Overcoming Bispecific Antibody Purification Challenges with CHT™ Ceramic Hydroxyapatite

Introduction

Bispecific antibodies (bsAbs) belong to a class of next generation therapeutics that have been engineered to contain an additional antigen or epitope recognition site, as compared to a traditional monoclonal antibody (mAb). This additional recognition site enables dual targeting: two different biological targets are held in proximity, potentiating novel treatment options that could not be achieved by application of a single mAb. The first bsAb was FDA approved in 2014 and to date (2023), 9 different bispecific molecules have been approved with most applications in oncology[2].

BsAbs have been broadly classified into three categories based on their structure, namely fragment-based bsAbs (no Fc region), asymmetric bsAbs and symmetric bsAbs.

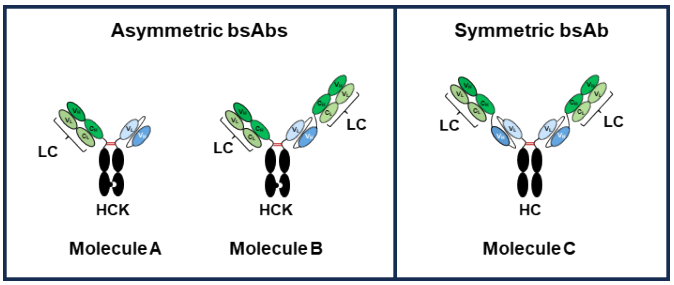

Figure 1. Bispecific antibodies studied, where Molecules A and B are asymmetric bsAbs and molecule C is a symmetric bsAb. Molecule A, "1 + 1" valency; Molecule B, "1 + 2" valency; Molecule C, "2 + 2" valency. Abbreviations: LC = Light chain; HCK = Heavy chain knob; scFv-FcH = scFv-Fc hole; VH-CH1-scFv-FcH = VH-CH1-scFv-Fc hole; and VH-CH1-scFv-FcW = VH-CH1-scFv-Fc wild type

Asymmetric bsAbs are derived from different parental mAbs and to prevent random chain pairing, technologies such as knobs-into-holes (Figure 1, Molecules A & B) and CrossMAb™ technology have been developed, where each half of an asymmetric bsAb can contain one or two antigen recognition sites. Symmetric bsAbs (Figure 1, Molecule C) have two different antigen recognition sites on each half of the molecule, and therefore are generally tetravalent (2+2).

As seen in Figure 1, antigen recognition sites can be in the traditional form with the CH1 domain present (Molecule A left arm) or single-chain variable fragments (Molecule A right arm), to give a single valency on each half of the molecule. Double valency can be introduced through a combination of both as seen in Molecule B (right arm) or Molecule C (both arms).

Downstream processing of bsAbs face numerous challenges, of which, propensity for aggregation is a major hurdle. BsAbs tend to be less stable than their parental mAbs, with "chromatography-induced aggregation" being observed in chromatography processes[3]. Cation exchange chromatography (CEX) is a commonly employed strategy for aggregate removal in mAb purification. This application note summarizes a recent study that investigated the benefits of CHT™ Ceramic Hydroxyapatite, versus the traditional CEX resin in bsAb purification[1].

Ceramic Hydroxyapatite

CHT™ is a bioceramic, spherical and macroporous form of hydroxyapatite known for its physical and chemical robustness. Functioning as both the ligand and support matrix, its multimodal resin features metal affinity interactions with calcium sites (C-sites) and cation exchange interactions with phosphate sites (P-sites). These sites allow for unique and precise separation of an extremely broad range of therapeutic modalities.
CHT™ Type II is manufactured by HOYA Technosurgical Corporation and distributed globally by Bio-Rad Laboratories, Inc. Hercules, CA USA.

CHT™ Type II Characteristics

Table 1. CHT™ Type II Characteristics*

Functional groups	Ca^{2+}, PO_4^{3-}, OH^-
Particle sizes	20, 40, and 80 µm (nominal)
Recommended linear flow rate	50 –1,000 cm/hr
Operating pH range	6.5 – 14
Sanitization	1–2 N NaOH
Autoclavability (121ºC, 20 min)	Yes
Packing density (g/ml packed bed)	0.63 g/ml Type II
Dynamic binding capacity	>12.5 mg lysozyme/g
Nominal pore diameter	800–1,000 Å
Maximum operating pressure	100 bar (1,500 psi)

**Referenced from Bio-Rad Bulletin 5667*

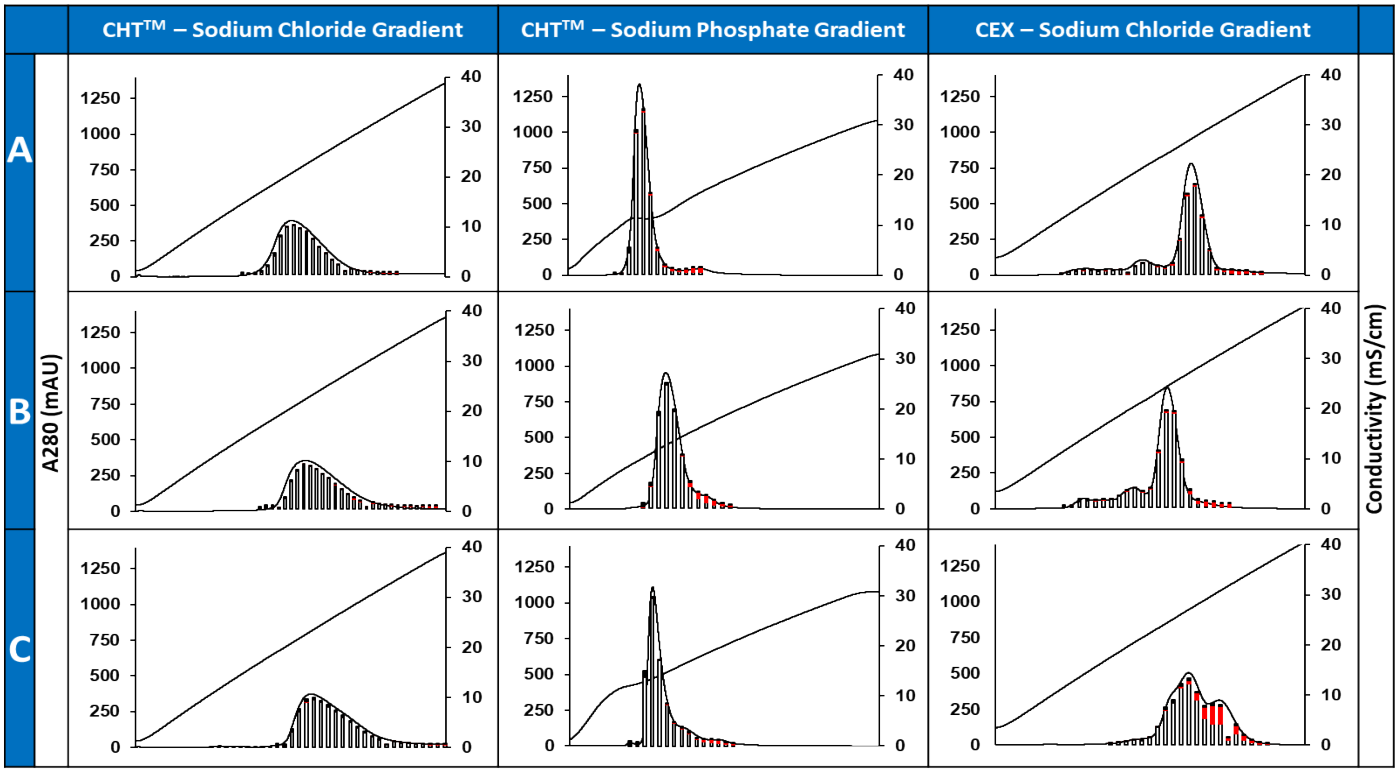

Figure 2. Elution profiles of Molecule A (top), Molecule B (middle) and Molecule C (bottom) when eluted from CHT™ using a sodium chloride gradient (left) or sodium phosphate gradient (middle) and eluted from Capto™ SP ImpRes using a sodium chloride gradient (right). Each plot is an overlay of the UV280 chromatogram (mAU, left y-axis) with each elution fraction's HMWs (red), monomer (white) and LMWs (black) distribution as a bar graph and conductivity (mS/cm, right y-axis), during the elution phase (40 CV, x-axis).

BsAb Culture

Molecules A, B and C (Figure 1) were produced in stably transfected CHO K1 cells, grown in protein-free medium supplemented with 1 g/L sodium carbonate, 6 mM glutamine and 0.1% Pluronic F-68. Glucose feed additions were performed when necessary[1].

Aggregate Removal

Table 2. Experimental Details

Column:	Tricorn™ 5/50 cm bed height
Resin:	- CHT™ Type II 40 µm - Capto™ SP ImpRes
Sample:	MabSelect PrismA™ purified cell culture harvest, ≤ 5 mS/cm
CHT™ NaP Gradient:	- 10 mM Sodium Phosphate, pH 6.8 - 400 mM Sodium Phosphate, pH 6.8
CHT™ NaCl Gradient:	- 10 mM Sodium Phosphate, pH 6.8 - 10 mM Sodium Phosphate, 400 mM Sodium Chloride, pH 6.8
CEX NaCl Gradient:	- 50 mM Sodium Acetate, pH 5.5 - 50 mM Sodium Acetate, 400 mM Sodium Chloride, pH 5.5
Flowrate:	150 cm/hr
System:	ÄKTA™ Avant 25

In the study[1], bsAb aggregate removal by CHT™ was compared with a typical 40 µm CEX resin, Capto™ SP ImpRes (Cytiva). Elution of bsAbs loaded onto CHT™ was carried out through a sodium chloride gradient (mixed-mode elution profile) or a sodium phosphate gradient (similar to CEX elution mode) , whereas elution of the CEX resin used a sodium chloride gradient (Figure 2). The eluate was fractionated (1 CV/fraction) and fractions which had a higher purity than the load material were pooled. Yields were calculated based on total pooled material and based on HPLC-SEC analysis of these pools. It was observed that both types of CHT™ gradients had a better HMW reduction than CEX for all molecules (Table 3). It was also observed that the occurrence of "chromatography-induced aggregation" was mitigated when CHT™ was used, possibly due to the presence of calcium ions at C-sites (Figure 3).

Other Impurities Removal

The study also observed that CHT™ with a sodium chloride gradient achieved the best HCP clearance, whereas LMW and HCDNA removal was comparable with CEX. Increased phosphate concentration was required for large LMW removal in the sodium chloride gradient for CHT™ (Table 3).

Table 3. Yield, HMW, HMW Reduction, LMW, HCP and HCDNA of post protein A eluate and pooled fractions obtained from CHT™ and CEX gradient elution

	Yield	HMW	HMW Reduction*	LMW	HCP (ppm)	HCDNA (ppm)
Post Protein A Eluate						
Molecule A	N.A.	4.7%	N.A.	1.7%	5 839	39
Molecule B		4.1%		4.2%	4 578	17
Molecule C		2.3%		6.3%	1 304	7
CHT™ Sodium Chloride Gradient#						
Molecule A	76.4%	0.5%	90.1%	1.0%	62	0.009
Molecule B	73.9%	0.2%	96.3%	2.5%	12	N.D
Molecule C	67.8%	0.3%	85.5%	4.3%	35	0.012
CHT™ Sodium Phosphate Gradient						
Molecule A	82.2%	1.8%	61.5%	1.7%	378	N.D
Molecule B	80.4%	0.9%	78.5%	3.0%	155	N.D
Molecule C	53.6%	0.4%	82.9%	1.9%	70	N.D
CEX Sodium Chloride Gradient						
Molecule A	80.3%	3.2%	31.7%	1.0%	301	0.008
Molecule B	69.4%	2.9%	30.5%	2.3%	43	0.006
Molecule C	31.4%	2.0%	10.5%	5.1%	243	0.026

*HMW Reduction was calculated based on (Δ of HMW%) / (Post Protein A Eluate HMW%)
Data from CHT™ Sodium Chloride Gradient, Molecules A and B were obtained with a modified post load wash to improve LMW removal[1]

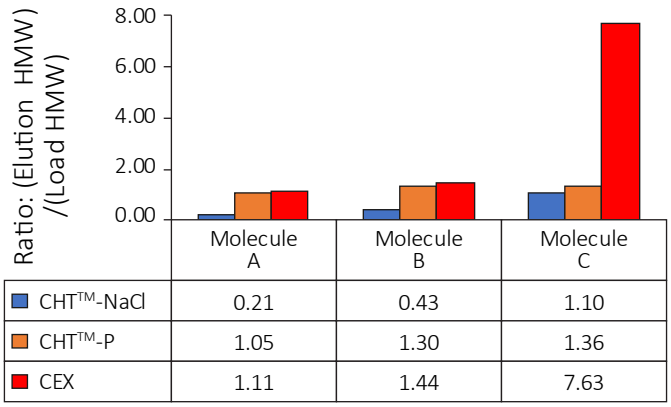

Figure 3. Observed "chromatography-induced aggregation", defined by the ratio of [HMW content in all elution fractions] / [HMW content in load], based on HPLC-SEC analysis

Conclusion

The above study has shown that CHT™ is effective in purification of both asymmetric and symmetric bsAbs, which resulted in products with at least 97% purity and consistently achieved high aggregate removal with both sodium chloride and sodium phosphate elution.

Notably, "chromatography-induced aggregation" was mitigated in post-CHT™ products but occurred in post-CEX products. CHT™ with NaCl elution achieved the best HCP clearance, LMW and HCDNA impurity removal was comparable to CEX.

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Acknowledgements

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