The use of dynamic control in continuous chromatography

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The use of dynamic control in continuous chromatography

The ÄKTA™ pcc continuous chromatography system can be used for purification of target proteins in continuous downstream processes using periodic counter-current chromatography (PCC). PCC enables greater use of the chromatography resin capacity, allowing sample loading to much higher levels compared with what is possible in traditional batch chromatography.

This application note shows how the dynamic control functionality of ÄKTA pcc can be used to adjust for variations in binding capacity of the chromatography resin. This function can also be used for changes in feed composition when, for example, perfusion cell culture is being employed. Dynamic control supports the process analytical technology (PAT) initiative defined by the U.S Food and Drug Administration (FDA).

Introduction

The interest in continuous bioprocessing is rapidly growing. This approach can provide gains in productivity and save costs. PCC is a suitable technical solution to enable protein purification in a continuous manner. Continuous chromatography is especially suitable for purification of unstable molecules, as the short process time helps ensure stability of your target product. In addition, continuous chromatography can significantly reduce facility footprint.

Basic principle of PCC

PCC employs three or more chromatography columns to create a continuous purification step. In a PCC setup, columns are switched between the loading step and non-loading steps comprising column wash, elution, cleaning in place (CIP), and equilibration. At a predefined level of breakthrough, the primary column in the loading zone is disconnected from the loading zone and the load is redirected to the next column. In parallel, the disconnected, column will be washed, eluted, and regenerated.

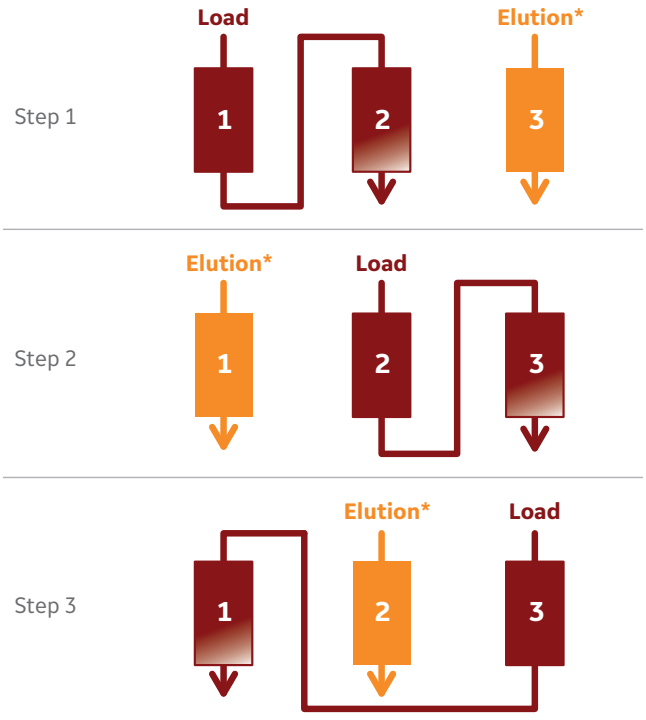

Fig 1. The principle of 3C PCC. Step 1: column 1 and 2 are loaded with sample (red). Step 2: column 1 has reached the defined level of breakthrough (BT) and is disconnected from the loading zone to be eluted, while column 2 becomes the first column in the loading zone. Step 3: column 2 has reached the defined BT level and column 3 becomes the first column in the loading zone. This procedure is repeated in a cyclic manner to achieve a continuous operation. *Elution phase in this figure includes wash, elution, strip, CIP, and re-equilibration.

Figure 1 gives an overview of a three-column PCC (3C PCC) operation. PCC increases use of available resin capacity by enabling loading to much higher breakthrough levels compared with batch chromatography (Fig 2).

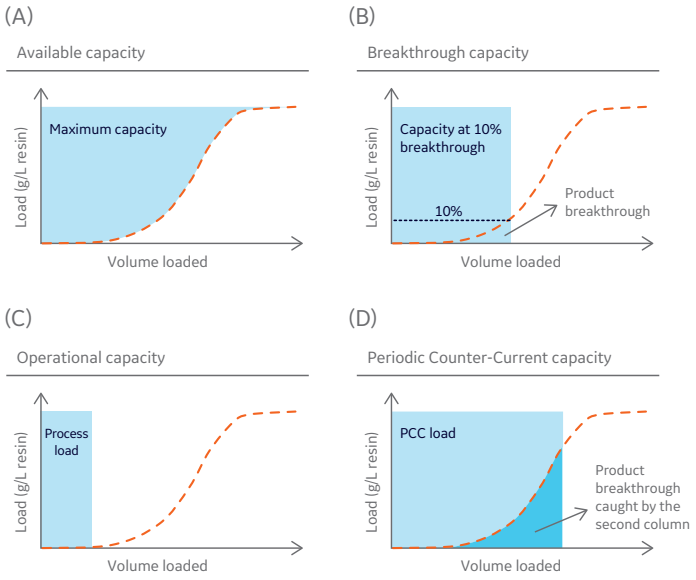

Fig 2. Capacity utilization for batch vs PCC. (A) Total available capacity of a chromatography resin. (B) The capacity typically measured during process development experiments. (C) The capacity typically utilized in manufacturing after adding safety factors. (D) Example of capacity utilized when implementing PCC. Note that product breakthrough is captured by the next column in the loading zone.

PCC using ÄKTA pcc chromatography system

ÄKTA pcc allows for continuous purification of target molecules. ÄKTA pcc is based on the well-established ÄKTA platform of chromatography systems designed to simplify system interaction and operational handling. Dynamic control functionality is a key feature of ÄKTA pcc. The dynamic control function allows monitoring and control of the column saturation level, which enables adjustments with regard to load in case of changes in feed composition and/or chromatography resin capacity. The system can also be operated with static control, that is, with predefined, fixed column switch times.

Principle of dynamic control

The principle of dynamic control is based on the relative difference in UV signals before and after the column at breakthrough. A schematic overview of the flow path of ÄKTA pcc is shown in Figure 3. The difference between the baseline UV and the UV signal at 100% breakthrough for a fully saturated column is defined as ΔUVmax (Fig 4). The level of breakthrough is defined as the percentage of ΔUVmax, where the desired level is process-dependent. The ÄKTA pcc system uses UV detectors assigned to the process stream and not to the separate columns. Hence, each breakthrough curve is generated based on signals from two UV detectors (Fig 5). ÄKTA pcc is controlled by UNICORN™ system control software. Dynamic control based on the ΔUV principle makes the system highly responsive to variability in the product stream, as can be the case with perfusion cell culturing, or in column performance.

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 chromatography resin capacity and concentration of monoclonal antibody (mAb) in the feed were used to verify the dynamic control functionality.

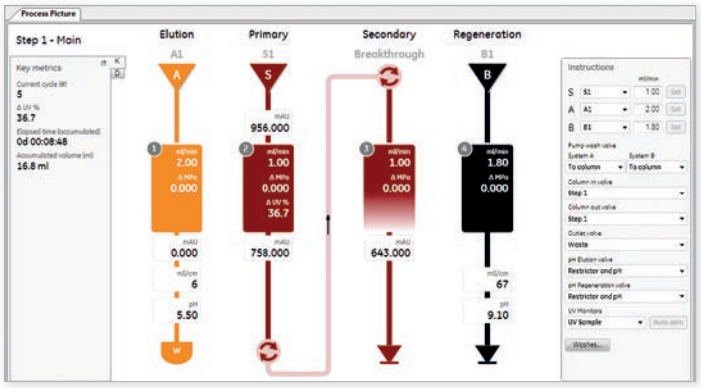

Fig 3. Process picture for ÄKTA pcc.

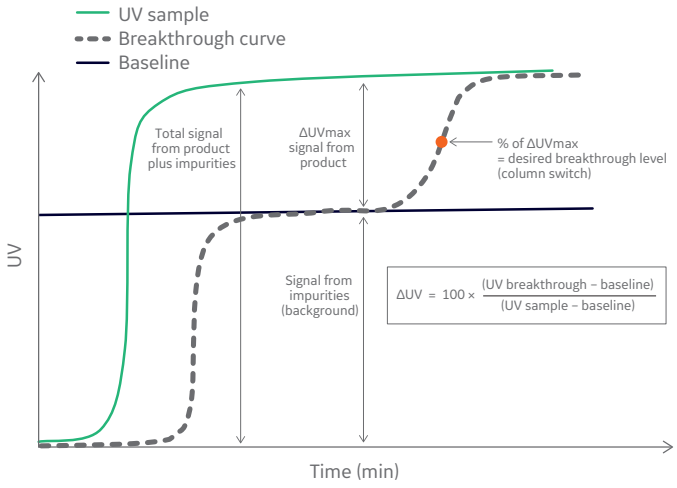

Fig 4. Overview of the two-step breakthrough, displaying the central UV signals used for dynamic control by the ÄKTA pcc system.

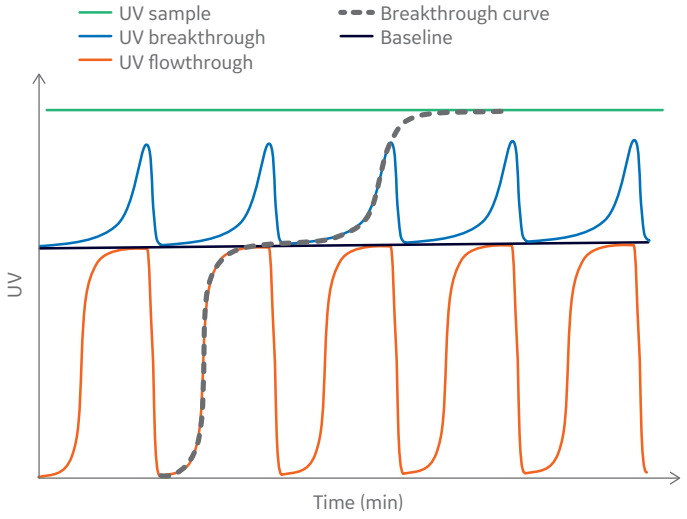

Fig 5. UV signal detectors used for dynamic control by ÄKTA pcc.

Materials and methods

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

Table 1. Running conditions for PCC experiments

Step	Buffer	Column volumes
Equilibration	Phosphate buffered saline, pH 7.4	5
Feed	Variable	Breakthrough (BT) level*
Wash 1	Phosphate buffered saline, 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	100 mM NaOH	3

*40% or 50% BT as listed per experiment.

Variable feed concentration

Studies of the ability of dynamic control to adjust for variations in the concentration of product in the feed were conducted by using both a stepwise and a gradient concentration change. For these experiments, HiTrap™ MabSelect SuRe™ LX, 5 mL columns were used.

The experiment with a stepwise change of the mAb concentration was performed by switching between two different concentrations of 1.5 and 1.8 g mAb/L during the run. Loading was performed to 50% breakthrough at 2.5 min residence time. Testing of the gradient concentration change was performed by loading using a gradient change from low (1 g mAb/L) to high (2 g mAb/L) mAb concentration, using BSA as background. Loading was performed to 40% breakthrough at 3.5 min residence time.

Variation in chromatography resin capacity

Studies of the ability of dynamic control to adjust for variable chromatography resin capacity were conducted using one HiScreen™ MabSelect SuRe column and two HiScreen MabSelect SuRe LX columns. The concentration of mAb in the feed used was 4.5 g/L. Loading was performed to 50% breakthrough at 5 min residence time.

Results

Variable feed concentration

To show the system's ability to adjust for changes in feed concentration, two different 3C PCC cases were considered:

1. Continuous change in feed concentration
2. Alternation between two feed concentrations

The dynamic control function of ÄKTA pcc was able to adjust for differences in feed concentration. As can be seen for the breakthrough signal (blue) in Figure 6A, the time, UV absorbance level, and steepness of the UV curve change as the concentration is changed between 1.8 mg/mL to 1.5 mg/mL. Still, breakthrough is consistently maintained at the defined ΔUV (pink) by the dynamic control functionality, and the difference in concentration is reflected only in the time before the breakthrough occurs. Consistency in sample load can be seen in the low variation in the amount of eluted mAb, with relative variations of less than 3% (Fig 6B).

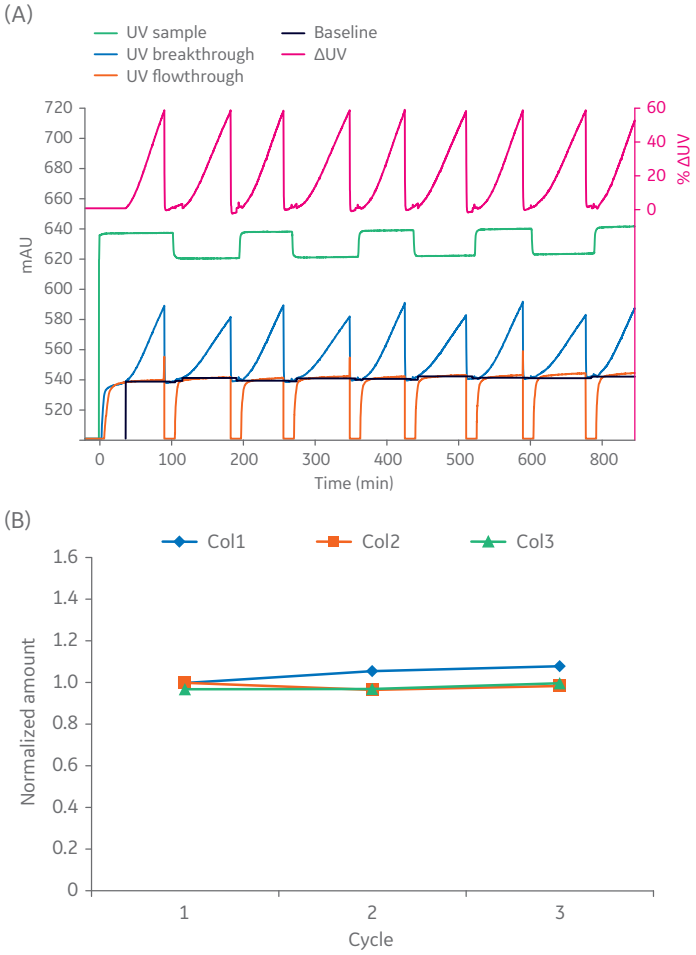

Fig 6. (A) Switching between feed concentrations of 1.5 and 1.8 g mAb/L during load onto MabSelect SuRe LX at a 2.5 min residence time. (B) Amount eluted mAb per column (Col1 to Col3) when switching between feed concentrations of 1.5 and 1.8 g mAb/L during load onto MabSelect SuRe LX at a 2.5 min residence.

As can be seen for the breakthrough signal (blue) in Figure 7A, a similar pattern can be observed when using a mAb concentration gradient from 1 g/mL to 2 g/mL. The time, UV absorbance level, and steepness of the UV curve closely follow the changes in mAb concentration, while breakthrough is consistently maintained at the defined ΔUV (pink). Also in this case, the variation in the amount of eluted mAb is low, with relative variations of less than 3% (Fig 7B).

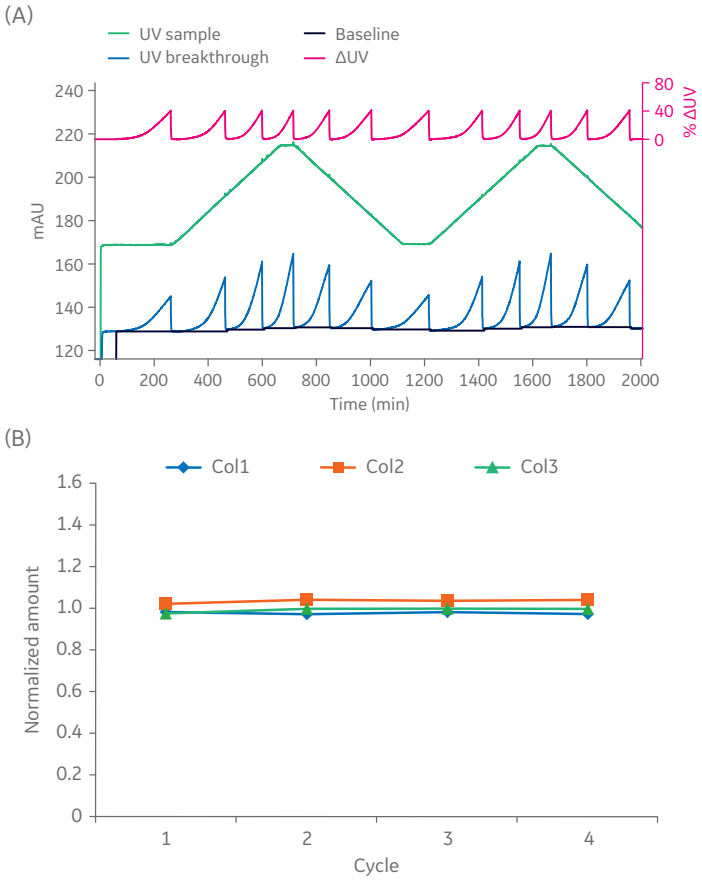

Fig 7. (A) Dynamic control when using a feed concentration gradient from 1 to 2 g mAb/L during load onto 5 mL HiTrap MabSelect SuRe LX columns at a 3.5 min residence time. (B) Amount eluted mAb per column (Col1 to Col3) when using a gradient from 1 to 2 g mAb/L during load onto MabSelect SuRe LX at a 2.5 min residence time.

Variation in chromatography resin capacity

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 equipped with one MabSelect SuRe column (Col1) and two MabSelect SuRe LX columns (Col2 and Col3) (Fig 8). A decrease in resin binding capacity can occur over time. Variations in resin binding capacity can also be due to differences in resin volume between packed column beds. MabSelect SuRe chromatography resin has lower binding capacity compared with MabSelect SuRe LX and was used here to mimic such a situation.

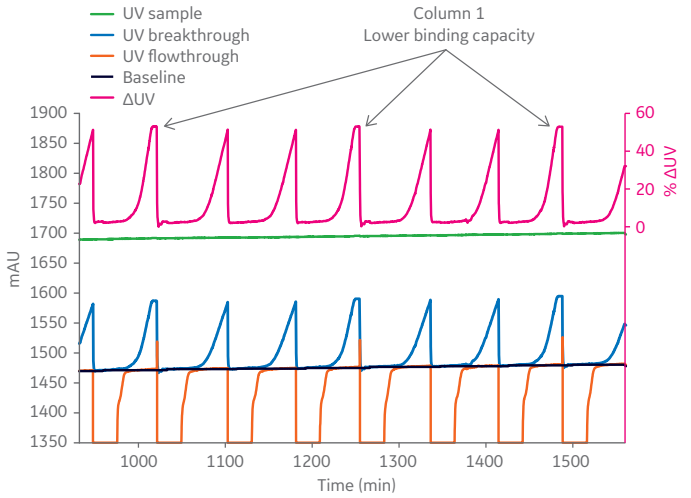

Fig 8. Difference in DBC between MabSelect SuRe (Col1) and MabSelect SuRe LX (Col2 and Col3) resin at 5 min residence time for a mAb concentration of 4.5 g/L. MabSelect SuRe reaches the set ΔUV faster than MabSelect SuRe LX, shown as the ΔUV signal plateau occurring every third load (arrows).

The dynamic control function of the ÄKTA pcc system detects the lower dynamic binding capacity (DBC) of Col1 (MabSelect SuRe) as the breakthrough occurs earlier on this column. In this case, the method was designed to halt the load until a new column is ready to be added to the loading zone. The halt in loading can be seen as a different peak shape in the chromatogram, as there is no change in UV during the halt and the UV peaks flatten out. These peaks, representing the breakthrough of the MabSelect SuRe column, are marked by arrows in the chromatogram.

The lower DBC of the MabSelect SuRe resin is reflected in the amount of eluted antibody from this column during each cycle (Fig 9). The dynamic control was able to adjust for differences in resin DBC between the columns. Despite the difference in DBC between the resins, the relative variance with regard to the amount eluted mAb per column was less than 3%. 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.

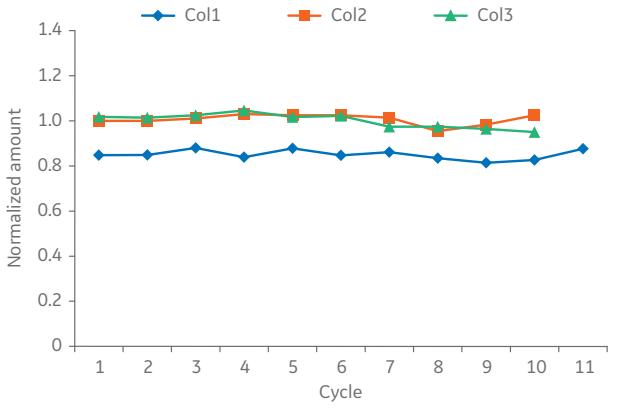

Fig 9. Amount eluted mAb per column (Col1 to Col3) and cycle.

Conclusions

The use of the dynamic control function enabled operation of the ÄKTA pcc system under process conditions where either the feed concentration or the chromatography resin capacity was changed. If static control with fixed load times had been used, changes in mAb concentration in the feed or binding capacity of the resin would have resulted in variations in eluted product. An increase in mAb concentration or decrease in binding capacity would have resulted in product loss. With dynamic control, on the other hand, differences in chromatography resin performance were detected and adjusted for. The ability of dynamic control to adjust for variations in feed concentration makes ÄKTA pcc well-suited for processing of perfusion cell culture feeds, where changes in the feed composition can occur. For additional examples of using dynamic UV control as a real time PAT tool, please see Chmielowski, *et al.* (1). They used this functionality to handle variations in cell culture feeds with titers between 3 and 31 g/L.

Reference

1. Chmielowski, R. A. *et al.* Definition and dynamic control of a continuous chromatography process independent of cell culture titer and impurities. *J Chromatogr A* **1526**, 58–69 (2017).

Ordering information

Product	Product code
ULTA Pure HC 0.2 µm, 4" TC	KMP-HC9204TT
HiScreen MabSelect SuRe column, 4.7 mL	28926977
HiScreen MabSelect SuRe LX columns, 4.7 mL	17547415
HiTrap MabSelect SuRe LX columns, 5 mL	29157185

ÄKTA pcc is offered through direct sales as a predefined custom system.
For quotes or more information, please contact your local sales representative.

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