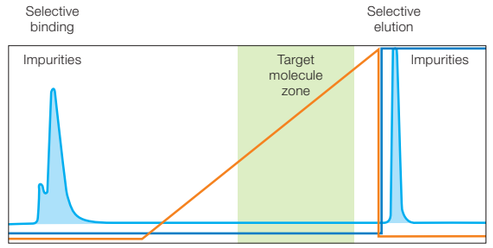
Objective

Upstream improvements in expression systems continue to impact the level of downstream product- and process-related impurities. This includes aggregate formation, which influences biotherapeutic efficacy and immunogenicity.

Traditional ion exchange media could not separate monomers from aggregate to acceptable levels for mAb S. This could indicate an intrinsic hydrophobicity of mAb S. A selection of mixed-mode media were screened and those with a binding capacity of >30 mg/ml were evaluated for aggregate clearance using a bind and elute strategy.

Bind and Elute Strategy

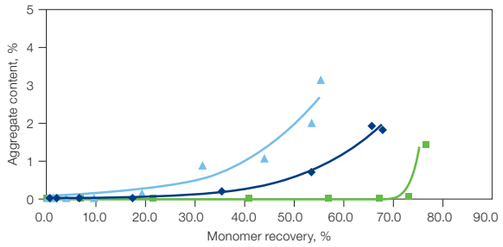


Bind and elute strategies allow enhanced selectivity compared to flow-through methods as both low- and high-binding impurities can be removed. Important factors to consider in addition to selectivity are the number of buffers, ease of buffer preparations, and overall elution volumes.

High Recovery at Low Aggregate Content

For CHT, aggregate breakthrough was not detected until over 70% pure monomer recovery was achieved. In contrast, aggregate breakthrough occurred at less than 40% monomer recovery for both Capto adhere and Capto adhere ImpRes Media.

Monomer Recovery from Gradient Elution Methods



A bind and elute strategy was employed for the three media. Sodium chloride and pH gradients were performed based on DoE studies for optimized separation of aggregate and monomer. The highest total recovery, at low aggregate content (>0.5%) was achieved with CHT. CHT Column, 1 ml, 0–1000 mM sodium chloride gradient (■); Capto adhere Column, 1 ml, pH 8–5 gradient (▲); Capto adhere ImpRes Column, 1 ml, pH 8–5 gradient (◆).

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Optimized Purity and Recovery of a Monoclonal Antibody Using Mixed-Mode Chromatography Media

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Abstract

Purity, recovery, and elution volume are important factors and parameters when screening multiple media (resins) for the development of efficient, robust, and economical production processes. Among a selection of mixed-mode media evaluated for the purification of mAb S, CHT™ Ceramic Hydroxyapatite provided the best monomer recovery at 83% in the smallest elution volume with a target purity of 99.5%. Using a bind and elute strategy provided enhanced purification power compared to flow-through modes.

mAb S Purification performance comparison. mAb S recovery using a bind and elute strategy with CHT, Capto adhere, and Capto adhere ImpRes.

Target Monomer Content Purity Specification, %	Chromatography Media	Monomer Recovery, %	Eluate Volume, CV	10% DBC of mAb S, mg/ml
99.5	**CHT**	**83**	**5**	**47**
99.5	Capto adhere	49	14	31.9
99.5	Capto adhere ImpRes	62	14	70.9

DBC, dynamic binding capacity

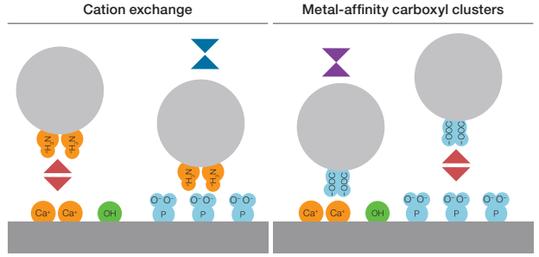
Advantages of Using Mixed-Mode Chromatography

A variety of advantages of mixed-mode interactions have been observed beyond simply finer discrimination between product and impurities. They include:

- Greater resolution compared to unimodal interactions
- Improved yield and activity via advantageous use of charge-charge repulsion between ligand and protein
- Large design space for protein binding
- Minimal feed manipulation prior to binding
- Mild operating conditions preserve activity

Design Principles of CHT

CHT is a spherical, macroporous form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which is formed from the chemical combination of calcium and phosphate salts. Unlike most other chromatography media, hydroxyapatite is both the matrix and the ligand, providing multiple modes of interaction. Proteins interact at the negatively charged phosphate (P) site via cation exchange and at the positively charged calcium (C) site via metal affinity. Multiple interactions between stationary and mobile phases can lead to unique selectivity and facilitate separation of closely related proteins and contaminants.



Schematic representation of CHT binding mechanism. Biomolecule (●); metal affinity (⧗); electrostatic repulsion (⧫); electrostatic attraction (⧗).

Screen CHT today. Request a sample at bio-rad.com/info/screen

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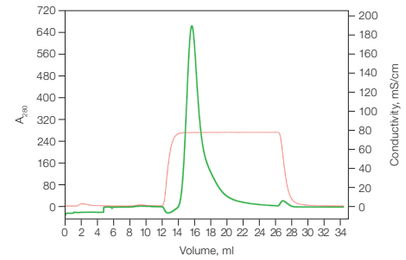
Highest Recovery in Smallest Eluate Volume

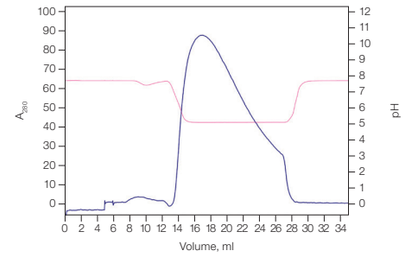
Following DoE optimization of each media, methods were converted to step elution with a minimum 99.5% monomer purity specification. Each 1 ml column was injected with 1 ml of affinity-purified mAb S (2.8 mg in equilibration buffer) at a flow rate of 300 cm/hr.

CHT: step elution at 550 mM NaCl



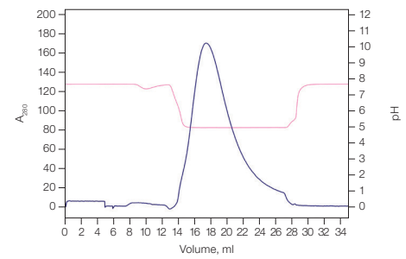
Equilibration: 10 mM sodium phosphate, pH 7.0, 10 CV
Wash: 10 mM sodium phosphate, pH 7.0, 5 CV
Elution: 10 mM sodium phosphate, 550 mM sodium chloride, pH 7.0, 15 CV

Capto adhere: step elution at pH 5.5



Equilibration: 50 mM sodium phosphate, pH 8.0, 10 CV
Wash: 50 mM sodium phosphate, pH 8.0, 5 CV
Elution: 50 mM sodium acetate, pH 5.5, 15 CV

Capto adhere ImpRes: step elution at pH 5.4



Equilibration: 50 mM sodium phosphate, pH 8.0, 10 CV
Wash: 50 mM sodium phosphate, pH 8.0, 5 CV
Elution: 50 mM sodium acetate, pH 5.4, 15 CV

Conclusion

CHT outperformed the other mixed-mode media, providing the best monomer recovery at 83% in the smallest eluate volume, 5 CV, with a target purity of 99.5%. For the hydrophobic and mildly acidic mAb S molecules, CHT is the chromatography media of choice for a robust production process with minimal loss of monomeric target molecules.

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