Rapid process development of a CIEX step for a biosimilar

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Introduction

When establishing purification processes for a biosimilar molecule it is important to develop steps that are scalable, selective, and cost-effective.

A cation exchange (CIEX) chromatographic step was developed as part of a collaborative project, where expertise in the field of downstream purification and bioprocessing was combined with specific biosimilar requirements. A standardized workflow was adapted using a high-throughput process development (HTPD) approach: chromatography resin and running conditions were selected based on experience and adapted to meet requirements. By providing the process development support, aggressive timelines could be addressed, which resulted in an accelerated progression of bioprocess design to reduce timelines.

The aim was to select the optimal resin from three candidates and provide initial running conditions for the chosen resin. The goal was to reduce aggregate levels to approximately 0.6% after the polishing step in conjunction with obtaining the highest yield possible for the monomer. HTPD experiments were performed in both in 96-well plates and small-scale columns.

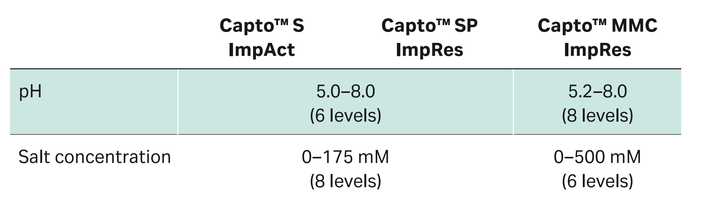

Fig 1. The standardized workflow applied for evaluating and selecting resins for a polishing step of a biosimilar molecule. The first part consists of screening of a wide area of conditions in a format that allows increased throughput and provides process understanding. The final part involves development of a chromatographic method to identify running conditions in an "easy-to-scale-up" approach.

Materials and methods

Tested conditions for the HTPD screening

Single-resin plates were used for a broad-range screening of SBC. Table 1 shows the experimental parameters used.

Screening plates were used for screening of elution conditions. Table 2 shows the experimental parameters used.

Table 1. Experimental parameters for SBC screening in single-resin plates, duplicate runs of all factors

	Capto™ S ImpAct	Capto™ SP ImpRes	Capto™ MMC ImpRes
pH	5.0–8.0 (6 levels)		5.2–8.0 (8 levels)
Salt concentration	0–175 mM (8 levels)		0–500 mM (6 levels)
Incubation time	60 min		
Sample conc.	3.4–5.1 g/L		
Analysis	UV absorbance		

Table 2. Experimental parameters used for elution screening, duplicate runs of all factors

	Capto™ S ImpAct	Capto™ SP ImpRes	Capto™ MMC ImpRes
Binding condition 1	40 mM phosphate, 10 mM NaCl, pH 6.2	60 mM acetate, 10 mM NaCl, pH 5.5	60 mM acetate, 10 mM NaCl, pH 5.8
Binding condition 2	25 mM phosphate, 10 mM NaCl, pH 7.2	25 mM phosphate, 10 mM NaCl, pH 7.2	25 mM phosphate, 10 mM NaCl, pH 7.0
Salt concentration in elution step	10–290 mM (8 levels)		10–920 mM (8 levels)
Incubation time	120 min		
Sample load	20 g/L resin		
Analysis	UV absorbance for yield SEC for aggregate		

Chromatographic method screening

Column experiments were performed with an ÄKTA™ avant 25 system at 5.4 minutes residence time using a Tricorn™ 5/100 column with a volume of 2 mL (10 cm bed height).

Results and discussion

Binding study

Contour plots were used for evaluating SBC and visualizing trends. They also provided information of which binding conditions to test for the subsequent elution studies as well as possible conditions for column method screening. As seen in Figure 2, Capto™ S ImpAct resin had the highest SBC of the resins tested.

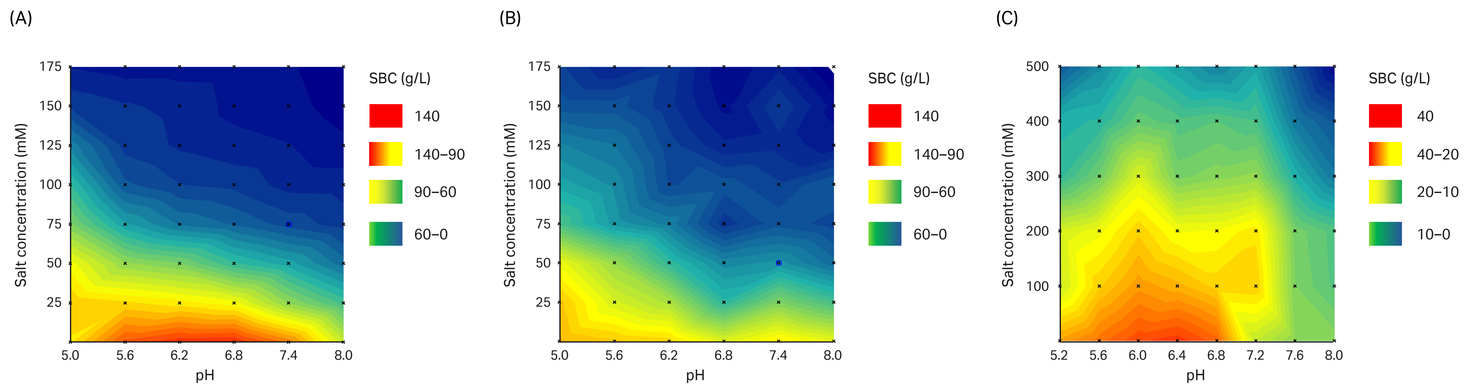

Fig 2. Evaluation of SBC for (A) Capto™ S ImpAct; (B) Capto™ SP ImpRes; (C) Capto™ MMC ImpRes.

Elution screening

The first elution fraction was evaluated for yield and aggregate content (Fig 3). Capto™ S ImpAct and Capto™ SP ImpRes showed most promising aggregate removal of all tested candidates. In addition, it was seen that lower pH was beneficial for aggregate removal. As Capto™ S ImpAct had the highest SBC, this resin candidate and the corresponding conditions were chosen for further optimization.

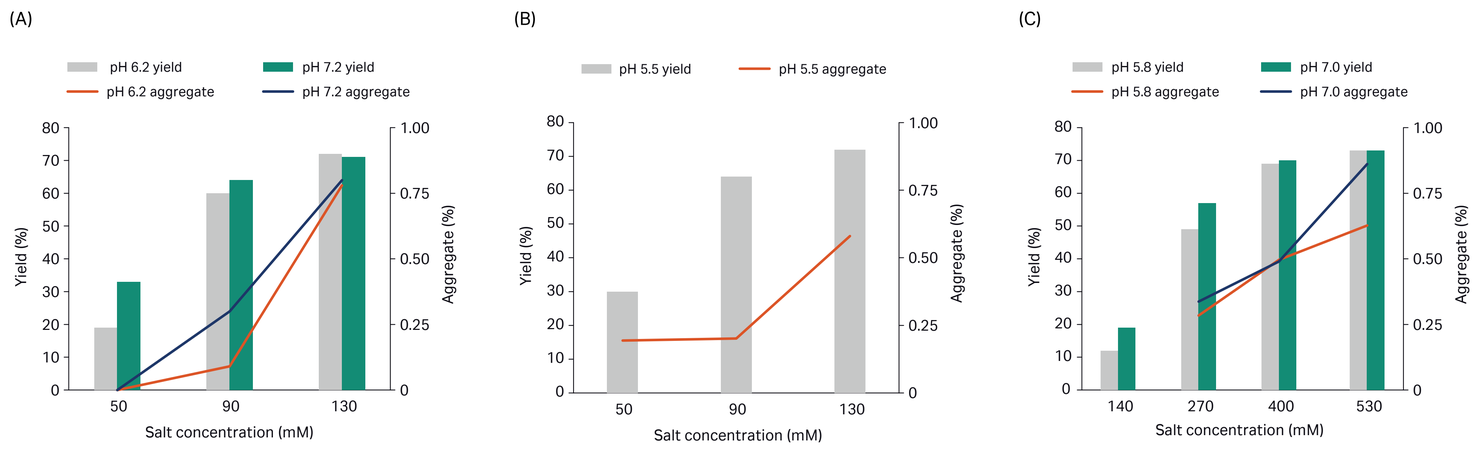

Fig 3. Yield (bars) and aggregate content (lines) for (A) Capto™ S ImpAct; (B) Capto™ SP ImpRes; (C) Capto™ MMC ImpRes.

Chromatographic method development

To aim for an increased productivity, the dynamic binding capacity was investigated. The 10% breakthrough ($Q_{B10\%}$) was determined by performing frontal analysis at two different pH values (pH 5.5 and 6.0) for Capto™ S ImpAct (Fig 4). The highest binding capacity ($Q_{B10\%}$ = 145 g/L resin) was obtained at pH 6.0.

The selectivity of Capto™ S ImpAct between monomers and aggregates was also verified at pH 5.5 and pH 6.0. The two conditions showed similar selectivity, but higher capacity, at pH 6.0. This condition was therefore finally selected. To improve the productivity, high sample loads (80 and 100 g/L resin) were evaluated at pH 6.0. Results showed that a sample load of 100 g/L resin (70% of $Q_{B10\%}$) gave excellent aggregate and host cell protein (HCP) removal (Fig 5 and Table 3). This fulfilled the biosimilarity requirements at a high sample load, resulting in a polishing step with high productivity.

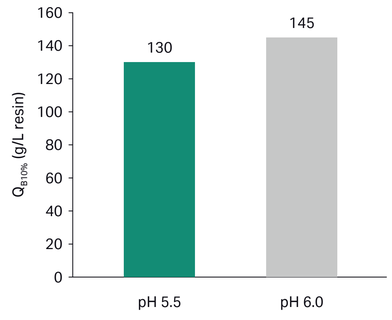

Fig 4. Dynamic binding capacity for Capto S ImpAct at different pH values.

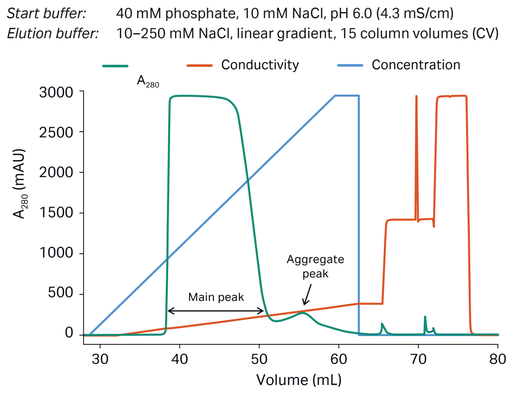

Fig 5. Chromatogram from elution gradient experiment at a sample load of 100 g/L resin.

Table 3. Main peak yield, aggregate content, and HCP results for Capto S ImpAct

	Yield (%)	Aggregate (%)	HCP (ppm)
Start sample	N/A	2.2	55
Sample load: 80 g/L resin	95	0.5	13
Sample load: 100 g/L resin	97	0.5	14

Conclusion

The close collaboration enabled rapid process development:

- Quick identification of a suitable resin candidate using Fast Trak standardized workflow
- Initial running conditions for further process development
- Improved process understanding

Wins from the collaboration:

- Short project duration: 4 weeks
- Support with resin selection and identification of conditions and critical parameters
- Access to expertise to help develop an adaptable and optimal purification process
- Know-how and technology support to get it right first time

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