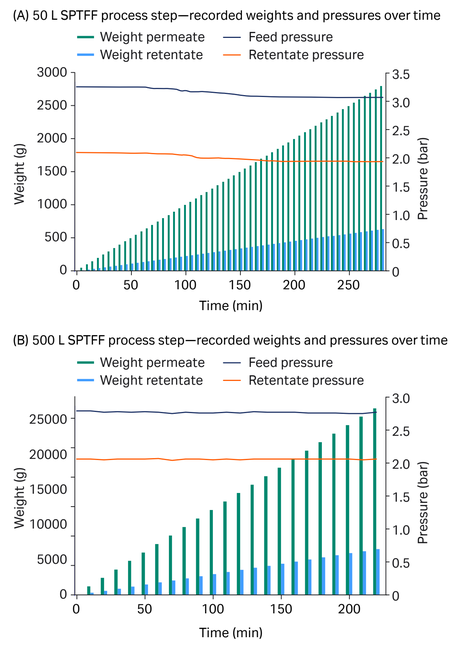

Fig 6. SPTFF process pressures and weight of retentate and permeate, 50 L (A) and 500 L (B).

Sterile filtration was performed on a 240 cm^2 Supor Prime capsule at a flux of 400 LMH. No flow decay was seen and the step yield was 100%.

The final bulk product fulfilled the acceptance criteria listed in Table 1 and both process scales showed reproducible results.

Table 1. Analytical results for the final bulk product

Assay	50 L	500 L	Acceptance criteria
HCP (ng/mg drug product)	15	22	< 100
DNA (ng/mL)	0.002	< 0.001	< 10 ng/dose for mAb (US FDA)
Aggregates (%)	3.3	4.8	mAb-dependent
Charge variants (% main)	38	37	Consistent profile
Titer (mg/mL)	240	223	> 200 g/L
Protein A (ng/mg drug product)	0.5	0.4	Not available
Turbidity (FNU)	2.8	3.8	As measured
Viscosity (cP)	41–43	30–32	As measured
Total process yield (%)	71	75	≥ 60

Conclusions

- Depth filter train of PDK7 plus PDCX filters generated very low turbidity.
- Successful virus filtration using Pegasus Protect and Pegasus Prime as a filter train connected on an ÄKTA pilot 600 and ÄKTA ready chromatography systems at constant pressure for the 50 and 500 L process scale.
- Successful demonstration to formulate the mAb quickly and gently on T-series cassettes with 30 kDa Delta regenerated cellulose membrane on both ÄKTA flux 6 and ÄKTA readyflux at steady TMP.
- Achieved final concentration above 200 g/L on an SPTFF unit with a concentration factor above 5.
- Final filtration on Supor Prime was successful and showed a high capacity for high-concentration mAb formulations at 390 L/m^2.
- The level of aggregate for the 50 L and 500 L scales at 3.3% and 4.8% respectively, is slightly high for a mAb solution but expected given that no optimization of the formulation buffer was performed.
- Successful scale-up using our ReadyToProcess single-use products demonstrated.

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CY57504-05Feb26-PO