Optimal Multimodal Chromatography (MMC) Resin Enhanced Host Cell Proteins (HCPs) Reduction during Monoclonal Antibody Purification

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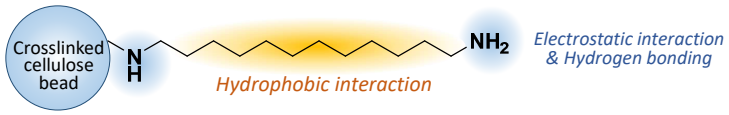
Abstract

JNC has developed a novel MMC resin for post rProtein A mAb capture polish processing to remove HCPs. Cellulose beads with various pore sizes were coated with the unique ligand comprised of long alkyl chain and primary amines. The impact of pore size was evaluated for HCPs removal in mAb flow-through polishing format and demonstrated that a larger pore size bead architecture was able to remove HCPs from higher mAb loading samples. Results from this study show that an optimized MMC resin has the potential to remove HCPs from rProtein A elution fractions more efficiently in flow-through mode processing.

Introduction

Host cell proteins (HCPs) are known as process related impurities of biopharmaceutical products such as monoclonal antibody (mAb) cultured in Chinese hamster ovary (CHO) cell culture. Some HCPs show unwanted immune responses themselves, and others have enzymatic activities which have the potential to degrade product molecules. An efficient HCP removal polishing step post rProtein A capture in downstream is required. Multimodal chromatography (MMC) resins are increasingly being used for mAb purification polishing processes because of their efficient removal of HCPs and mAb aggregates from post rProtein A elution fractions. In our previous study, the ligand comprised of long alkyl chain and primary amines showed excellent HCPs removal (Figure 1). In this poster, we will describe the impact of base beads pore size on HCPs removal during mAb purification process using a flow-through polishing format. In addition, we will demonstrate the HCPs removal efficacy of various loading condition with the optimized MMC prototype resin.

Figure 1 MMC prototype structure



Impact of pore size of base matrix on HCP capacity

The impact of base bead pore size was examined with five different crosslinked cellulose beads whose surface was coated with the MMC ligand. Prototype 5 has over 1 µm continuous pore structure (Figure 2). 1010 mg/mL-resin of mAb was applied to 0.3 mL pre-packed columns and breakthrough peak behavior of HCPs monitored (Figure 3). A summary of mAb purification and removal of HCP is shown in Table 2.

Figure 2 SEM images of base matrices

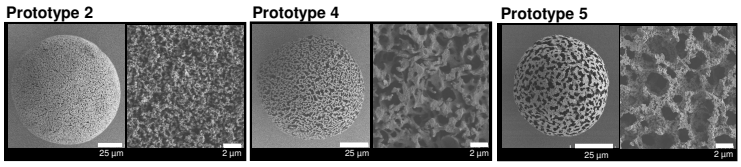
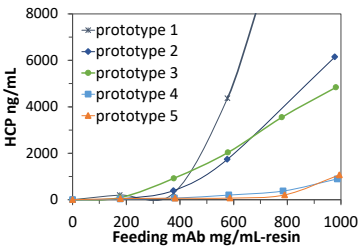


Figure 3 Breakthrough curves of HCPs.



mAb purification procedure
System: AKTA avant25 (Cytiva), Column: Tricorn 5/20 (5 mm ID, 15 mm L, 0.3 mL, Cytiva), Flow rate: 0.075mL/min (Residence time 4 min), Loading buffer: 20 mM Tris-HCl, NaCl, pH7.0, 6.0 mS/cm, mAb solution: 10 mg/mL in loading buffer after protein A purification (Cellufine SPA-HC, JNC) and virus inactivation (pH3.4, 1h), Feed volume: 101 CV (1010 mg mAb/mL resin), FT and column wash fractions are collected and analyzed.
Analysis
mAb concentration: 280 nm absorption (Bio spec nano, Shimazu),mAb monomer purity: HPLC-SEC (TSKgel SuperSW mAb HR), HCPs concentration: CHO HCP ELISA kit 3G (Cygnus)

Table 1 Summary of mAb purification in flow-through study.

	Average particle diameter (µm)	Estimated pore size from iSEC (nm)	mAb monomer purity in feed (%)	HCPs in feed (ppm)	mAb monomer purity in FT pool (%)	mAb monomer yield (%)	HCPs in FT pool (ppm)
Prototype 1	87	132	97.5	2421	97.9	97.3	297
Prototype 2	89	252	97.2	2474	98.3	99.7	133
Prototype 3	89	410	97.2	2474	98.1	100.5	99
Prototype 4	91	444	97.2	2474	97.6	100.1	7
Prototype 5	81	849	97.2	1718	97.7	99.4	8

✓ **Prototype 4 and 5 showed excellent HCPs clearance and high % recovery of mAb monomer as seen by SEC analysis at an initial load of 1000 mg of post-rProtein elution fraction.**

Influence of Loading condition

Various loading conditions regarding residence time and conductivity were examined using prototype 5 to investigate the HCP-reduction dependence on loading condition in flow-through.

Figure 4 Influence on loading conditions (left: processing residence time, right: conductivity of mAb solution) for HCPs removal ability of prototype 5.

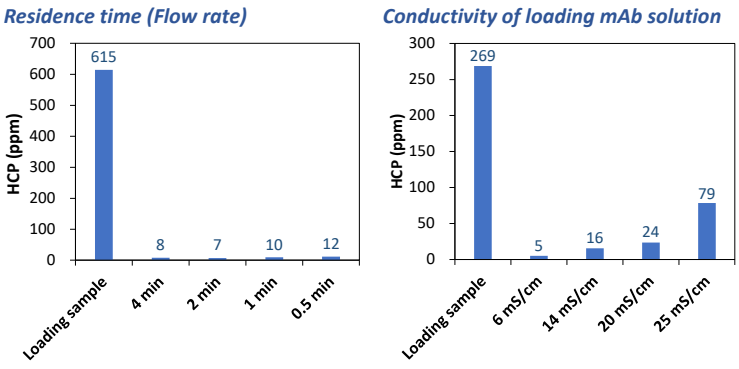


Table 2 Summary of mAb purification with prototype 5 under various loading (600 mg-mAb/ mL-resin applied)

Conductivity (mS/cm)	Residence time (min)	mAb monomer purity in feed (%)	HCPs in feed (ppm)	mAb monomer purity in FT pool (%)	mAb monomer yield (%)	HCPs in FT pool (ppm)
6	4.0	98.2	615	98.0	99.4	8
6	2.0	98.2	615	97.7	99.5	7
6	1.0	98.2	615	98.2	99.3	10
6	0.5	98.2	615	97.6	98.4	12
6	4.0	98.8	269	99.4	99.1	5
14	4.0	98.8	269	99.3	101.6	16
20	4.0	98.8	269	98.7	99.0	24
25	4.0	98.8	269	98.6	101.0	79

✓ **The HCP removal ability of prototype 5 is independent of residence time owing to its large continuous pore structure.**
✓ **The loading conductivity influences the HCPs reduction because the ligand has the electrostatic interaction moieties.**

Comparison to commercially available MMC resins

The new MMC resin was compared with commercially available resins.

Figure 6 Breakthrough curves of HCPs in the comparison study

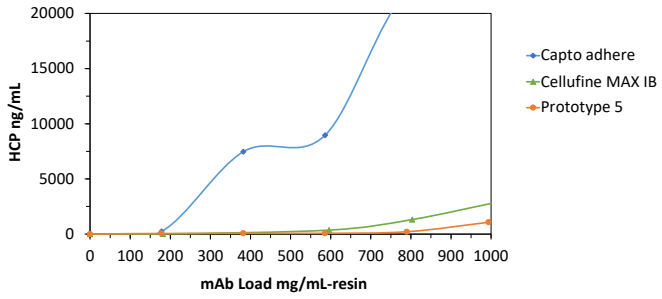


Table 3 Summary of comparison between prototype 5 and commercial resins in mAb purification (1010 mg-mAb/1 mL-resin applied)

	mAb monomer purity in feed (%)	HCPs in feed (ppm)	mAb monomer purity in FT pool (%)	mAb monomer yield (%)	HCPs in FT pool (ppm)
Prototype 5	97.2	1718	97.7	99.4	8
Cellufine MAX IB	96.7	2229	97.8	96.3	59
Capto adhere	97.2	1718	97.6	95.6	513

✓ **Prototype 5 removed HCPs more efficiently than commercial MMC resins**

Conclusion

✓ **JNC has developed a MMC resin with a unique ligand and pore structure for retaining HCPs in a post rProtein A mAb capture polishing step.**
✓ **The pore size influenced the HCP retention ability of the MMC resin.**
✓ **Large pore structure enabled reduced processing time.**

Reference: C. Mori *et al., Journal of Chromatography A*, 1732 (2024) 465202.
Learn about Cellufine™ technologies at https://www.jnc-corp.co.jp/fine/en/cellufine/