Use of a continuous chromatography system for both resin screening and scale-up studies

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Abstract

The aim of this study was to demonstrate the usefulness of a periodic counter-current chromatography (PCC) system for both resin screening and scale-up possibilites within the same system. Furthermore, the applicability of dynamic control functionality in PCC runs with different sample concentration was shown. The use of the new MabSelect™ PrismA protein A resin, with improved dynamic binding capacity (DBC) and alkaline stability, enabled the use of high concentrations of NaOH for cleaning in place (CIP), thereby reducing the risk of column contamination during the extended PCC runs.

Introduction

The cost pressure on biopharmaceuticals drives the industry towards exploring various process intensification options such as continuous or connected biomanufacturing.

The aim of this study was to demonstrate different PCC examples in downstream processing.

The principles of three-column (3C) and four-column (4C) PCC are shown in Figure 1.

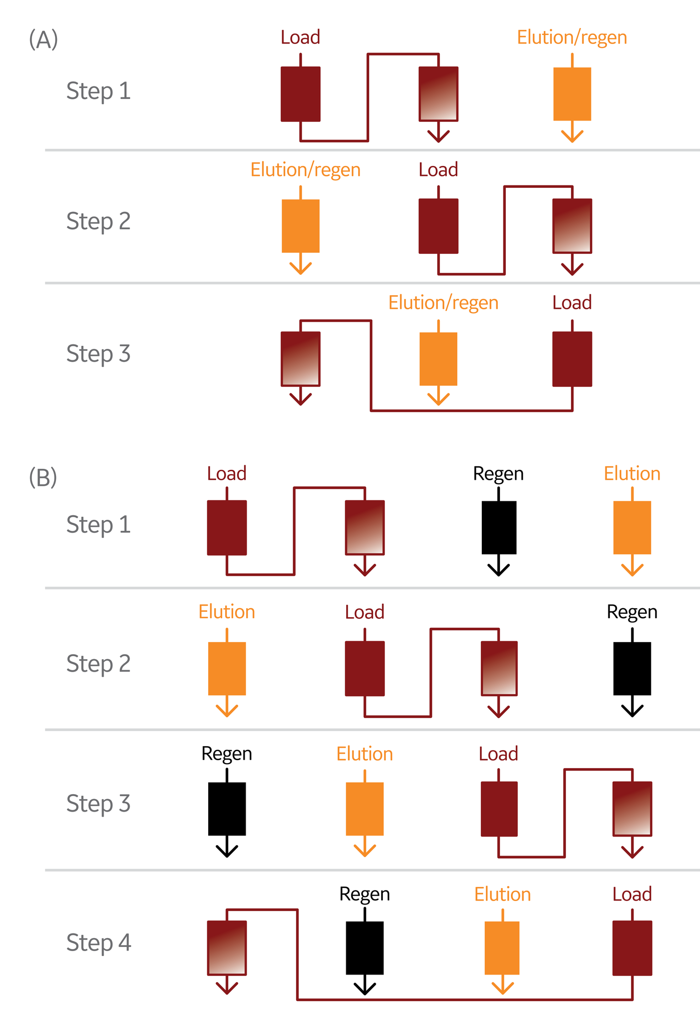

Fig 1. Working principle of one PCC cycle in (A) 3C PCC and (B) 4C PCC setups. One cycle consists of three loops in 3C PCC and four loops in 4C PCC. A loop is defined as when one column has been loaded, eluted, and regenerated, while a cycle is when all columns have been loaded, eluted, and regenerated.

Purification of mAb by PCC

A monoclonal antibody (mAb) was purified on columns packed with MabSelect PrismA resin using the ÄKTA pcc chromatography system. The columns were subjected to CIP with 1 M NaOH in each cycle. The sample titers were varied between 1.1, 4.9, and 9.0 g/L of mAb in cell culture supernatant, and loaded to 50%, 40%, and 50% breakthrough, respectively.

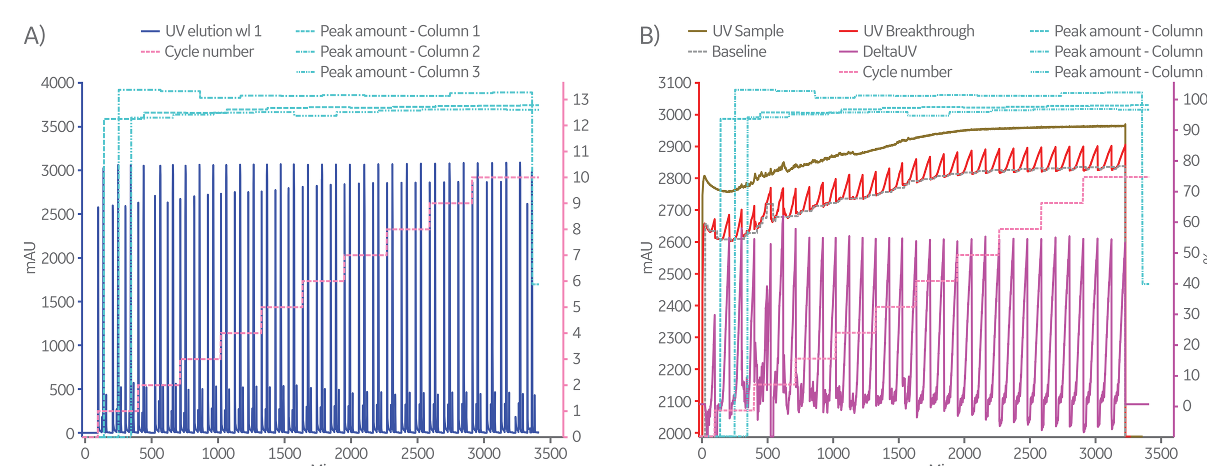

Fig 2. Chromatograms for 31 loops, showing (A) elution peaks and (B) delta UV dynamic control. HiTrap columns (2 × 1 mL connected in series, 5 cm bed height) were used in a 3C PCC setup. Sample concentration was 1.1 g mAb/L.

↓~50x scale up

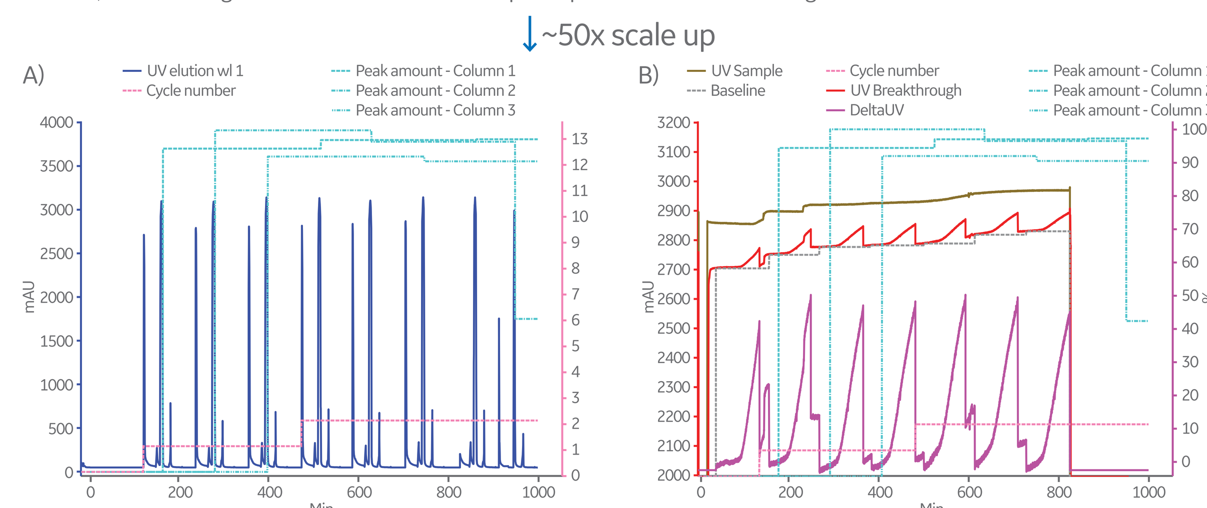

Fig 5. Chromatograms for seven loops, showing (A) elution peaks and (B) delta UV dynamic control. AxiChrom 50 columns (94 mL column volume [CV], 5 cm bed height) were used in a 3C PCC setup. Sample concentration was 1.1 g mAb/L.

Chromatograms are shown in Figures 2–4. The dynamic control functionality enabled consistent sample application, even when the sample background and titer varied. The trend curves show real time values for sample load volume and elution peak areas, ensuring consistent operation over time.

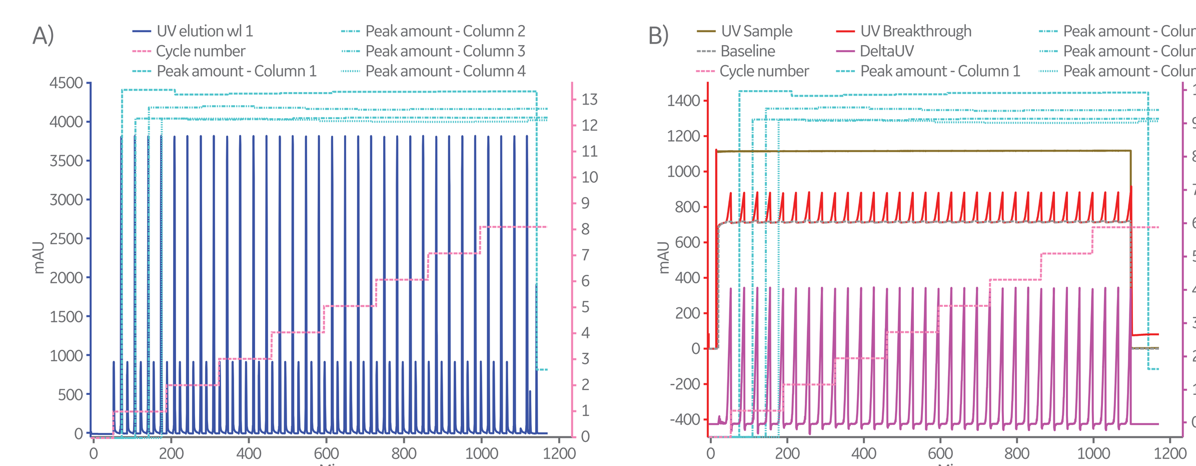

Fig 3. Chromatograms for 32 loops, showing (A) elution peaks and (B) delta UV dynamic control. HiTrap columns (2 × 1 mL connected in series, 5 cm bed height) were used in a 4C PCC setup. Sample concentration was 4.9 g mAb/L.

Table 1. Recovery of mAb for different feed concentrations and scales

Sample conc.	PCC	CV (mL)	Loops	Recovery (%)
1.1 mg mAb/mL	3C	2	31	94
1.1 mg mAb/mL	3C	94	7	93
4.9 mg mAb/mL	4C	2	32	94
9.0 mg mAb/mL	4C	2	32	96

A scale-up from 2 × 1 mL HiTrap™ columns to 94 mL AxiChrom™ columns was performed, and the results were comparable between scales. The chromatograms are shown in Figure 5. Table 1 shows recovery of mAb for the different runs.

Fig 4. Chromatograms for 32 loops, showing (A) elution peaks and (B) delta UV dynamic control. HiTrap columns (2 × 1 mL connected in series, 5 cm bed height) were used in a 4C PCC setup. Sample concentration was 9.0 g mAb/L.

Experimental

Chromatography system: ÄKTA™ pcc, 3C or 4C configuration

Chromatography resins: MabSelect PrismA, MabSelect SuRe™, MabSelect SuRe LX, and MabSelect SuRe pcc

Sample: filtered, mAb-containing cell culture supernatant

Method outline:

Sample application: sample load to preset breakthrough levels (delta UV)

Wash 1: 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0

Wash 2: 50 mM sodium acetate, pH 5.5

Elution: 50 mM sodium acetate, pH 3.5

CIP: 0.5 or 1 M NaOH

Equilibration: 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0

Analysis:

Concentration of mAb was determined by surface plasmon resonance using a Biacore™ system.

Lifetime study

ÄKTA pcc was used in a 4C PCC setup for a CIP study with four different protein A resins over 100 cycles.

Column load was set to 10% breakthrough level, and the DBC for mAb was reflected by the load volume to reach this set point. A decrease in DBC could therefore be monitored as a decrease in sample load volume. The relative remaining DBC for four different protein A resins over the 100 cycles is illustrated in Figure 6. In this way, the ÄKTA pcc system can be used for resin screening by running multiple columns at the same time.

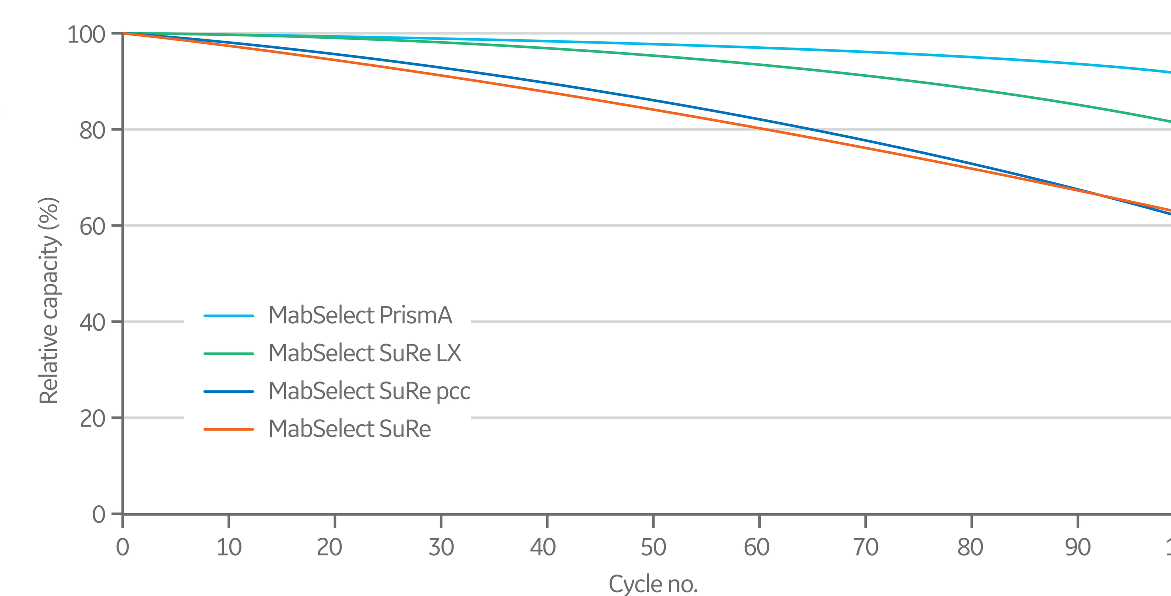

Fig 6. Relative remaining DBC (%) based on the respective sample load volume after repeated purification of mAb (up to 100 cycles), including cleaning with 0.5 M NaOH at 15 min contact time each cycle.

Conclusions

This work shows the usability of PCC in different applications, such as resin screening and mAb purification, at different scales, and with different sample concentrations. PCC technology, together with high-capacity and alkaline-stable resins, has the potential to increase productivity, while reducing the size of the equipment needed for clinical and commercial productions. Compared with batch chromatography, PCC uses smaller parallel columns that are continuously feed by the same chromatography system.

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