Viral Clearance Capability of POROS Hydrophobic Interaction Chromatography resins

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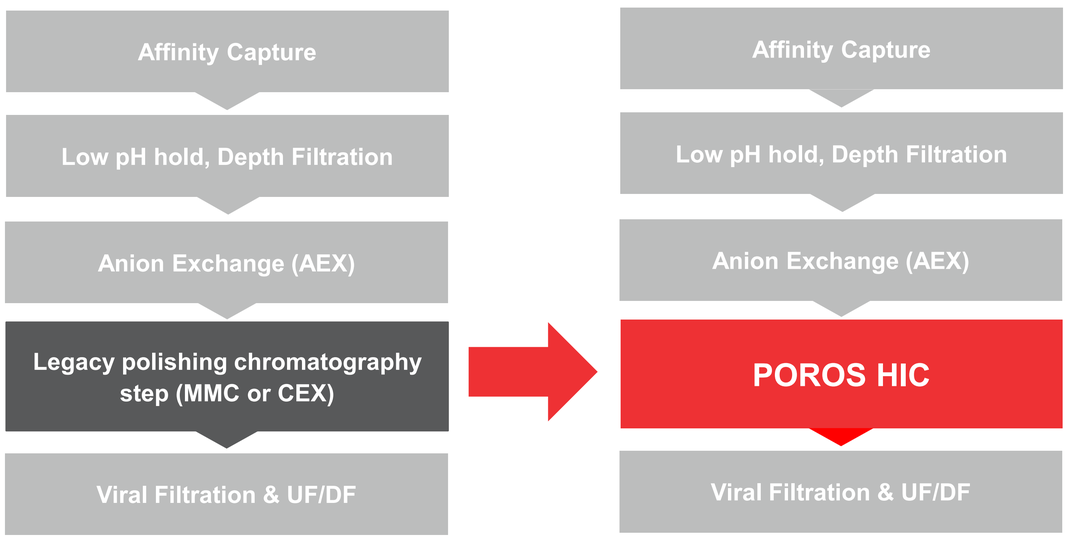
INTRODUCTION

Product-related impurities and viral contamination are major concerns in the production of biologics. In a previous study, we demonstrated how Thermo Scientific™ POROS™ Hydrophobic Interaction Chromatography (HIC) resins, used either in bind and elute (B/E) or flow-through (FT) mode, are an efficient alternative to cation exchange resins (CEX) for monoclonal antibody aggregate removal and process productivity improvement. Additionally, next to specific viral reduction steps, POROS HIC resins can contribute to the overall viral clearance of the process and help reach the 12 LRVs (Log Reduction Value) required by regulatory agencies.

The present study highlights the viral clearance performance of POROS HIC resins, either in FT or B/E mode, in the purification process of two monoclonal antibodies.

STUDY DESIGN

The viral clearance study was conducted on two monoclonal antibodies (mAb A and mAb B), routinely polish-purified on a Mixed-Mode resin for mAb A and on a CEX resin for mAb B. When the anion exchange step is performed, both mAbs still exhibit a high level of aggregates (~10%). On the legacy processes, mAb A and B were loaded at 6 minutes residence time, respectively at 25 and 45 g/L resin. In the present study, legacy chromatography polishing steps were substituted by POROS HIC, as depicted below:



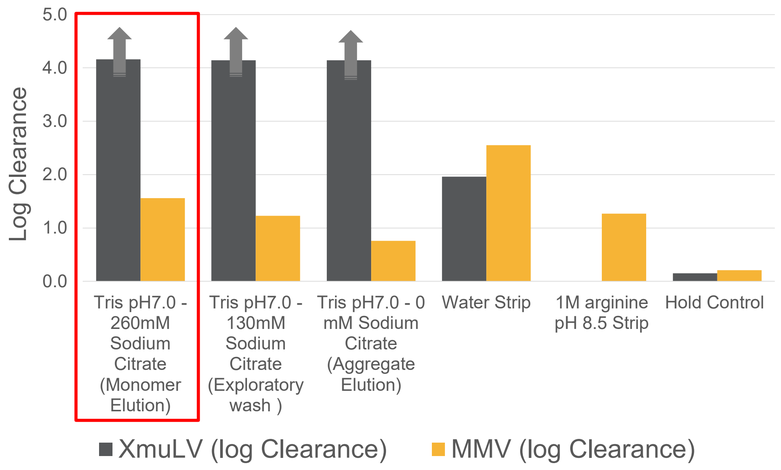
The study was performed according to the matrix below (see table). mAb A was evaluated in B/E mode on Benzyl and in FT mode on Benzyl Ultra, while mAb B was processed in B/E more on Ethyl and Benzyl Ultra.

Thermo Scientific™ POROS™ HIC resin	Resin hydrophobicity	mAb A	mAb B
POROS™ Ethyl	+	-	B/E (exp#3)
POROS™ Benzyl	+++	B/E (exp#1)	-
POROS™ Benzyl Ultra	+++++	FT (exp#2)	B/E (exp#4)

In each experiment, the feedstream was spiked with Mouse Minute Virus (MMV – non-enveloped virus) and Xenotropic Murine Leukemia-related virus (XMuLV – enveloped virus). Flowthrough, wash, elution and strip fractions were eventually assessed for viral content to calculate resulting LRVs. Fractions containing the product of interest are circled in red.

PROCESS OPTIMIZATION FOR mAb A – VIRAL CLEARANCE

Exp#1: POROS BENZYL IN B/E MODE

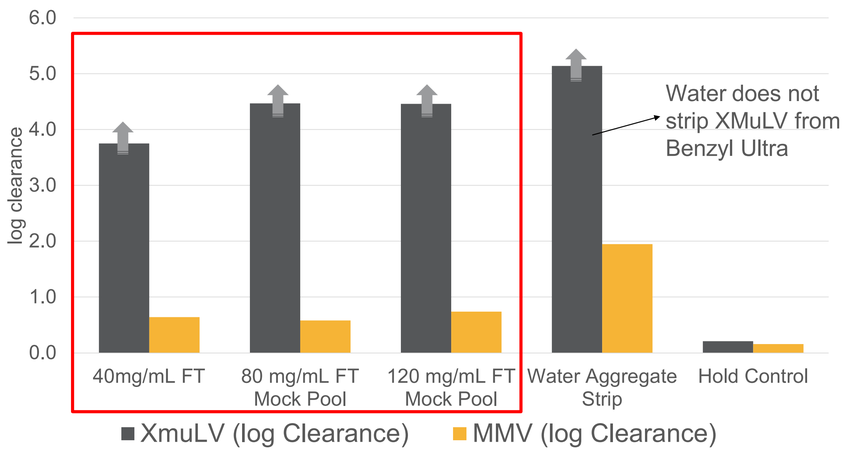


Process details

Column: 0.8x10 cm / CV = 5 mL
Load Buffer: Tris pH7, 575 mM NaCitrate – MabA at 3 mg/mL
Flow rate: 2.5 mL/min
Residence time: 2 min
Load density: 32 mg/mL

- ✓ **Complete XMuLV (>4 LRV) clearance in mAb A pool**
- ✓ **Partial MMV clearance in mAb A pool**
- ✓ **Water partially and Arginine fully strips XMuLV from POROS Benzyl**

Exp#2: POROS BENZYL ULTRA IN FT MODE



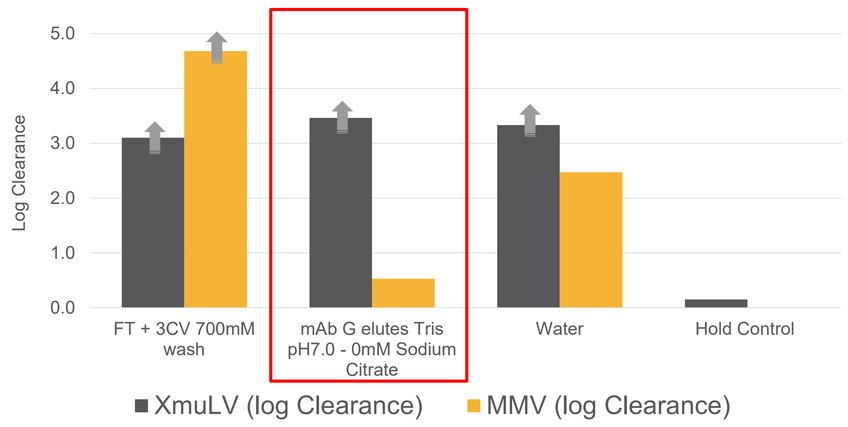
Process details

Column: 0.5x5 cm / CV = 1 mL
Load Buffer: Tris pH7, 2mS/cm – MabA at 2.5 mg/mL
Flow rate: 0.5 mL/min
Residence time: 2 min
Load density: up to 120 mg/ml

- ✓ **Complete XMuLV (>4 LOG) clearance in mAb A Flow-Through**
- ✓ **Minimal MMV (~0.5 LOG) clearance in mAb A Flow-Through**

PROCESS OPTIMIZATION FOR mAb B – VIRAL CLEARANCE

Exp#3: POROS BENZYL ULTRA IN B/E MODE

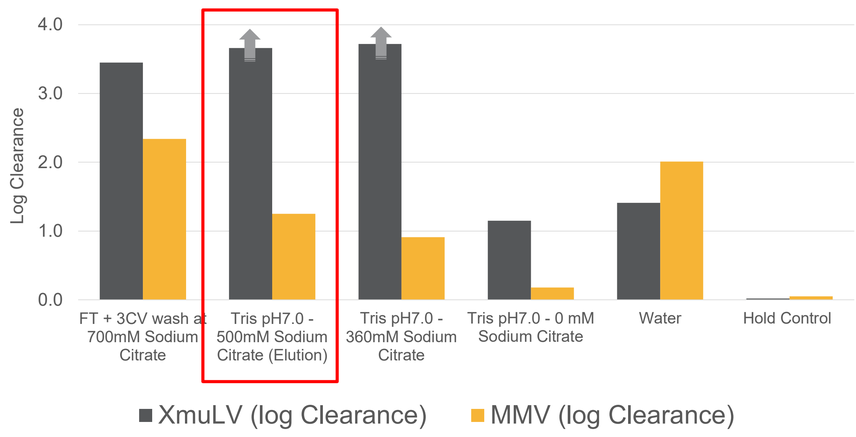


Process details

Column: 0.8x10cm / CV = 5mL
Load Buffer: Tris pH7, 575mM NaCitrate – Mab B
Flow rate: 2.5 mL/min
Residence time: 2 min

- ✓ **Complete XMuLV (>3 LRV) clearance in mAb B elution pool**
- ✓ **Minimal MMV (~0.5 LRV) clearance in mAb B elution pool**
- ✓ **Buffer with no salt elutes MMV**

Exp#4: POROS ETHYL IN B/E MODE



Process details

Column: 0.8x10cm / CV = 5mL
Load Buffer: Tris pH7, 575mM NaCitrate – Mab B
Flow rate: 2.5 mL/min
Residence time: 2 min

- ✓ **Complete XMuLV (>3.5 LRV) clearance in mAb B pool**
- ✓ **Partial MMV (1.3 LRV) clearance in mAb B pool**
- ✓ **Buffer with no salt elutes MMV and XMuLV**

CONCLUSIONS

- **Complete XMuLV clearance (> 3.5 LRV) on all POROS HIC resins in both Bind/Elute (Benzyl & Ethyl) as in flow-through mode (Benzyl Ultra)**
- **Partial MMV clearance due to lower hydrophobicity of the virus**
- **Despite moderate MMV log reduction, these data indicate MMV log reduction could be improved with further optimization**

TRADEMARKS/LICENSING & INTENDED USE

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