Host cell protein removal by Seitz™ PDC3 depth filter after virus inactivation in mAb processing

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Introduction

Host cell proteins (HCPs) are process-related protein impurities from host cells used to produce biopharmaceutical molecules. HCPs have the potential to affect product quality, safety, and efficacy. We can employ multiple steps along the course of purification to remove HCPs, including clarification of post-virus inactivation (VI) solution. Typically, pH adjustment at this step causes precipitate formation, so filtration is needed to remove these precipitates prior to loading onto a polishing chromatography column. By implementing a charged depth filter, we can not only remove the precipitate but also reduce the HCP levels. Our objective for this study was to remove > 90% of HCPs using a Seitz™ PDC3 depth filter, a dual-layer filter consisting of two stages of Seitz Supra EK1P filter.

Materials and methods

We were provided unfiltered batches of post-VI feed material from CHO cell line by InhibRx for filter testing. Viral inactivation occurred between pH 3.5 to 4.0 with a 1 h hold. We then adjusted the post-VI material to pH 5.5. Precipitates were visually observed after the pH was raised, and the filtration was performed at room temperature. Below, we have listed the post-VI feed material characteristics:

- pH adjusted to pH 5.5.
- pI of mAb was pH 9.5, indicating the mAb carried net positive charge at pH 5.5, and was less likely to bind onto filter matrix.
- Initial mAb concentrations were 16.70 mg/mL in test 1 and 15.85 mg/mL in test 2.

Seitz PDC3 depth filter

Seitz depth filters are constructed with a cellulose-based porous matrix integrated with additives and resins. The resins create varying wet strengths and surface charges on specific single-layer filters. Depth filters can be constructed with either a single specific layer of media, or dual layers of those specific single layers. Supra EK1P is a single-layer depth filter with a positively charged filter media sheet. The Seitz PDC3 depth filter is a dual layer depth filter constructed with two layers of positively charged Supra EK1P filter membrane (Table 1).

Table 1. Depth filter specification and operating parameters

Filter grade	Filter media	Retention rating	Effective filtration area	Tested flux (L/m²/h)
Seitz PDC3 (EK1P/EK1P)	Cellulose-based with positive charge	0.2 to 0.5 µm	22 cm²	150

Depth filters were tested using a constant flow method

We used peristaltic pumps to deliver constant flow, flushed the depth filters with 100 L/m² of water at 300 L/m²/h (LMH) and equilibrated with 2 × holdup volumes of buffer. After equilibration, we left the buffer in the depth filters prior to loading the post-VI feed material. When filtration started, the first holdup volume was collected as Fraction 0, which contains mostly buffer. We kept this fraction separate from the filtrate pool. Remaining fractions were collected every 20 L/m². Differential pressure across each filter was monitored and recorded, and we collected the filtrate mass on a balance to reflect volume (assume density as 1 g/mL). We ran the tests until the designated throughput, preferably with a > 90% reduction of HCP.

Results

Test 1: Reduction in HCP by 94% in a feed with 5472 ppm HCP

After VI and pH adjustment, we found that the Seitz PDC3 depth filter reduced 94% of HCPs at 140 L/m² of throughput. In total, Seitz PDC3 depth filter (22 cm²) removed 2.5 x 10^7 ng of HCPs from the solution at 140 L/m² of throughput, while maintaining 96.5% of mAb recovery. Post-filtration buffer flushing could further increase the total mAb recovery.

Table 2. Test 1 HCP reduction percentage, HCP removal capacity, and mAb recovery using the Seitz PDC3 depth filter at different throughput

Sample	mAb recovery (%)	Pool HCP (ppm)	Total HCP input (ng)	Total HCP output (ng)	HCP reduction (%)	HCP removal capacity (ng/m²)	Achieved throughput (L/m²)
Pre-Seitz PDC3 filter	100	5472	0	0	0	0	-
Post-Seitz PDC3 filter - 1	-	138	1.9 x10^7	5 × 10^5	97	8.4 × 10^9	100
Post-Seitz PDC3 filter - 2	-	210	2.3 × 10^7	9 × 10^5	96	1 × 10^{10}	120
Post-Seitz PDC3 filter - 3	96.5	333	2.7 × 10^7	1.6 × 10^6	94	1.5 × 10^{10}	140

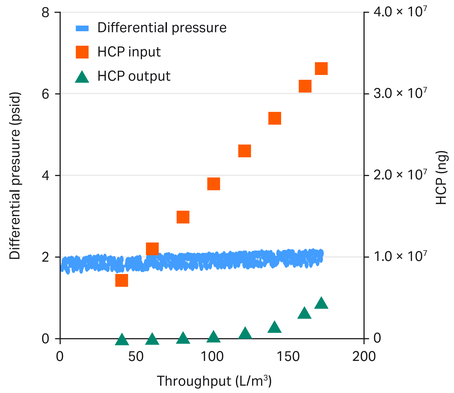


Fig 1. Post-VI clarification analysis for Test 1 using the Seitz PDC3 filter, showing differential pressure and HCP total input/output in relation to throughput.

Test 2: Reduction in HCP by 98% in a feed with 3551 ppm HCP

After test 1, a few parameters were slightly modified by InhibRx at the harvest and clarification steps, prior to conducting the second post-VI clarification test (Table 3). In this test, we found the Seitz PDC3 depth filter decreased 94% of HCPs at 200 L/m² of throughput, and this could be increased to 98% HCP removal at 140 L/m² of throughput.

In total, the Seitz PDC3 depth filter (22 cm²) removed 2.1 × 10^7 ng of HCPs from the solution at 200 L/m² of throughput, while maintaining 92.1% of mAb recovery. A post-filtration buffer flush could further increase the mAb recovery.

Table 3. Test 2 HCP reduction percentage, HCP removal capacity, and mAb recovery using the Seitz PDC3 depth filter at different throughput

Sample	mAb recovery (%)	Pool HCP (ppm)	Total HCP input (ng)	Total HCP output (ng)	HCP reduction (%)	HCP removal capacity (ng/m²)	Achieved throughput (L/m²)
Pre-Seitz PDC3 filter	100	3551	0	0	0	0	-
Post-Seitz PDC3 filter – 1	94.4	81	1.6 × 10^7	4.0 x 10^5	98	7.3 × 10^9	140
Post-Seitz PDC3 filter – 2	92.4	126	1.8 × 10^7	7.0 x 10^5	97	8.0 × 10^9	160
Post-Seitz PDC3 filter - 3	92.1	240	2.3 x 10^7	1.5 x 10^6	94	9.7 × 10^9	200

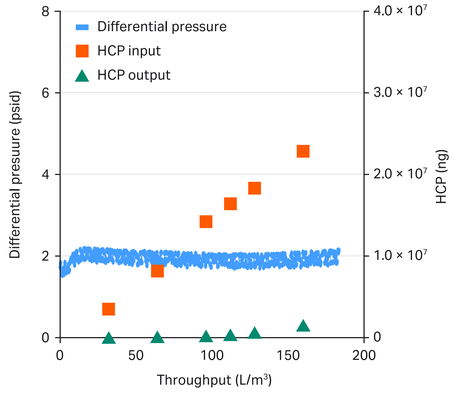


Fig 2. Post-VI clarification analysis for Test 2 using the Seitz PDC3 filter, showing differential pressure and HCP total input/output in relation to throughput.

Conclusions

- The Seitz PDC3 depth filter decreased HCP levels in a post-VI mAb solution by 94% to 98%, while delivering a throughput of 140 to 200 L/m² for the post-VI feed materials.
- This study indicates that the Seitz PDC3 depth filter can potentially remove approximately 9 g of HCPs per square meter of filter membrane.
- By eliminating the majority of HCPs during this post-VI clarification stage, the load on subsequent polishing chromatography steps can be greatly reduced.

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