High-capacity capture of a recombinant growth factor directly from refold solution using salt tolerant cation exchange chromatography

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Introduction

Growth factors, a class of molecules with eminent therapeutic potential, are commonly expressed in *E. coli* as inclusion bodies, therefore needing homogenization, clarification, solubilization, and refolding prior to being able to move into traditional downstream purification operations. The refold pool presents a challenge for conventional chromatography media and often requires significant dilution or buffer exchange in order to achieve reasonable binding capacities and separations. The advent of salt tolerant resins offers an opportunity to intensify these processes by allowing direct connection to the refold pool.

In this work, we evaluated Eshmuno® CPS resin - a new salt tolerant tentacle resin targeting the purification of biomolecules from feeds at high salt concentration - for capture of a recombinant human growth factor produced in *E. coli*. As a strong cation exchanger without hydrophobic groups, the new resin facilitates easy process development with straightforward binding and elution conditions.

A cost analysis will compare salt tolerant with conventional cation exchanger for the growth factor purification.

Feed Characteristics

- rec. growth factor after solubilisation and refold in ≈50 mM Tris, ≈120 mM NaCl, ≈10 mM $CaCl_2$, ≈0.5 M $CuSO_4$, ≈0.3 M GuaHCl, pH 8.0, 36 mS/cm
- titer: 2 g/L (after ~10X concentration using 5kD cut-off TFF UF, no diafiltration)

Methods

DBC Measurement

- column dimension: 20 mm L x 8 mm ID, CV = 1.0 mL
- run buffers:
  buffer A: 50 mM Tris/HCl + 120 mM NaCl, pH 8.0
  buffer B: 50 mM Tris/HCl + 2 M NaCl, pH 8.0
- load: undiluted feed, titer 2 g/L, pH 8.0, 36 mS/cm, 3 min residence time
- wash: 10 CV buffer A at 238.7 cm/h (2 mL/min)
- elution: 10 CV buffer B at 238.7 cm/h
- CIP/re-equilibration:
  20 CV 1 M NaOH at 119.4 cm/h (1 mL/min)
  15 CV buffer B at 238.7 cm/h
  20 CV buffer A at 238.7 cm/h
- fractionation/sampling:
  flow-through + wash, fraction size 2 mL
  eluate pool, a single 17 mL fraction
- analytics: RP-HPLC (purity, titer)
- evaluation: determination of 10 % breakthrough level of rh growth factor, calculation of the corresponding dynamic binding capacity ($DBC_{10\%}$) from load volume

Purification Protocol

- column dimension: 200 mm L x 5 mm ID, CV = 3.93 mL
- run buffers: buffer A, buffer B
- load: undiluted feed, titer 2 g/L, pH 8.0, 36 mS/cm, to 54 – 58 % of $DBC_{10\%}$ (due to limited feed), at 400 cm/h, 3 min residence time
- wash: 10 CV buffer A at 180 cm/h
- gradient elution:
  phase 1) 15 – 100 % B in 21 CV at 400 cm/h
  phase 2) 2 CV 100 % B at 400 cm/h
- CIP/re-equilibration:
  3 CV 1 M NaOH upflow at 120 cm/h
  (30 min residence time)
  3 CV buffer B upflow at 120 cm/h
  10 CV 100 % buffer A at 180 cm/h
- fractionation:
  flow-through + wash, fraction size 40 mL
  eluate fractions, fraction size 2 mL
- analytics: RP-HPLC (purity, titer), HCP ELISA
- evaluation: purity and yield

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Results

Dynamic Binding Capacities

DBC was measured for Eshmuno® CPX resin, a standard cation exchange (CEX) resin, and the new salt tolerant Eshmuno® CPS resin, both built on MilliporeSigma's proprietary tentacle technology, as well as Resin C, a conventional CEX resin not bearing MilliporeSigma's tentacles (Figure 1).

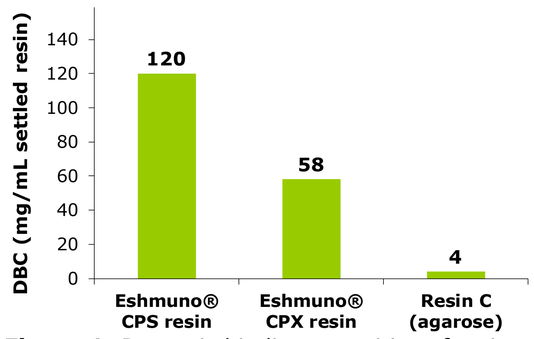

Figure 1: Dynamic binding capacities of cation exchange resins at 10 % breakthrough for rh growth factor at 36 mS/cm.

Purification

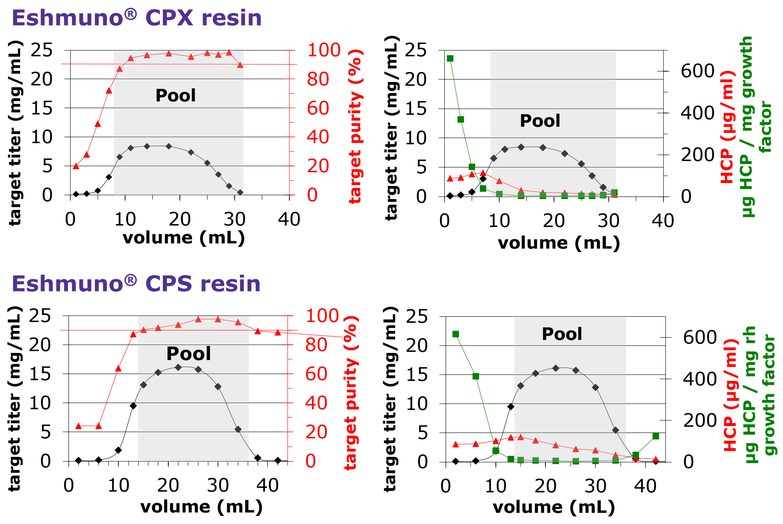

Figure 2: Eluate profiles.

Eshmuno® CPX resin showed a favourable separation profile for HCP and rh growth factor: The HCP maximum eluted at the ascending flank of the growth factor peak. With Eshmuno® CPS resin the HCP peak was shifted further into the growth factor peak. Purity (by HPLC) and yield for the indicated pools were similar for both resins, namely 96 % purity and 93 % yield for Eshmuno® CPX resin and 95 % purity and 89 % yield for Eshmuno® CPS resin (Figure 2). The quantitation of HCP is given in Figure 3.

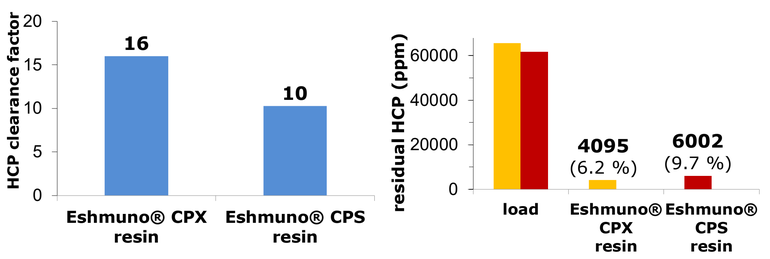

Figure 3: HCP reduction.

Cost Analysis

Cost and productivity calculations were carried out using a simplified cost model based on Xenopoulos et al., 2015 (J. Biotechnol. 213, 42-53, A new, integrated, continuous purification process template for monoclonal antibodies: Process modeling and cost of goods studies). The calculations compare three scenarios: Scenarios 1 and 2 comprise Eshmuno® CPS and Eshmuno® CPX resin, respectively, with the real case data of the purifications described in this poster. Scenario 3 employs the virtual case of a conventional CEX resin with inline diluted f with a residual low conductivity of about 5 mS/cm (requires 8 fold dilution) with parameters which are reasonable from experience. Basis for the calculation is a 2000 L batch with a titer of 2 g/L and a packed bed height of 20 cm. The column diameters used reflect the required resin volumes, and do not take into account the constraint of real existing column sizes. This focuses on the economics of the operation in general and allows a direct comparison of the different scenarios. Further selected input parameters for the calculations are listed in Table 1.

Table 1: Selected input parameters for cost calculation of different CEX capture scenarios.

Resin	Loading capacity (g/L CV)	Step yield (%)	Feed dilution factor	Column diameter (cm)	Cost of purified water (PW) ($/L)	Buffer cost ($/L)	Labor cost ($/h)	Cost of PW for dilution ($/batch)	Process time for loading (h)
Eshmuno® CPS, high salt	84	89	0	55	1.5	2.0	100	0	2.1
Eshmuno® CPX, high salt	40	93	0	79	1.5	2.0	100	0	1.0
Conventional CEX, plus inline dilution	90	93	8	53	1.5	2.0	100	21000	18.0

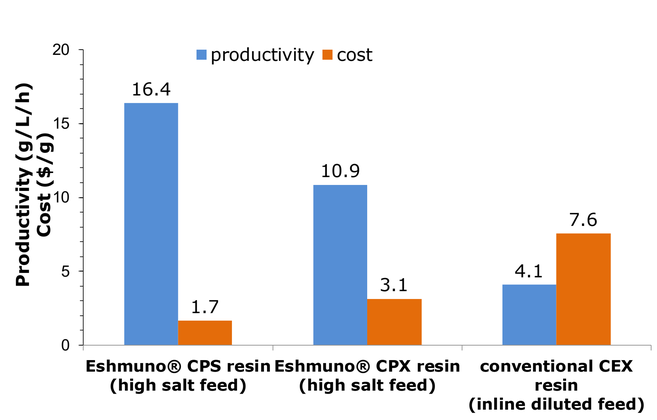

Figure 4: Costs and productivities associated with application of different types of cation exchange resins in rh growth factor purification.

Use of the standard tentacle cation exchanger compared to the conventional CEX resin results in a 2.7 fold increase in productivity and a 2.4 fold reduction of the cost per gram of target produced in the capture step. Application of the salt tolerant resin gains an additional 50 % of productivity and a further 1.9 fold cost reduction. The decisive negative impact in scenario 3 with the conventional cation exchanger is the large water volume required for feed dilution. This causes a much longer loading time and in turn the reduced productivity, and also presents a huge cost driver.

Conclusion

- Superior performance of Eshmuno® CPS resin at high salt concentration achieved two times higher binding capacity compared to standard tentacle cation exchanger.
- A conventional ion exchange resin almost completely failed under these conditions.
- Higher column loading at high salt conditions was associated with some lower selectivity towards impurity removal.
- The bulk of HCP (90 %) was still removed, though.
- Cost analysis highlights the cost-effectiveness of the new approach of salt tolerant ion exchangers compared to conventional ion exchange resins which can only be operated at low to modest salt concentrations.
- Cost per gram of growth factor produced were 4.5 times lower, and productivity was 4 times greater.
- In addition to the advanced capture step itself, the option of directly connecting capture to refolding allows intensification of the overall downstream process.

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