Optimizing Reusability and HCP Reduction: Cellufine MLP^{TM} MMC in Monoclonal Antibody Purification

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1. Abstract

JNC has developed a novel MMC medium utilizing cellulose beads with pore structures exceeding 1 µm (Cellufine MLP™) and a ligand composed of primary amines and a long alkyl chain. Although the MMC prototype has demonstrated high HCP removal and mAb recovery in a flow-through polishing format, it has been challenging to remove strongly bound molecules from the medium using a 0.5 M NaOH clean-in-place (CIP) step. In this study, we focused on improving the reusability of the MMC prototype while maintaining enhanced HCP removal by modifying the ligand structure. We discovered that modification of the interface between the base resin and the ligand is important for improving media reusability. The results indicate that the optimized MMC ligand and unique base bead with large pore structures have the potential to enhance the mAb purification process while maintaining efficient HCP removal.

2. Introduction

Mixed-mode chromatography (MMC) media are widely used for downstream processing in monoclonal antibody (mAb) production due to their efficient ability to remove impurities such as host cell proteins (HCPs) and mAb aggregates. In our previous study, a ligand composed of a long alkyl chain and primary amines (Figure 1) showed excellent HCP reduction in flow-through mAb polishing. Moreover, the pore size of the base bead influences the resin's HCP retention ability, and beads with pore diameters greater than 500 nm demonstrated high-level HCP removal even with over 1 g of mAb loaded per 1 mL of column (Table 1, Figure 3). We have now developed a novel MMC medium using our latest Cellufine MLP, which has a pore size exceeding 1 µm as its base matrix (Figure 2).

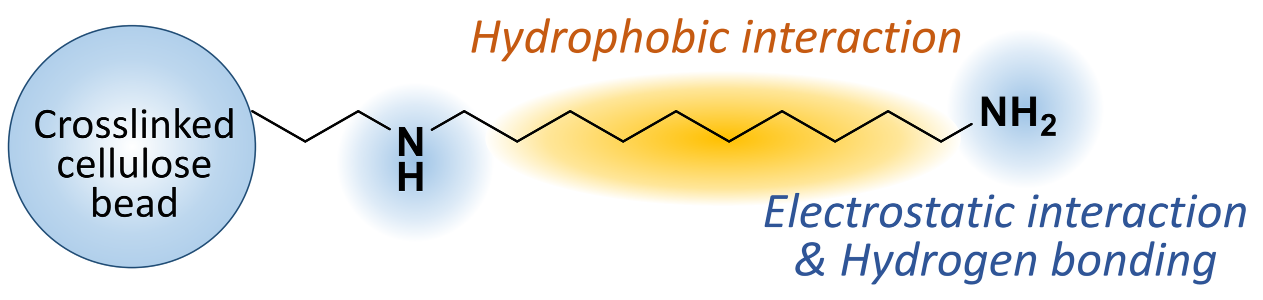


Figure 1: MMC prototype structure

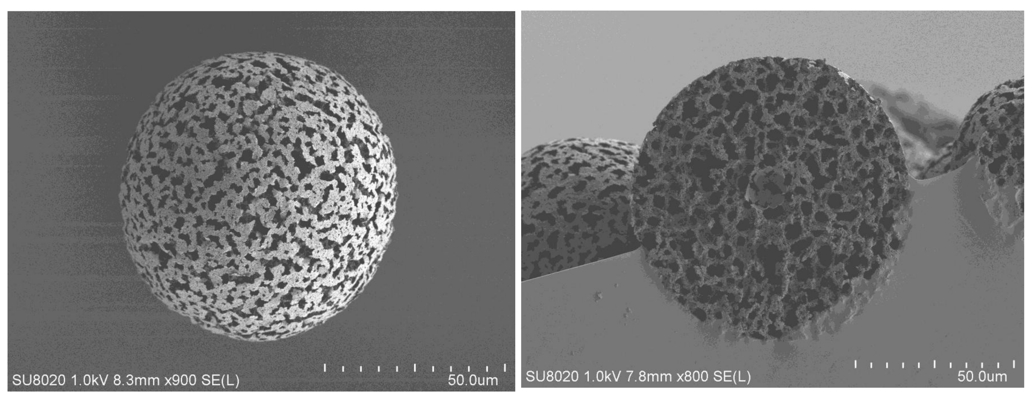


Figure 2: SEM images of Cellufine MLP

Table 1: Summary of comparison between MMC prototype with different pore size and commercial resins in mAb purification (1010 mg-mAb/1 mL-resin applied, pH 7.0, 6 mS/cm)

	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)
MLP MMC prototype	849	97.2	1718	97.7	99.4	8
Cellufine MAX IB	86	96.7	2229	97.8	96.3	59
Capto adhere	no data	97.2	1718	97.6	95.6	513

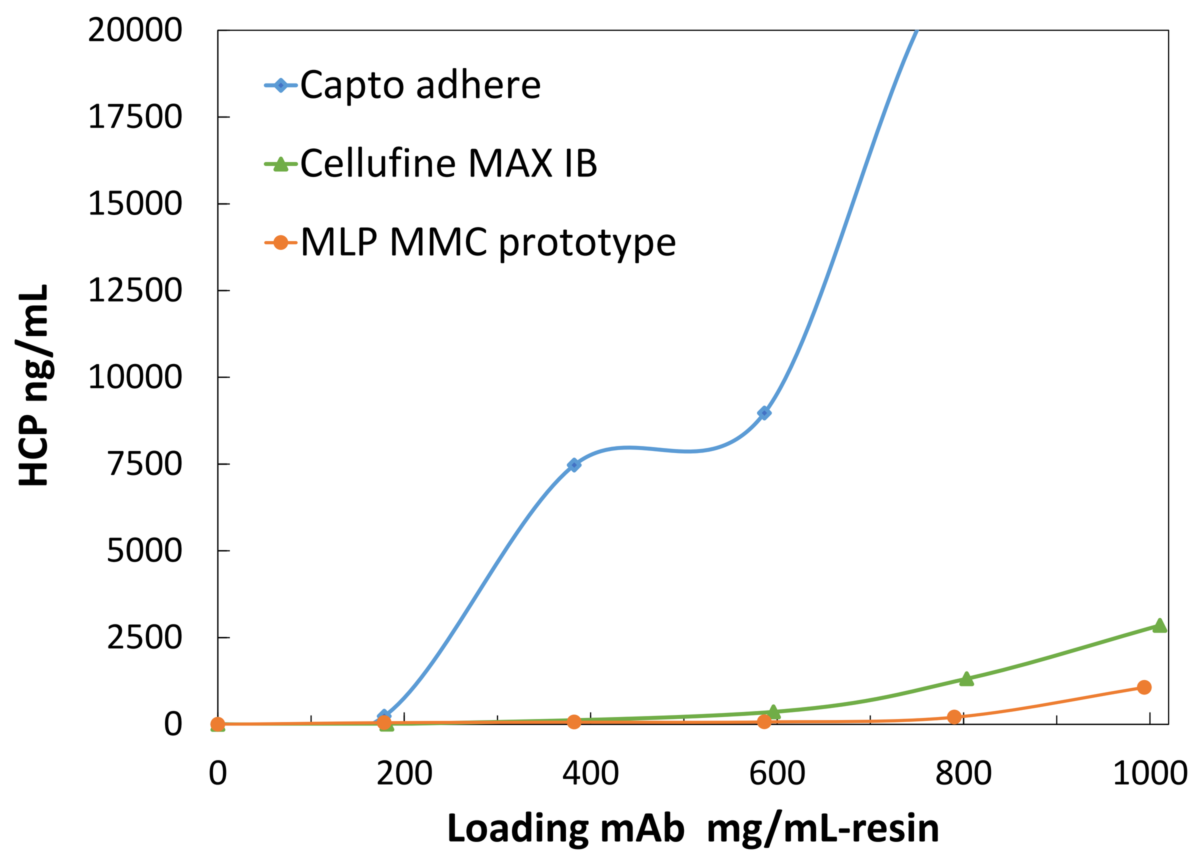


Figure 3: Breakthrough curves of HCPs in the flow-though mAb polishing study

3. Resin Reusability Study of MLP MMC Prototype

Although our MLP MMC prototype shows excellent performance in mAb purification, the reusability of the resin is low because strongly bound proteins are difficult to remove during the clean-in-place (CIP) step. We conducted a CIP study using γ-globulin from human serum as a model protein. The DBC value decreased gradually by repeated column usages (Figure 4).

CIP study

Chromatography conditions

System: AKTA avant25, Column: Super Edge 1mL mini column (6.7 mmID × 30 mmH = 1 mL), Resin: MLP MMC prototype, Flow rate: 0.5 mL/min (CIP step: 0.25 mL/min)

Step	Solution	Volume (CV)
Equilibrium	20 mM Tris-HCl, 100 mM NaCl, pH7.5	10
load	10 mg/mL γ-globulin from human serum	25
wash1	20 mM Tris-HCl, 100 mM NaCl, pH7.5	10
elution	20 mM Tris-HCl, 2 M NaCl, pH7.5	10
wash2	0.1 M AcOH	5
wash3	20 mM Tris-HCl, 100 mM NaCl, pH7.5	3
CIP	0.5 M NaOH	5
Equilibrium	20 mM Tris-HCl, 100 mM NaCl, pH7.5	20

(a) Chromatogram of the 0.5 M NaOH CIP study

(b)10% DBC of γ-globulin

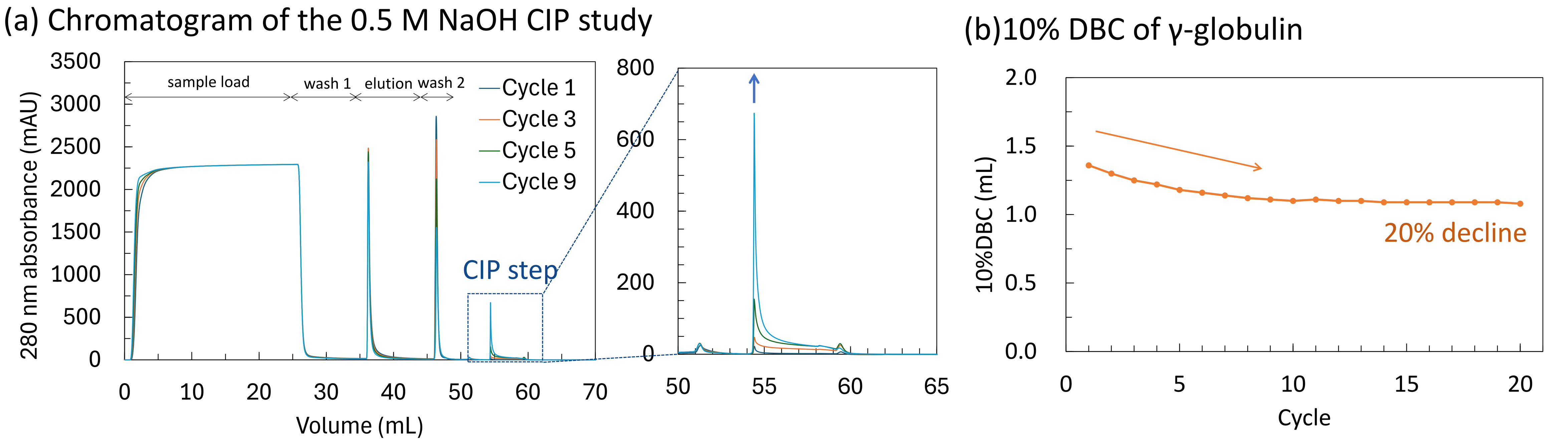
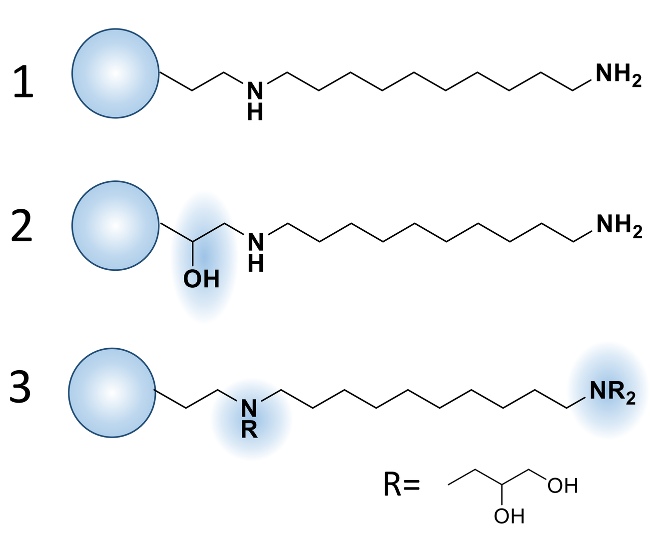


Figure 4: Results of the CIP study of MLP MMC prototype (a) Chromatograms of cycle 1, 3, 5, 9 with 0.5 M NaOH CIP, (b) 10% DBC of each cycle

4. Optimization of Ligand Structure

The impact of ligand structure on resin reusability was investigated by conducting CIP studies using γ-globulin. The mAb purification performance of prototypes 1, 2, and 3 was also evaluated using a monoclonal antibody solution derived from CHO cells after the protein A capture process.

MMC prototypes



1. CIP study

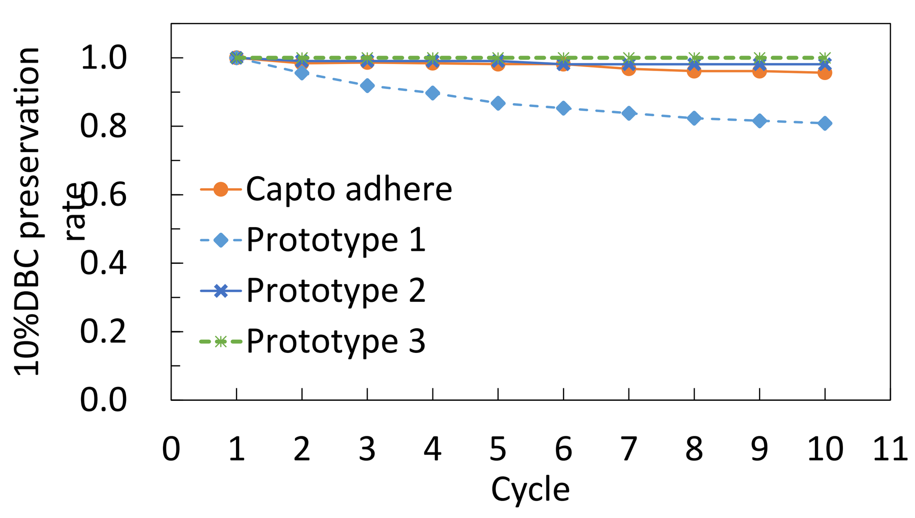


Figure 5: Results of the CIP study of the MLP MMC prototype 1, 2, 3, and Capto adhere.

2. mAb purification performance

Table 2: Summary of comparison among MMC prototypes in mAb purification (1010 mg-mAb/1 mL-resin applied)

	mAb purity (%)	mAb yield (%)	HCPs (ppm)
Loading	98.2	-	4201
Prototype 1	98.0	100	27
Prototype 2	98.1	100	33
Prototype 3	98.4	100	104

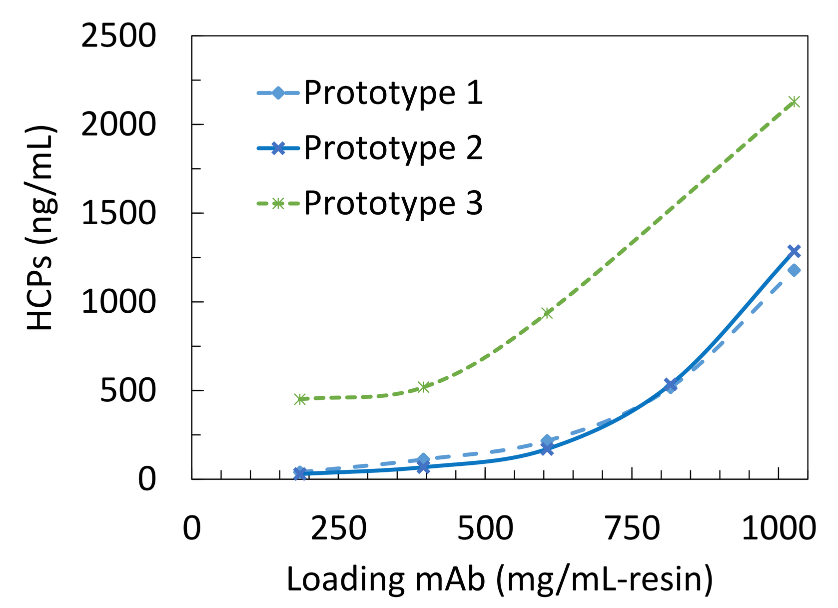


Figure 6: Breakthrough curves of HCPs in the flow-though mAb polishing study

✓ Prototype 2 achieved improved reusability while maintaining excellent mAb purification performance.

5. Conclusion

JNC found that the classification of amine and the interface structure between the base resin and the ligand of the MMC medium influence the resin's reusability. The primary amine structure was found to be essential for maintaining mAb purification performance. These results demonstrate that the optimized MMC ligand structure holds promise for robust and efficient mAb purification.

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Reference: C. Mori *et al., Journal of Chromatography A*, 1732 (2024) 465202.

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