Simplifying Progress

Efficient Separation of Antibody Light Chains From Bispecific Antibody Monomer Using Mixed Mode Resins

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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 resins 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 resins: 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

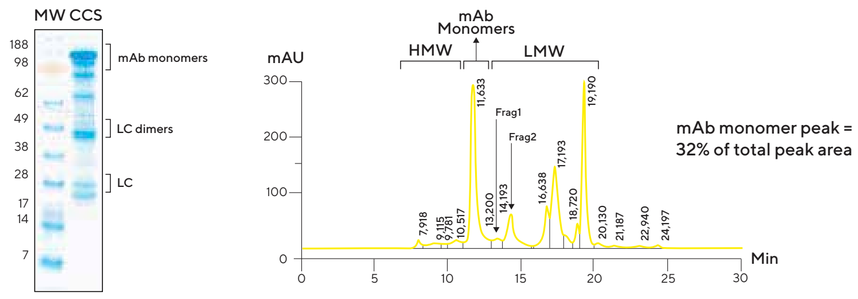
mAb Feedstock Properties and Performance Evaluation Approach

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

Table 1: *Properties of CCS Containing (κλ)-Bispecific mAb*

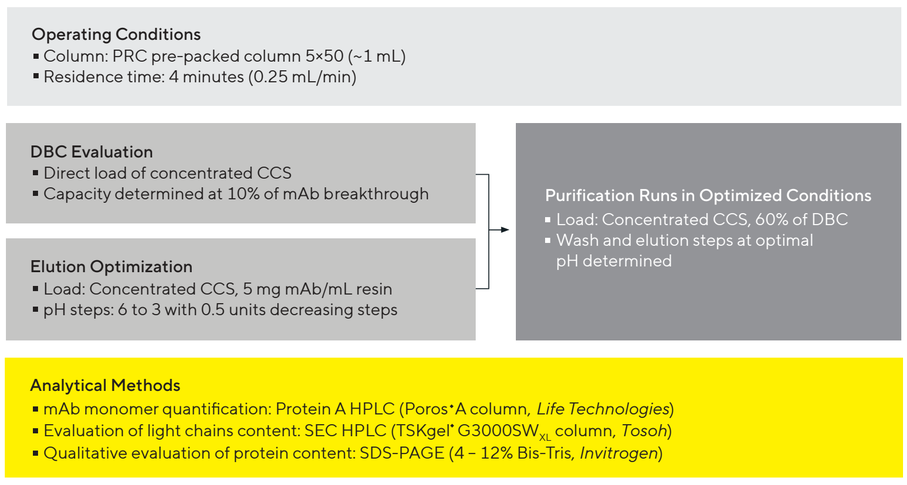
Conditions	mAb Monomer	HCP
pH 7.4, 15 mS/cm	2 mg/mL	393,000 ppm

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



Note. HMW = High molecular weight species, LMW = Low molecular weight species, LC = mAb light chain

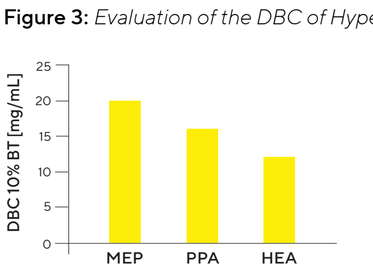
Figure 2: *Resin Performance Evaluation Approach*



Evaluation of the DBC of Mixed Mode Resins

- Good DBC obtained with MEP and PPA HyperCel resins

Figure 3: *Evaluation of the DBC of HyperCel Mixed Mode Resins*



On-Column Optimization of Elution Conditions for LMW Species Elimination

Screening of Elution pH for LMW Species Elimination

Figure 4: *Evaluation of the DBC of HyperCel Mixed Mode Resins*

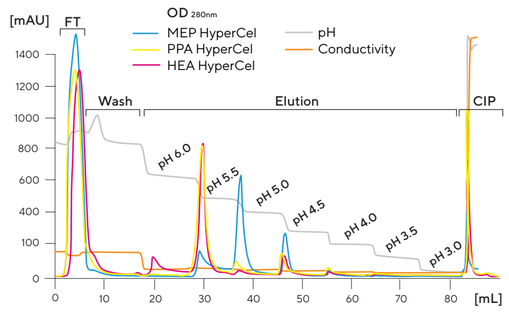
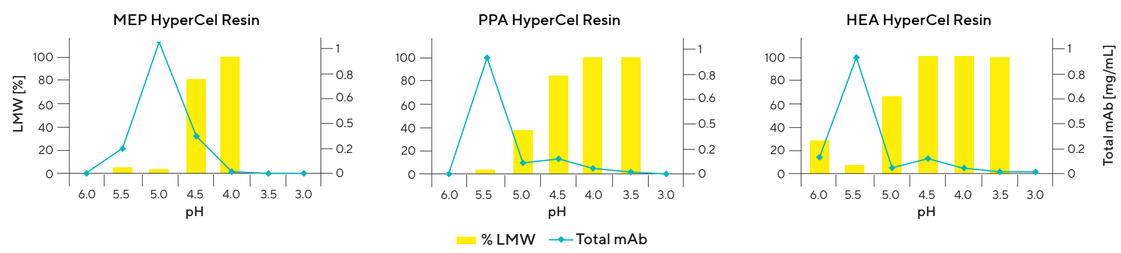


Figure 5: *Total mAb and LMW Species Content According to Elution pH Steps for MEP, PPA and HEA HyperCel Resins*



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

Conclusions on the Optimization of mAb Capture with Mixed Mode Resins

Table 2: *Summary of Mixed Mode resin performance during mAb purification optimization*

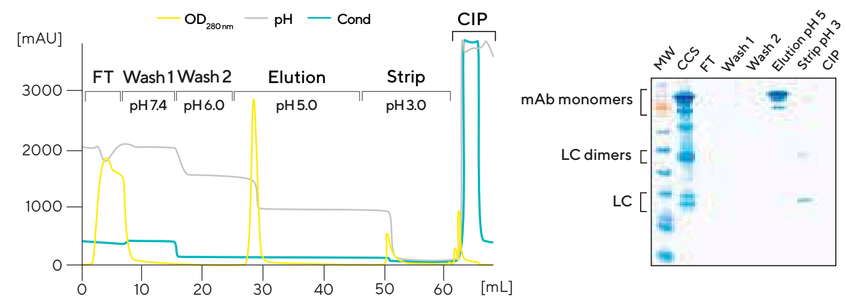
HyperCel Resins	DBC [mg/mL]	Optimal Elution pH	Monomer Purity [%]
MEP	20	5.0	96.2
PPA	16	5.5	96.7
HEA	12	5.5	92.6

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

Capture of κλ-Bispecific Antibody Using Optimised Conditions on Mixed Mode Resins

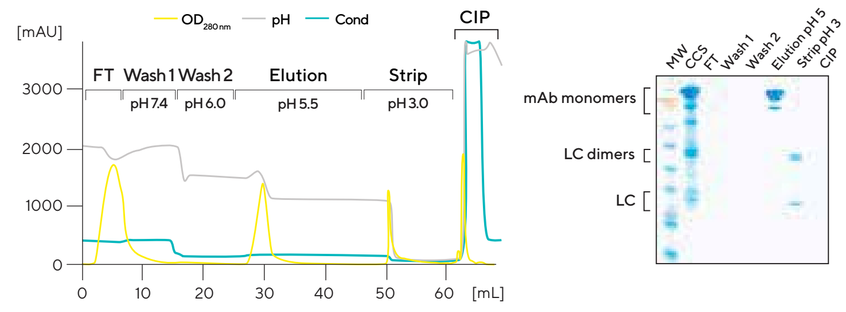
MEP HyperCel Resin
Specific elution of mAb monomers at pH 5.0, LMW species in pH 3.0 strip fraction.

Figure 6: *mAb Capture on MEP HyperCel Resin with Optimized Conditions: Chromatogram and SDS-PAGE Analysis*



PPA HyperCel Resin
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 Resin 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 Resins for the Capture of κλ-Bispecific Antibody*

HyperCel Resins	Elution	Elution Volume [CV]	$DBC_{10\%BT}$ [mg/mL Resin]	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 resins 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 resin
- Possible operation of resins in flow through mode to process higher mAb quantities
- Mixed Mode chromatography: A powerful tool to address future challenges in purification of emerging biomolecules