Efficient Separation of Antibody Light Chains from Bispecific Antibody Monomer Using Mixed-Mode Sorbents

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INTRODUCTION

Bispecific Antibodies

- One of the most promising classes of next generation therapeutic molecules
- Growing number of such products expected on the market
- Unique challenges in purification compared to monoclonal antibodies (MAbs)

Present Study

- Use of mixed-mode sorbents for the purification of bispecific antibodies, focusing on the separation of low molecular weight (LMW) species, especially MAb light chains (LC) from monomeric form
- Evaluation of three mixed-mode sorbents: MEP, HEA and PPA HyperCel™ for purification of a kappa-lambda (κλ)-bispecific monoclonal antibody (MAb) kindly supplied by NovImmune (Switzerland)
  - *Determination of dynamic binding capacity (DBC) for capture of MAb*
  - *Screening of pH elution conditions on column to separate LMW species from MAb monomer*

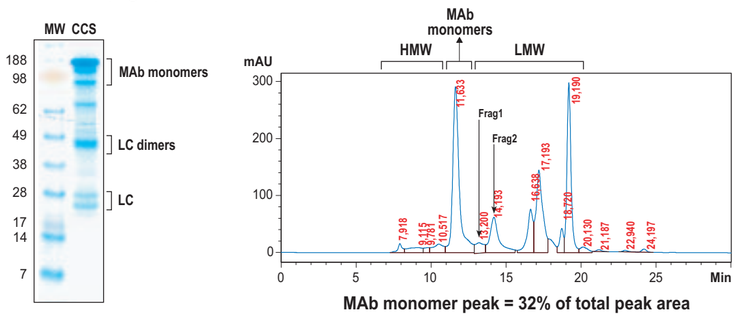
MAb FEEDSTOCK PROPERTIES AND PERFORMANCE EVALUATION APPROACH

- Significant amount of LMW species in cell culture supernatant (CCS): Challenge in monomer purification

Table 1
Properties of CCS Containing κλ-Bispecific MAb

Conditions	MAb Monomer	HCP
pH 7.4, 15 mS/cm	2 mg/mL	393,000 ppm

Figure 1
Characterization of MAb Fragments in CCS with Non-Reducing SDS-PAGE (Left) and SEC HPLC (Right)



HMW = High molecular weight species, LMW = Low molecular weight species, LC = MAb light chain

Figure 2
Sorbent Performance Evaluation Approach

Operating Conditions
- **Column: PRC prepacked column 5x50 (~1 mL)**
- **Residence time: 4 minutes (0.25 mL/min)**

DBC Evaluation
- **Direct load of concentrated CCS**
- **Capacity determined at 10% of MAb breakthrough**

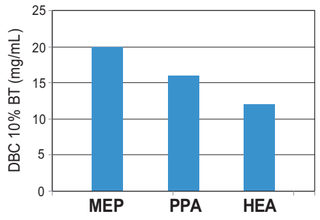
Elution Optimization
- **Load: Concentrated CCS, 5 mg MAb/mL sorbent**
- **pH steps: 6 to 3 with 0.5 units decreasing steps**

Purification Runs in Optimized Conditions
- **Load: Concentrated CCS, 60% of DBC**
- **Wash and elution steps at optimal pH determined**

Analytical Methods
- **MAb monomer quantification: Protein A HPLC** (Poros* A column, *Life Technologies)*
- **Evaluation of light chains content: SEC HPLC** (TSKgel* G3000SW$_{XL}$ column, *Tosoh)*
- **Qualitative evaluation of protein content: SDS-PAGE** (4-12% Bis-Tris, *Invitrogen)*

EVALUATION OF THE DBC OF MIXED-MODE SORBENTS

Figure 3
Evaluation of the DBC of HyperCel Mixed-Mode Sorbents



- **Good DBC obtained with MEP and PPA HyperCel sorbents**

ON-COLUMN OPTIMIZATION OF ELUTION CONDITIONS FOR LMW SPECIES ELIMINATION

Screening of Elution pH for LMW Species Elimination

Figure 4
Chromatograms of Elution Optimization by pH Steps on the Three Mixed-Mode Sorbents

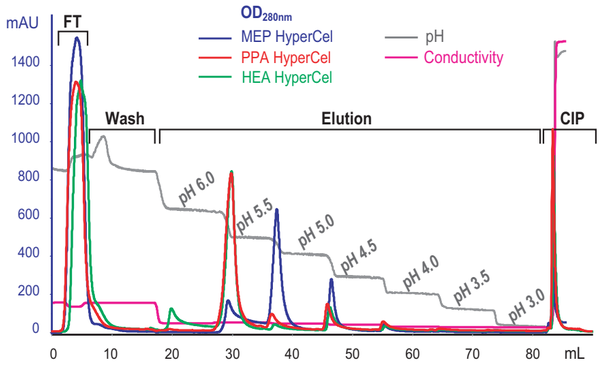
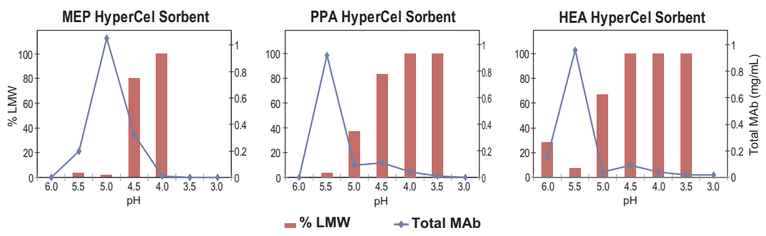


Figure 5
Total MAb and LMW Species Content According to Elution pH Steps for MEP, PPA and HEA HyperCel Sorbents



- **Separation of LMW species according to pH (LMW eluted at lower pH)**

Conclusions on the Optimization of MAb Capture with Mixed-Mode Sorbents

Table 2
Summary of Mixed-Mode Sorbent Performance During MAb Purification Optimization

HyperCel Sorbents	DBC (mg/mL)	Optimal Elution pH	Monomer Purity (%)
MEP	20	5.0	96.2
PPA	16	5.5	96.7
HEA	12	5.5	92.6

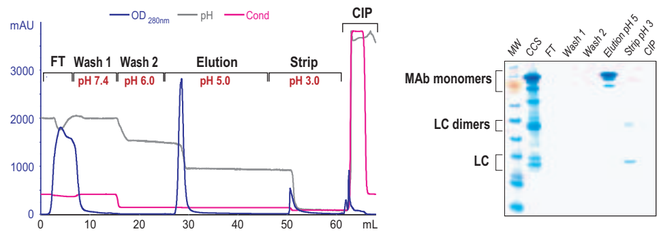
- **MEP HyperCel sorbent:** Best sorbent for DBC and elimination of LMW species
- **PPA HyperCel sorbent:** Good alternative for elution at mild pH

CAPTURE OF κλ-BISPECIFIC ANTIBODY USING OPTIMIZED CONDITIONS ON MIXED-MODE SORBENTS

MEP HyperCel Sorbent

- Specific elution of MAb monomers at pH 5.0, LMW species in pH 3.0 strip fraction

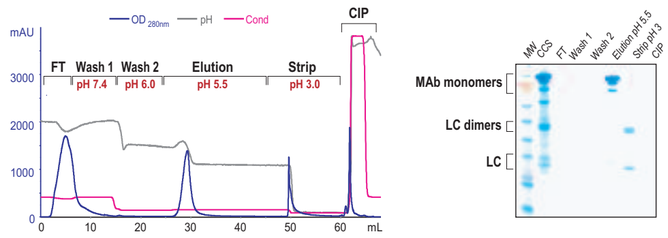
Figure 6
MAb Capture on MEP HyperCel Sorbent With Optimized Conditions: Chromatogram and SDS-PAGE Analysis



PPA HyperCel Sorbent

- Specific elution of MAb monomers using mild elution of pH 5.5, LMW species in pH 3.0 strip fraction

Figure 7
MAb Capture on PPA HyperCel Sorbent With Optimized Conditions: Chromatogram and SDS-PAGE Analysis



- **Good performance of MEP and PPA HyperCel sorbents for monomer recovery, elimination of LMW and HCP contaminants**

Table 3
Summary of Performance of Mixed-Mode Sorbents for the Capture of κλ-bispecific Antibody

HyperCel Sorbents	Elution	Elution Volume (CV)	DBC$_{10\%BT}$ (mg/mL Sorbent)	Monomer Recovery (% Load)	Monomer Purity (%)	HCP* (Log Red)
MEP	50 mM Na acetate, pH 5.0	2.6	20	94.0	**97.4**	1.50
PPA	50 mM Na acetate, pH 5.5	4.7	16	91.0	**95.9**	1.63

**Initial HCP content: 393,000 ppm*

CONCLUSION

- **Efficient light chains removal with MEP and PPA HyperCel sorbents in bind/elute mode: Up to 97% pure monomers after capture step**
- **High yield of monomer recovery (>90%) and efficient HCP removal (≥1.5 log red)**
- **Best performance (DBC, HCP removal, monomer purity and recovery) with MEP HyperCel sorbent**
- **Possible operation of sorbents in flow through mode to process higher MAb quantities**
- **Mixed-mode chromatography: A powerful tool to address future challenges in purification of emerging biomolecules**

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