The use of dynamic control in periodic counter-current chromatography

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The use of dynamic control in periodic counter-current chromatography

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Introduction

The interest in continuous bioprocessing is rapidly growing and the technique has proven to provide gains in productivity and savings in cost of goods. Periodic counter-current chromatography (PCC) is one suitable technological approach, enabling continuous downstream processing. However, implementation in downstream applications is still not common mainly due to perceived complexity in operation and process control strategies.

The dynamic control function for column saturation level is a key feature of the ÄKTA™ pcc 75 system.

Dynamic control enables adjustments with regard to load in the case of changes in feed composition and/or chromatography medium capacity.

This work aims to demonstrate the principle of dynamic control applied to a protein A capture step operated in a three-column PCC (3C PCC) setup. For this study, changes in both medium capacity and concentration of monoclonal antibody (MAb) in the feed were used to verify the dynamic control functionality.

Principle of dynamic control

A schematic overview of the flow path for ÄKTA pcc 75 is shown in Figure 1. The principle of dynamic control is based on the relative difference in UV signals before and after the column at breakthrough. The difference between the baseline UV and the UV signal at 100% breakthrough for a fully saturated column is defined as ΔUVmax (Fig 2). The level of breakthrough is defined as percentage of ΔUVmax, where the desired level is process dependent.

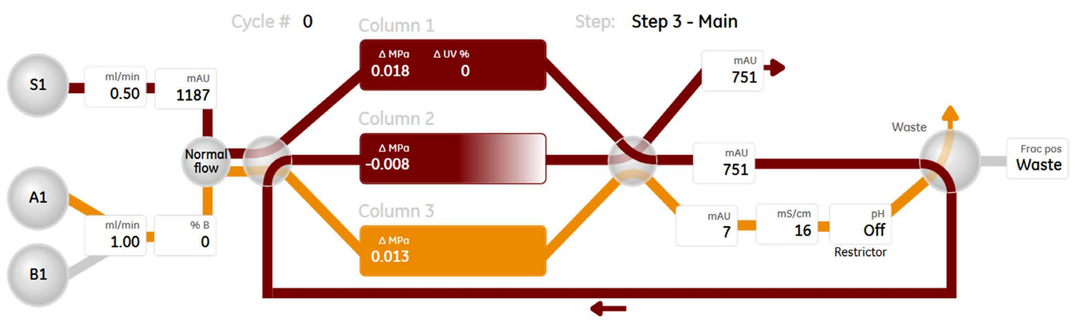

Fig 1. Process picture for the 3C PCC system setup.

The ÄKTA pcc 75 system uses UV detectors assigned to process streams and not to separate columns. Hence, each breakthrough curve is generated based on signals from two UV detectors (Fig 3).

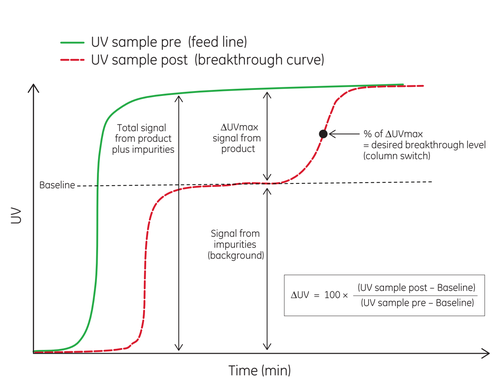

Fig 2. Overview of the two-step breakthrough, displaying the central UV signals used for dynamic control by the ÄKTA pcc 75 system.

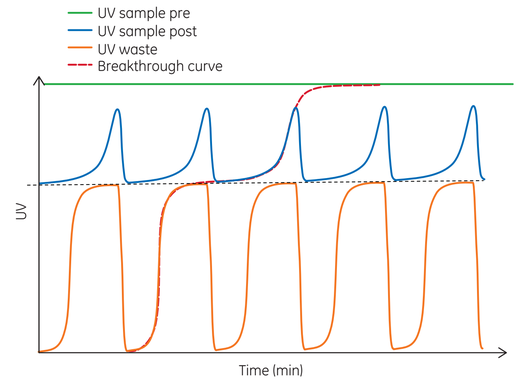

Fig 3. UV signal detectors used for dynamic control by ÄKTA pcc 75.

Results

Alternating between two feed concentrations

Figure 4 shows the ability of dynamic control to adjust for alternating feed concentrations when switching between two MAb concentrations. Consistency in sample load can be seen in the low relative variation in the amount of eluted MAb.

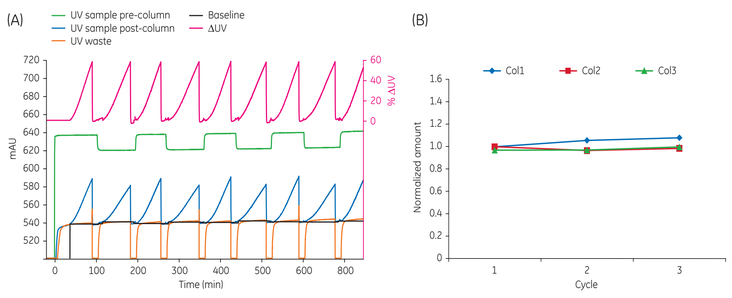

Fig 4. (A) Switching between feed concentrations of 1.5 and 1.8 g MAb/L during load onto MabSelect SuRe™ LX at a 2.5 min residence time. (B) Amount eluted MAb per column (Col1 to Col3) and cycle.

Continuous change in feed concentration

Figure 5 shows the ability of dynamic control to adjust for change in feed concentration by using a gradient increase/decrease in MAb concentration. As shown, the relative variation in the amount of eluted MAb is low.

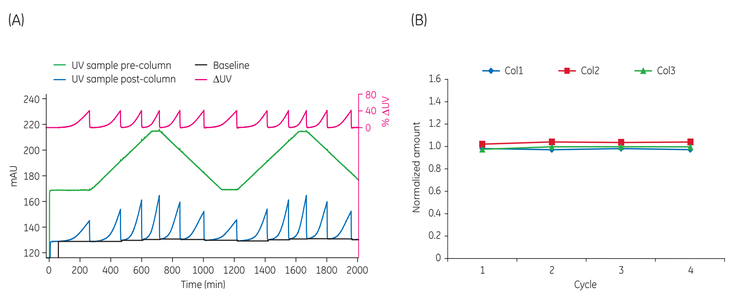

Fig 5. (A) Dynamic control when using a feed concentration gradient from 1 to 2 g MAb/L during load onto MabSelect SuRe LX columns at a 3.5 min residence time. (B) Amount eluted MAb per column (Col1 to Col3) and cycle.

Variation in column performance

To show the system's ability to adjust for changes in column performance, dynamic control was used to control a 3C PCC setup on ÄKTA pcc 75 equipped with one MabSelect SuRe column (Col1) and two MabSelect SuRe LX columns (Col2 and Col3) (Fig 6). The lower dynamic binding capacity (DBC) of MabSelect SuRe medium is reflected in the lower amount of eluted antibody from this column during each cycle.

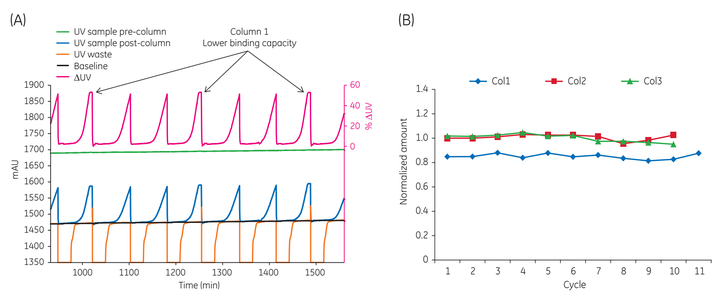

Fig 6. (A) Difference in DBC between column 1 (MabSelect SuRe) and columns 2 and 3 (MabSelect SuRe LX) media at 5 min residence time for a MAb concentration of 4.5 g/L. MabSelect SuRe reaches the set ΔUV faster than MabSelect SuRe LX, shown as the ΔUV signal plateau occurring every third load (arrows). (B) Amount eluted MAb per column (Col1 to Col3) and cycle.

Materials and methods

Cell culture harvest containing MAb was prepared by filtration using an ULTA™ Capsule HC 0.22 µm filter. The protein A capture step was performed using the predefined protocol outlined in Table 1 in a 3C PCC system setup.

Table 1. Running conditions for PCC experiments

Step	Buffer	Column volumes
Equilibration	PBS, pH 7.4	5
Feed	Variable	50% BT*
Wash 1	PBS, pH 7.4	5
Wash 2	50 mM acetate buffer, pH 6.0	1
Elution	50 mM acetate buffer, pH 3.5	4
Strip	100 mM acetate buffer, pH 2.9	2
Cleaning in place (CIP)	100 mM NaOH	3

* Breakthrough

Discussion

Variable feed concentration

The dynamic control in ÄKTA pcc 75 was able to adjust for differences in feed concentration. The variation per column with regard to both amount eluted MAb and eluate concentration was low.

Variation in column performance

The dynamic control was able to adjust for differences in DBC between the columns. Despite the difference in DBC between the chromatography media, the relative variance with regard to the amount eluted MAb per column was low.

The observed consistency in performance was enabled by the dynamic control function and would not have been achieved if the system instead was run based on predefined column switching times.

Conclusions

- The use of the dynamic control function enabled system operation under process conditions where either the feed concentration or the chromatography medium capacity was altered.
- The dynamic control enabled detection and adjustment for differences in chromatography medium performance, thus preventing yield losses and ensuring eluate consistency.
- The dynamic control enabled adjustments for variations in feed concentration, which makes ÄKTA pcc 75 well-suited for processing of perfusion cell culture feeds where changes in the feed composition can occur.

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