Increased lifetime of RPC resins in insulin production by clean-up using WorkBeads 40S

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Background

Recombinant DNA technology has long been used to produce therapeutic insulin. Since over 90% of the manufacturing cost is associated with recovery and purification, being competitive means maximizing the yield and the purity of the insulin target. The purity requirements are very stringent and are usually met by using high-resolution reversed phase chromatography (RPC) with silica-based resins. However, impurities from the feed often cause column fouling. Using sodium hydroxide for cleaning-in-place is limited for silica resins resulting in impurities that are difficult to remove.

Here, we introduce an upstream orthogonal purification step which significantly reduces the bioburden on the expensive high-performance RPC column while increasing the peptide yield, purity and column life-time.

Introducing IEX prior to RPC

A 72.5% pure human insulin precursor (Met-Lys-Human insulin) was purified in a three-step process using cation exchange chromatography (CIEX) prior to two RPC steps (Fig. 1). **WorkBeads™ 40S** was used in the CIEX capture step with a comparison to Capto™ SP ImpRes (Cytiva). RPC columns were used in both subsequent steps, but the conditions were different (Table 1).

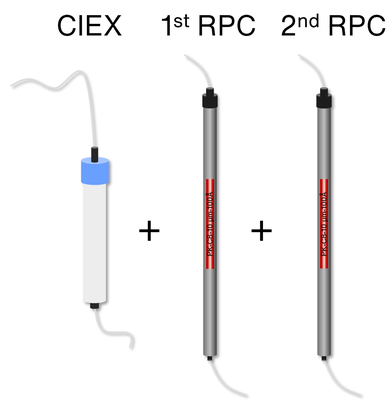

Figure 1. Three-step purification. CIEX followed by two RPC steps.

Result

- WorkBeads 40S gives increased purity to Capto SP ImpRes (Fig. 1)
- CIEX minimized impurities to be applied on the subsequent purification steps (Fig. 2)
- The final purity of the three-step process is 99.7% (Fig. 2)

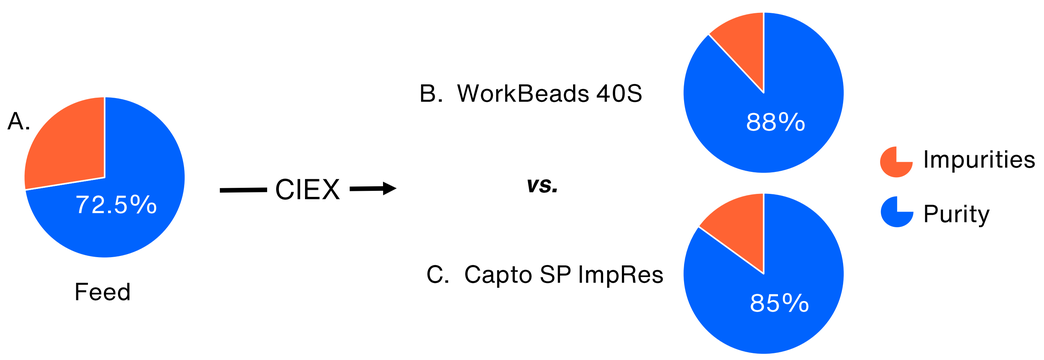

Figure 1. Purity of feed **(A).** CIEX comparison between WorkBeads 40S **(B)** and Capto SP ImpRes **(C)** for a pool yield of 90%.

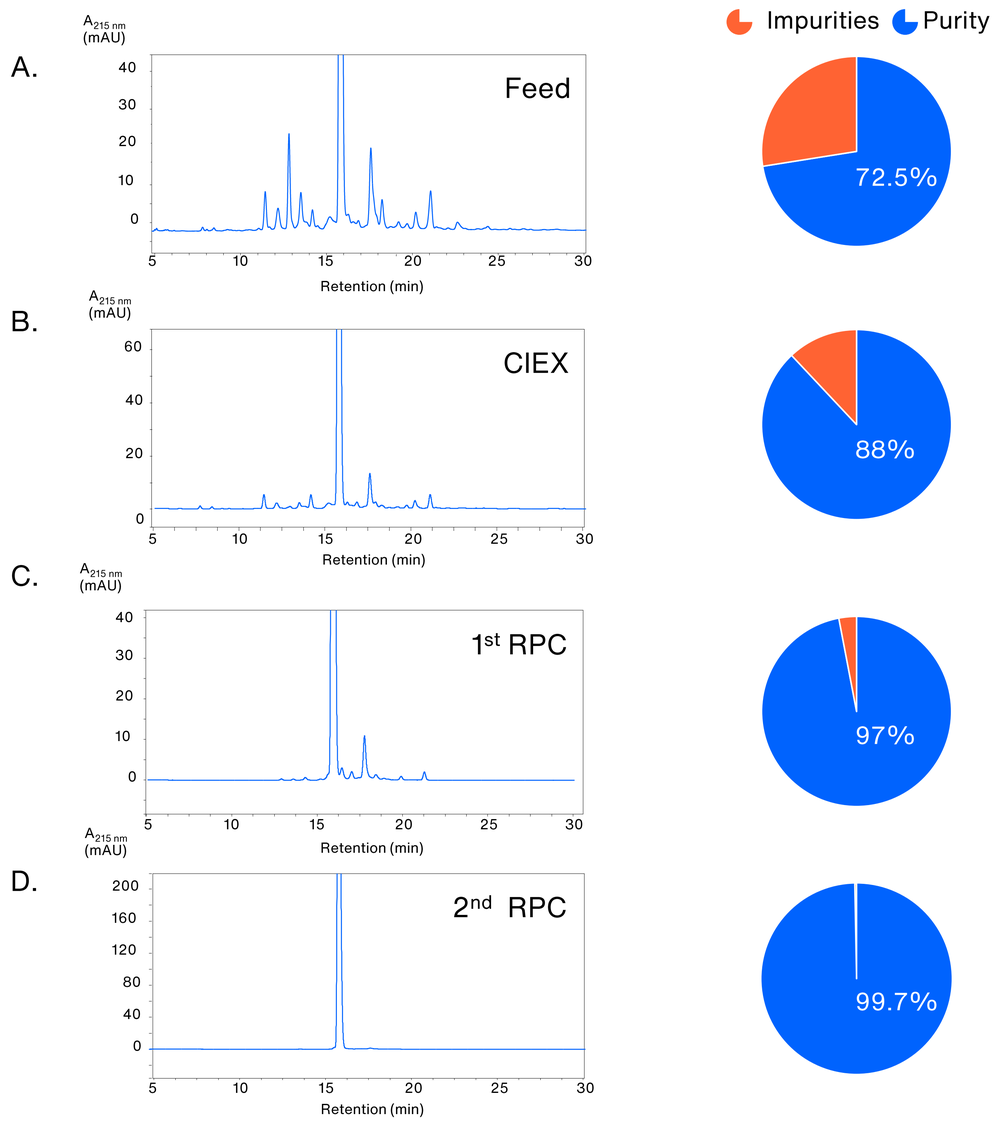

Figure 2. RPC analyses of purified pools from the optimized three-step purification and the purity progress. Feed **(A)**, step 1: eluted pool from WorkBeads 40S **(B)**, step 2: eluted pool: from RPC1 **(C)** step 3: eluted pool from RPC2, final product **(D)**. Blue area in the pie charts represents the purity percentage of human insulin precursor while impurities are orange.

Conclusion

Introducing CIEX prior to the high-performance silica-based RPC step, significantly reduced the bioburden from 27.5% to 12%. This minimizes the fouling of the RPC column and reduces the need for cleaning-in-place. Using WorkBeads 40S can thus extend the life-time of the RPC column.

WorkBeads 40S gave higher purity than Capto SP ImpRes in the introduced CIEX capture step, resulting in a more efficient capturing step and also improved selectivity.

The final purity for the process is 99.7%, well above the target of 99%.

Table 1. Three-step purification process of a recombinant human insulin precursor.

	Step 1: IEX	**Step 2: RPC**	**Step 3: RPC**
Resins	WorkBeads 40S (Bio-Works, Sweden)	PK-C8-10 µm-100Å (Osaka Soda, Japan)	PK-C8-10 µm-100Å (Osaka Soda, Japan)
Bed heights	24 cm	25 cm	25 cm
Feeds	Crude human insulin precursor	Pooled elution fraction from step 1	Pooled elution fraction from step 2
Loadings	20 g product/L resin	15 g product/L resin	15 g product/L resin
Eluents A	50 mM Na-acetate, pH 4.0: EtOH (7:3)	100 mM $(NH_4)_2SO_4/H_2SO_4$, pH 3.2	200 mM NH_4-acetate/Acetic acid, pH 5.5
Eluents B	50 mM Na-acetate, 1 M NaCl, pH 4.0: EtOH (7:3)	Acetonitrile	Acetonitrile
Gradients	0 - 20% B 2 in CV, 20% B in 1 CV, 20 - 70% B in 6.5 CV, 100% B in 20 CV	5 - 21% B in 1 CV, 21 - 27% B in 6 CV, 60% B in 2 CV	5 - 25% B in 1 CV, 25 - 28% B in 6 CV, 60% B in 2 CV
Flow rates	150 cm/h	180 cm/h	180 cm/h

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