Most trace contaminants have an acidic ionic electric potential (pH) and can be removed in a flow through mode of an AEX chromatography. From a hydrodynamic standpoint, membranes offer significantly higher flow rates and therefore smaller bed sizes as compared to packed resins that are diffusion limited (Figure 2a+b).

While flow-through columns are designed based on the flow rate rather than on binding capacity, membrane adsorbers with a more open structure provide a robust alternative. In addition, the disposability of membrane-based tools makes them very interesting also from a cleanliness as well as from a process economy standpoint.

A case study based economic assessment of the single-use membrane chromatography step is a part of this study.

Figure 1: A Generic Purification Strategy

Introduction:
Validated clearance of endogenous and adventitious viruses is essential for the manufacturing of cell culture derived biopharmaceuticals. Orthogonal mechanisms are desirable. In addition, the removal of host cell and process-related contaminants such as DNA, host cell proteins (HCP), endotoxins, dyes and residual affinity ligands like protein A is an inherent challenge of bioprocessing. In this study, a disposable virus and contaminant clearance concept including charged membrane adsorbers and nanofilters is evaluated.

Orthogonality was ensured by the combination of adsorption and size exclusion which justifies additive claims in validation studies. In a generic MAb purification platform a capturing phase with affinity chromatography (AC) followed by cation exchange chromatography (CEX) is established. The membranes, developed are designed to purify an effluent that is sufficient after these initial recovery steps and modern supports are proportionally capable of providing this resolution. In the polishing phase, however, additional safety with regard to pathogen and contaminant virus clearance is obtained with a strong focus on reliability and productivity at the same time.

Figure 1: A Generic Purification Strategy for MAbS

Results and Discussion:
For the flow through membrane chromatography (FT-MC) step, studies were designed to reflect the process scale conditions and performed with a 3.5 m (125 cm²) spiral wound scale down device. LRVs of > 5 were obtained for four model viruses (Figure 3). After elution of the virus fraction, the mass balance could be closed in 3 of 4 cases, providing the adsorptive mechanism of virus removal and excluding any size exclusion effect.

Figure 2a: Hydrodynamic Properties of Membranes vs Resins

As a consequence, a new device with spiral wound geometry and 1 m (36 cm²) bed was introduced recently (data not shown here).

Figure 2b: Size Exclusion Limits of Membranes vs Beads

For the positioning of the Q-FT polishing step in the overall process HCP removal is limiting. The ideal position of this step is therefore directly after a two column process as part of the final filtration train. In principle, the Q-membrane step can also be performed after protein A and applications even before capturing have been seen where the Q-membrane serves as a disposable "pre-column". In this case the requirement for additional membrane area have to be balanced with the overall process cost. In addition to HCP and DNA, removal of endotoxins and residual protein A are common. Initial results are indicating that a phenomenon anecdotally known as "golden antibody" (D. Stoughton, pers. communication) – apparently coming from co-purification of yellow degradation factors – can also be removed in this step (Joe Zhou, data not shown).

Figure 3: Membrane Chromatography Spiking Study with 4 Model Viruses

Overall conclusion: The study demonstrates that a disposable polishing concept can provide > 11 LRV for all virus classes as well as significant DNA and HCP removal and is compatible with a two column process for MAb Manufacturing.

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To further illustrate virus retention as a function of filter blocking, the same sample material was used for a flow decay study of the membrane. PPV and PPA as a surrogate model phage served as spiking material (Figure 7). No significant breakthrough of virus or phage and thus decrease in LRV was detected up to 90% of flow decay.

Figure 7: LRV as a Function of Flow Decay

A detailed cost model was developed to evaluate the use of a two-step chromatography strategy followed by a disposable virus and contaminant clearance platform. With the assumptions of the case study as input parameters, the results indicate that a disposable polishing approach provides overall cost savings of up to 70% at a LRV of 3 log9 (modified study [8]). The disposable MC option benefits from lower capital investment and significantly reduced material costs. Small disposable device volumes translate into 95% less buffer consumption. The breakeven for the use of FT-MC was determined to be above 0.6 kg/m² (2 kg/L). However, with eliminated cleaning, life time and carry-over validation a number of risks are minimized in the disposable option.

Figure 8: Process Economy of a Disposable Polishing Strategy

Table 1: TCID50/ml TCID 50/ml Vol. [ml] reduction (1000)(1000)

Para 3 150 – 300 Ss-RNA Yes 6.9 < 0.6 55 > 6.3
Reo3 60-80 ds-RNA No 5.7 < 0.23 55 > 5.5
Reo-3 75 – 80 5.35 ± 0.23 6.94 ± 0.24 100
Reovirus Type III 75 – 80 7.00 ± 0.31 6.94 ± 0.24 100
Pr: Pseudorabies virus
Run 1 Run 2 Recovery (%)

Flow decay study results for PPV and PPA spiking material. PPV study was performed at Analytik Jena. Test substances are an pathobiotic strain of PPV [3]. PPV solution: 100 mL PBS (0.34 g Na3HPO4, 10 mM NaH2PO4, 0.1 mM CaCl2, 0.6 M KCl; pH 6.5. Operating pressure is 2 bar.

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