ÄKTA pcc 75

CHROMATOGRAPHY SYSTEMS

ÄKTA™ pcc 75 chromatography system is developed for purification of target proteins in continuous downstream processes using periodic countercurrent chromatography (PCC) at process development scale (Fig 1). The technology employs three or four chromatography columns to create a continuous purification step. In a PCC setup, columns are switched between the loading and non-loading steps such as wash and elution. Continuous chromatography supports process intensification by reducing footprint and improving productivity. In addition, continuous chromatography is especially suited for purification of unstable molecules, as the short process time helps ensure stability of your target product.

Key benefits of ÄKTA pcc 75 include:

- Dynamic control functionality to automatically adjust for variations in resin binding capacity, differences in column volume, or changes in feed composition
- Real-time monitoring of process performance through trend curves for UV, amount of target molecule in elution peaks, and sample volume loaded on each column per cycle
- PCC method design tool to support transition from batch to continuous processing
- Post load wash functionality to avoid product loss when switching columns
- Extensive UV capabilities to cover a broad feed range

General system description

ÄKTA pcc 75 is based on the well-established ÄKTA platform of chromatography systems designed to simplify system interaction and operational handling. The system consists of the ÄKTA pcc instrument and UNICORN™ system control software. The instrument offers easy access to working areas using a swivel foot, and has a modular design, with all valves, monitors, and columns mounted on the wet side of the instrument. The wet side allows easy interaction with the system, and has a door and pump cover for safer handling during runs. A buffer tray on top of the instrument provides a large storage area for vessels and bottles (Fig 2). On the front side of the instrument, ÄKTA pcc 75 has a built-in, cooled fraction collector that provides secure product storage. A display on the front panel informs you of the current instrument and method state, and together with the process picture displayed on the system control computer, this allows you to easily monitor and control your runs.

Fig 1. ÄKTA pcc 75 chromatography system.

Fig 2. The wet side of ÄKTA pcc 75 allows easy interaction with the system.

Principles of PCC

Compared with batch operations, continuous chromatography can offer:

- Productivity gains through increased utilization of chromatography resin binding capacity
- Reduced footprint of system, buffer, and columns through increased production capacity
- Continuous bioprocessing by integration of upstream and downstream unit operations
- Process robustness and control by steady-state operation
- Reduced process time and less hold steps, for example, to ensure stability of delicate target molecules

In PCC, keeping two columns in the loading zone allows for overloading of the first column without the risk of product loss, as the breakthrough will be caught by the second column (Fig 3).

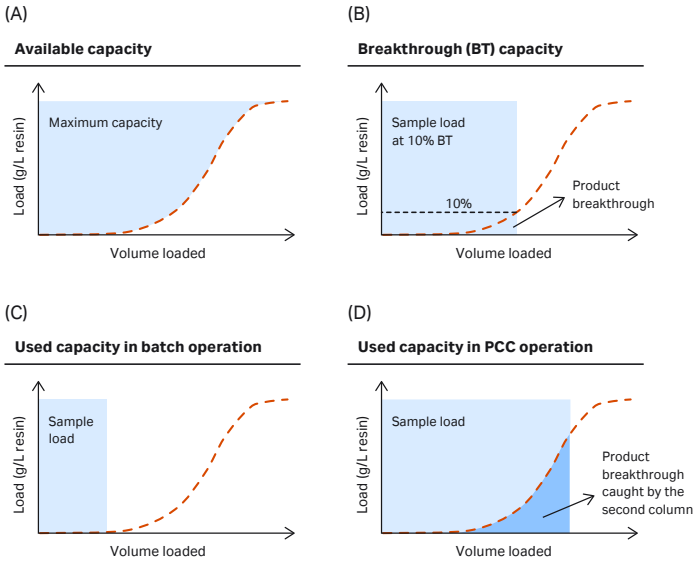


Fig 3. Capacity utilization for batch capture chromatography vs PCC. (A) Total available capacity of a chromatography resin. (B) The capacity typically measured during process development experiments. (C) The capacity typically utilized in manufacturing after adding safety factors. (D) Example of capacity utilized when implementing PCC. Note that product breakthrough is captured by the next column in the loading zone.

ÄKTA pcc 75 can be used in a three-column (3C) or four-column (4C) PCC setup. The 3C PCC setup features two parallel flows: one for loading of the two columns in the loading zone and one for the non-loading steps, for example, elution and regeneration of the third column (Fig 4). In step one, column 1 and 2 are in the loading zone. Column 1 can be overloaded without sample loss, as column 2 catches the breakthrough from column 1. In this way, the utilization of the resin binding capacity is maximized. In the second step, the overloaded column 1 is switched and column 2 becomes the first column and column 3 becomes the second column in the loading zone. The overloaded column 1 will now be subjected to the non-loading steps, such as elution and regeneration in a parallel workflow. In step three, the overloaded column 2 in the loading zone is switched. Now, column 3 becomes the first column and column 1 the second column in the loading zone, while column 2 is subjected to elution and regeneration in the parallel workflow. These three steps are repeated in a cyclic manner until required sample volume is reached (or until resin lifetime is reached and column needs to be repacked or exchanged).

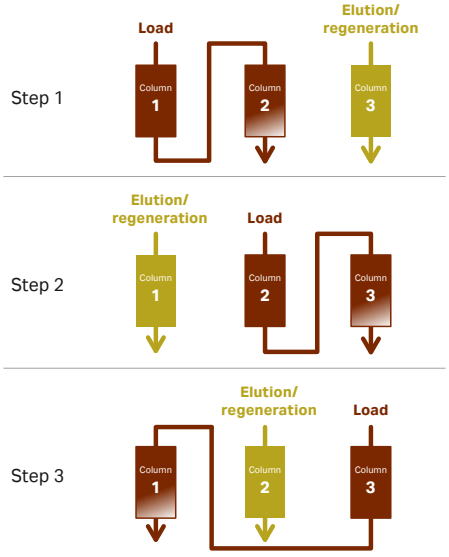


Fig 4. Principle of 3C PCC workflow.

The 4C PCC setup employs the same principle as the 3C PCC setup. However, as the non-loading steps can become limiting in a 3C PCC setup (Fig 4), these steps can be split on two columns and run in parallel utilizing a third flow path in the 4C PCC setup (Fig 5). A 4C PCC setup allows for balancing the loading and non-loading steps.

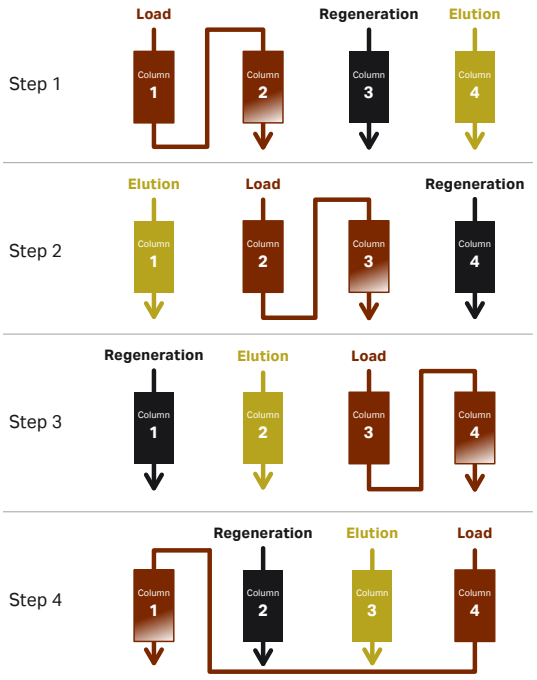


Fig 5. Principle of 4C PCC workflow.

Dynamic control

One of the key features of ÄKTA pcc 75 is the dynamic control function, in which UV detectors are used to monitor and dynamically control the switching of columns at a predefined level of sample breakthrough. The ability to control the column loading automatically makes the system highly responsive to variability in the product stream, as can be the case with perfusion cell culturing, or in column performance (e.g., compensation for loss of resin binding capacity, differences in bed volume between columns or in sample concentration over time). The principle of dynamic control is based on the relative difference in UV signals before and after the first column in the loading zone at breakthrough. A schematic overview of the flow path of ÄKTA pcc 75 is shown in the process picture in Figure 6. The difference between the baseline UV and the UV signal at 100% breakthrough for a fully loaded column is defined as ΔUVmax (Fig 7). The level of breakthrough is defined as percentage of ΔUVmax, where the desired level is process-dependent. The ÄKTA pcc 75 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 8).

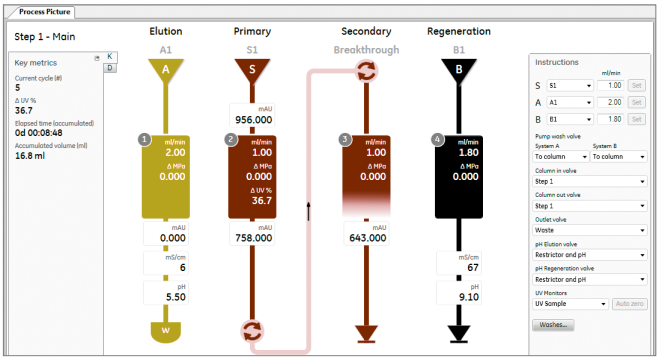


Fig 6. Process picture for the 4C PCC system setup.

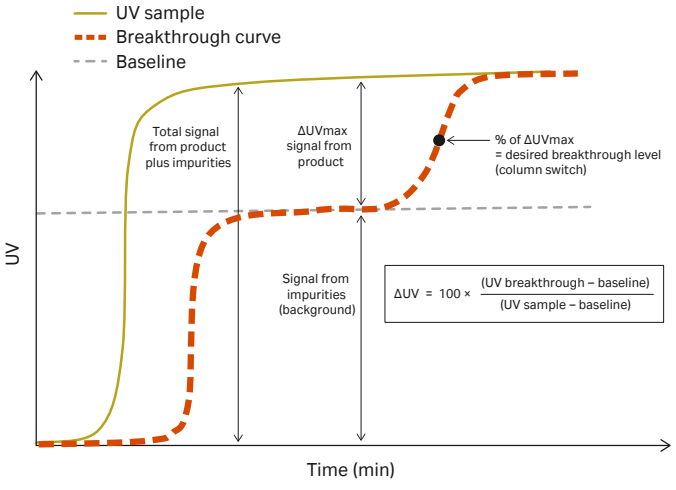


Fig 7. Overview of the two-step breakthrough, displaying the central UV signals used for dynamic control by the ÄKTA pcc 75 system.

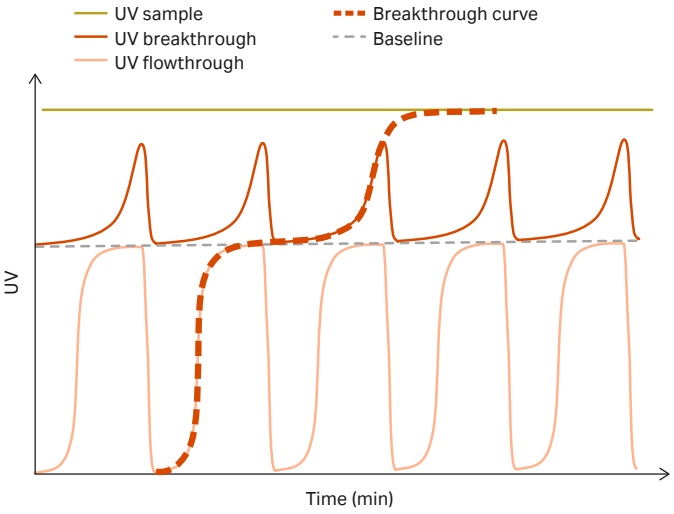


Fig 8. UV signal detectors used for dynamic control by ÄKTA pcc 75.

Trend curves

Real-time monitoring of process performance through trend curves adds an additional level of control of the continuous runs. Trending during runs is available for UV, amount of target molecule in elution peaks, and sample volume loaded on each column in each cycle.

PCC method design tool

Included with the ÄKTA pcc 75 is a PCC method design tool that supports transition from batch to continuous processing (Fig 9). Using a batch mode breakthrough curve, the tool enables you to theoretically vary your parameters to reach an optimal purification step based on your criteria. The method can, for example, be optimized for productivity or process time. Note that the PCC method design tool is a model and suggested process parameters need to be verified through actual laboratory runs.

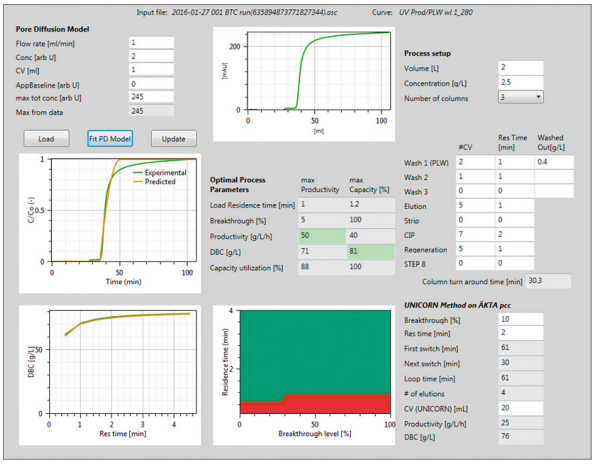


Fig 9. PCC method design tool.

Post-load wash

When the first column in the loading zone has reached its breakthrough level, there is a risk of losing unbound product if changing the flow to drain during wash of the overloaded column. As shown in Figure 10, this loss can be minimized by performing a post-load wash of the overloaded column into the regenerated column before adding the regenerated column to the loading zone.

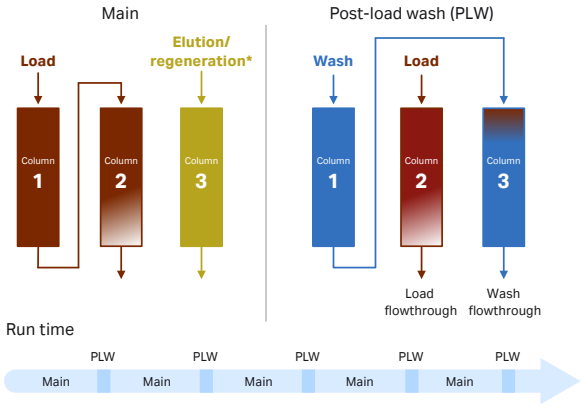


Fig 10. Post-load wash minimizes product loss during the wash step.
* Includes wash, elution, strip, CIP, re-equilibration.

UV capabilities

Monitors and sensors are included in all flow paths to keep control of your chromatography run (Fig 11). ÄKTA pcc 75 features extensive UV capabilities, including three to four UV LEDs (280 nm), of which two are used for dynamic control and one triple wave length UV for detection of elution peaks. Both 2 mm and 0.4 mm UV LED cells are available to support dynamic control of a broader feed range.

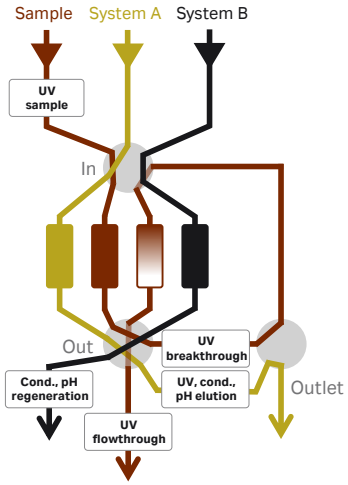


Fig 11. Positioning of monitors and sensors in the flow path of the ÄKTA pcc 75 system.

For example, the 0.4 mm cell covers samples with higher titers and background signals from the cell culture feed, as it lowers the absorbance level to be within the linear range of the detector and therefore gives reliable delta UV measurements (Fig 12).

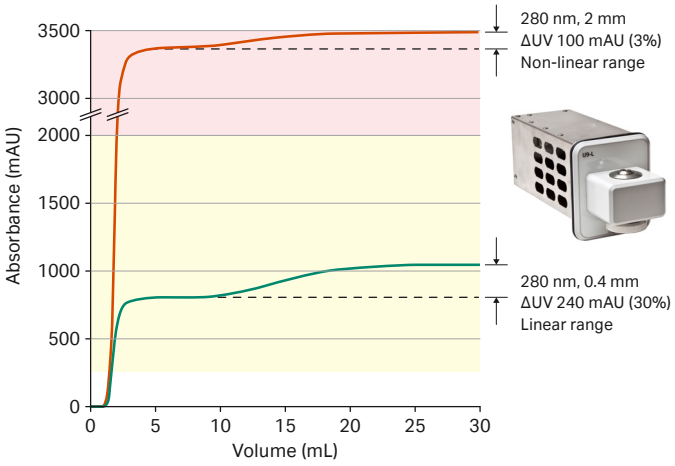


Fig 12. Different UV path lengths to cover a broader feed range.

UNICORN system control software

The well-established UNICORN software gives you real-time control of your chromatography system and includes valuable tools for increasing operational security, efficiency, and productivity. To learn more about the different features of the UNICORN software, please refer to data file 29135786AB.

Application examples

Continuous chromatography in downstream processing of a monoclonal antibody

PCC and straight-through processing (STP) technologies were evaluated in a continuous three-step monoclonal antibody (mAb) purification process. Antibody capture was performed using MabSelect SuRe™ LX protein A chromatography resin in a 3C PCC setup on the ÄKTA pcc 75 system. To assess robustness of the setup, 10 cycles were performed. The results showed consistent yield and purity over time. Using the 3C PCC setup, the capacity utilization could be increased by 56% as compared with an equivalent batch run for the mAb purified.

The capture step was followed by two polishing steps in an STP setup on the ÄKTA pure chromatography system. Using continuous chromatography, the results show similar yield and purity as can be expected when the individual unit operations are run in batch mode, while increasing utilization of the chromatography resin capacity, eliminating the need for intermediate hold-up tanks, and reducing equipment footprint.

For additional information, see application note 29170800AA.

Dynamic control to adjust for variable feed concentration

To show the ability of the dynamic control functionality of ÄKTA pcc 75 to adjust for changes in feed titers, an experiment was set up in which the feed was altered between two different mAb concentrations. As shown in Figure 13, the dynamic control functionality of ÄKTA pcc 75 was able to adjust for the difference in feed concentration during the run. As can be seen for the breakthrough signal (Fig 13A), the time, UV absorbance level, and steepness of the UV curve change as the mAb

concentration is changed between 1.8 mg/mL and 1.5 mg/mL. Still, breakthrough is consistently maintained at the defined ΔUV (Fig 13A) 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 13B).

For additional information, see application note 29169950AA.

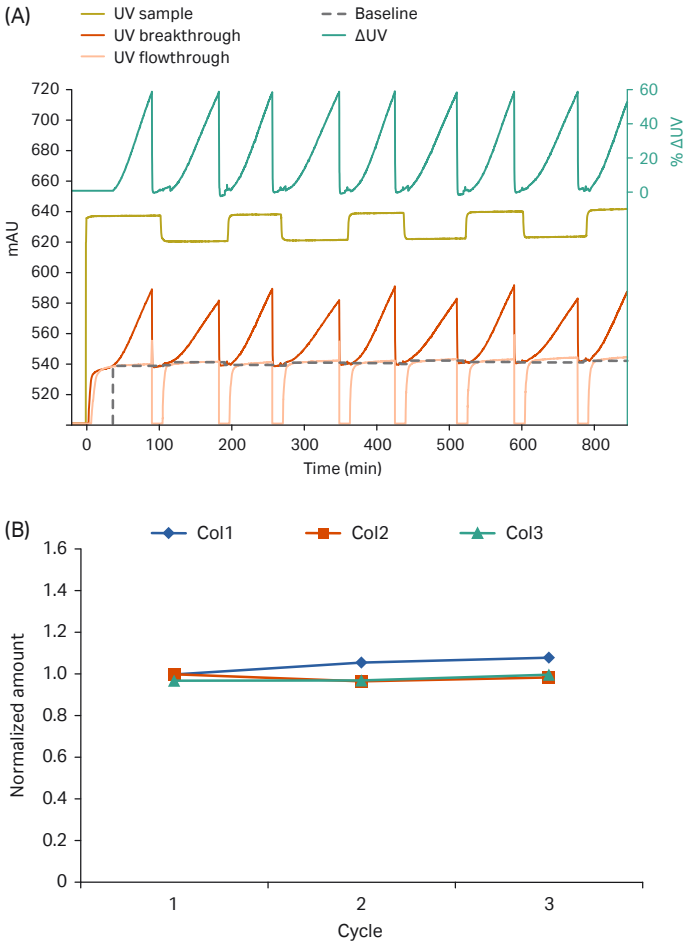


Fig 13. (A) Switching between feed concentrations of 1.5 and 1.8 g mAb/L during load onto MabSelect SuRe LX at a 2.5 min residence time. (B) Amount eluted mAb per column (Col1 to Col3) when switching between feed concentrations of 1.5 and 1.8 g mAb/L during load onto MabSelect SuRe LX at a 2.5 min residence.

Dynamic control to adjust for variations in chromatography resin capacity

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. To mimic such a situation, an ÄKTA pcc 75 equipped with one MabSelect SuRe column (Col1) and two MabSelect SuRe LX columns (Col2 and Col3) was used in a 3C PCC setup to show the system's ability to adjust for changes in column performance (Fig 14).

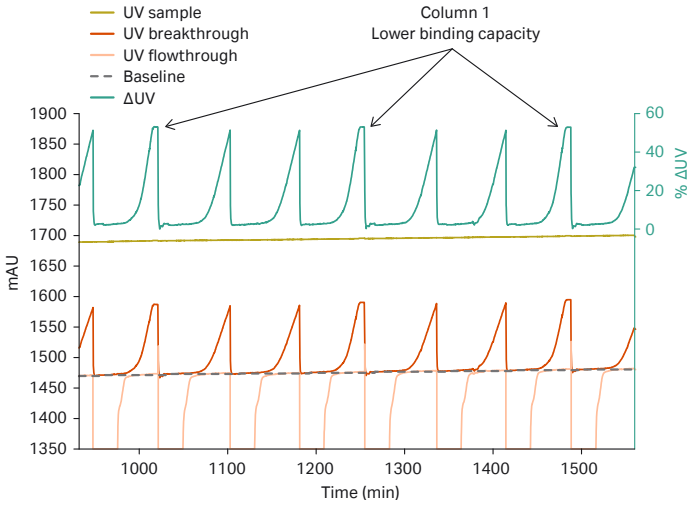


Fig 14. Difference in DBC between MabSelect SuRe (Col1) and MabSelect SuRe LX (Col2 and Col3) resins at 5 min residence time for a MAb concentration of 4.5 g/L. MabSelect SuRe resin reaches the set ΔUV faster than MabSelect SuRe LX, shown as the ΔUV signal plateau occurring every third load (arrows).

MabSelect SuRe resin has lower dynamic binding capacity (DBC) compared with MabSelect SuRe LX. The system detects the lower DBC of Col1 (MabSelect SuRe) as the breakthrough occurs earlier on this column. The dynamic control functionality was able to adjust for the difference 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%.

For additional information, see application note 29169950AA.

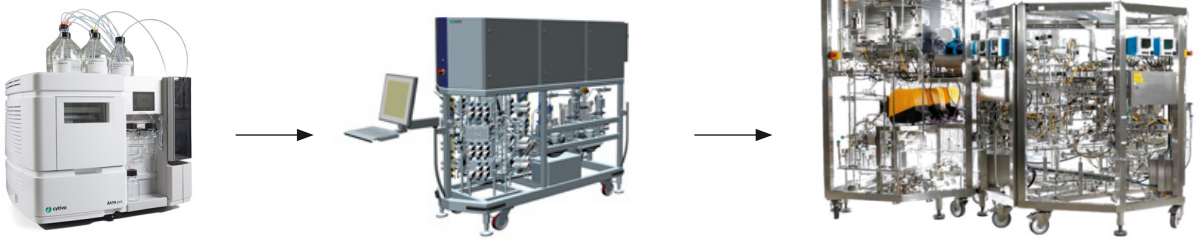
Integration of continuous upstream and downstream operations in mAb production

Process intensification is gaining interest as a strategy to reduce production costs, while improving product quality and throughput in the manufacturing of biopharmaceuticals. For a competitive production process, continuous or semi-continuous upstream and downstream processing can be employed. In a study, the integration of a high-performing upstream cell culture process with downstream purification utilizing emerging technologies such as PCC and STP was demonstrated. The developed mAb production and purification process, performed in a continuous manner, showed a performance equivalent to traditional processing performed in batch runs, while adding the benefits of reduced equipment footprint and eliminated need for intermediate hold-up steps.

For additional information, see poster 29208615AA.

Proven process scale-up

Cytiva's offering of process-scale PCC systems allows for scaling of the continuous chromatography process (Fig 15). Customized process-scale systems are available on request.



ÄKTA pcc 75

BioProcess™ pcc 3/8" SS
4 columns, multipath

BioProcess pcc ½" SS
4 columns, multipath, SIP

Size	Flow rate	No. of columns
ÄKTA pcc 75	1–75 mL/min	3 or 4 columns
BioProcess pcc, 4.7 mm PP or ¼" SS	1–60 L/h	3 or 4 columns
BioProcess pcc, 6 mm PP, 3/8" SS	4–180 L/h	3 or 4 columns
BioProcess pcc, 10 mm PP, ½" SS	15–600 L/h	3 or 4 columns
BioProcess pcc, 16.2 mm PP, ¾" SS	30–1000 L/h	3 or 4 columns
BioProcess pcc, 20.4 mm PP or 1" SS	45–2000 L/h	3 or 4 columns

Fig 15. Cytiva's scalable PCC solutions.

Related products

MabSelect SuRe pcc chromatography resin

MabSelect SuRe pcc affinity resin offers exceptional capacity at short residence time, making it well-suited for applications requiring fast mass transfer such as mAb capture in a continuous process.

The base matrix of MabSelect SuRe pcc combines a porosity optimized for mAbs with a rigidity that delivers good pressure/flow properties. These features provide the ability to run the medium at high flow rates, thereby increasing productivity of continuous capture steps. The small bead size also creates possibilities for high-resolution purification, for example, for purification of bispecific antibodies.

MabSelect SuRe pcc is part of Cytiva's program for custom designed resisn and is available in bulk as well as in prepacked columns. For additional information see, data file 29177558AA.

Key benefits of MabSelect SuRe pcc include:

- Exceptional binding capacity (e.g., ~ 60 g IgG/L medium at 2.4 min residence time)
- High productivity through capture of large mass of mAbs in a short period of time
- Highly concentrated elution pools for operating flexibility and small unit operations
- Allows cost-effective cleaning with 0.1–0.5 M NaOH over hundreds of purification cycles
- High ligand stability reduces ligand leakage
- Generic elution conditions for different mAbs, enabling platform purifications, increased productivity, and easy scale-up

Chromatography columns

Cytiva offers a variety of chromatography columns for use in PCC setups, such as the small-scale HiTrap™ and HiPrep™ prepacked columns and the larger-scale HiScale™ and AxiChrom™ columns. Recommended column sizes range from 1 mL columns to columns with inner diameters of 50 mm and bed heights of up to 10 cm.

System specifications

System specifications are listed in Table 1.

Table 1. ÄKTA pcc 75 system specifications

System configuration	Benchtop system, external computer
Control system	UNICORN 7 or later version
Connection between PC and instrument	Ethernet
Dimensions (W × D × H)	860 × 710 × 660 mm
Weight (excluding computer)	119 kg
Power supply	100 to 240 V~, 50 to 60 Hz
Power consumption	800 VA
Enclosure protective class	IP 21, wet side IP 22
Tubing and connectors	
Inlet	Teflon tubing, 2.9 mm i.d., 5/16–24 UNF connections
Pump to outlet valve	PEEK tubing, 1.00 mm i.d., 10–32 UNF connections
Environmental ranges	
Storage and transport temperature	-25°C to 60°C
Chemical environment	See User manual
Operating ranges	
Temperature	4°C to 35°C
Relative humidity	20% to 95%, non-condensing

Technical specifications

Pumps

Pump type	Piston pump, metering type
Flow rate setting	0.01 to 75 mL/min
Pressure range	0 to 2 MPa (290 psi)
Viscosity range	0.7 to 10 cP
Flow rate specifications	
Conditions	1.0 to 75 mL/min, 0.8 – 2 cP
Accuracy	± 1.5%
Precision	RSD < 0.5%

Mixer

Mixing principle	Chamber with a magnetic stirrer (3 column configuration)
Mixer volume	1.4 mL 5 mL 15 mL (accessory)

Note: 15 mL mixer chamber can be added on request. Mixer applicable for 3 column configuration only

Gradient formation

Gradient flow rate range	Binary: 1 to 75 mL/min (3 column configuration)
Gradient composition accuracy	Binary: ± 0.5%

Note: gradient formation functions available for 3 column configuration only.

Valves

Type	Rotary valves
Functions	Inlet A, Inlet B, Inlet Sample, Pump wash, Column, pH, Outlet

Number of inlets

Inlet A	8
Inlet B	8
Inlet Sample	8

Pressure sensors

Placement of sensors	Before and after columns
Range	0 to 2 MPa (290 psi)
Accuracy	± 0.015 MPa or ± 1.5% whichever is greater

Air sensors

Placement	Inlet A, Inlet B, Inlet Sample
Optional placement	After sample pump
Sensing principle	Ultrasonic

Elution UV monitor

Placement	After elution column
Wavelength range	190 to 700 nm in steps of 1 nm, up to 3 wavelengths simultaneously
Absorbance range	-6 to 6 AU
Linearity	within ± 2% at 0 to 2 AU
Operating pressure	0 to 2 MPa (290 psi)
Flow cells	2 mm optical path length, 2 µL cell volume 0.5 mm optical path length, 1 µL cell volume (accessory) 10 mm optical path length, 8 µL cell volume (accessory)

Note: 0.5 and 10 mm UV cell can be added to the quote on request

UV LED

Placement	Before first column in the loading zone (UV Sample), between first and second column in the loading zone (UV Breakthrough), and after the second column in the loading zone (UV Flowthrough) column (3 and 4 column configuration) After regeneration column (UV Regeneration) optional in (4 column configuration).
Wavelength range	Fixed 280 nm
Absorbance range	-6 to 6 AU
Linearity	280 nm: within ±5% at 0 – 2 AU
Operating pressure	0 to 2 MPa (290 psi)
Flow cells	2 mm optical path length 0.4 mm optical path length (accessory) 5 mm optical path length (accessory)

Note: 0.4 and 5 mm UV cell can be added on request

Conductivity monitor	
Placement	
After elution column	3 and 4 column configuration
After regeneration column	4 column configuration
Conductivity reading range	0.01 to 999.99 mS/cm
Accuracy	± 0.01 mS/cm or ± 2%, whichever is greater (within 0.3 to 300 mS/cm)
Operating pressure	0 to 2 MPa (290 psi)
Flow cell volume	22 µL

Temperature monitor	
Placement	
After elution column	3 and 4 column configuration
After regeneration column	4 column configuration
Reading range	0°C to 99°C
Accuracy	± 1.5°C within 4°C to 45°C

pH monitor	
Placement	
After elution column	3 and 4 column configuration
After regeneration column	4 column configuration
pH reading range	0 to 14
Accuracy	± 0.1 pH unit (within pH 2 to 12, temp. within 3°C from calibration temp.)
Operating pressure	0 to 0.5 MPa (72 psi)
Flow cell volume	129 µL

Outlet valve fractionation	
Placement	After elution column
Number of outlets	8
Delay volume (UV to outlet valve)	535 µL

Fraction collector	
Placement	After elution column
Number of fractions	up to 576
Vessel types	3, 8, 15 or 50 mL tubes
Deep-well plates	96 / 48 / 24
Bottles	250 mL
Vessel type selection	Automatic recognition
Fraction volumes	1 to 250 mL
Spillage-free modes	Accumulator
Protection of fractions	Covered vessels and climate control (settable 6°C to 20°C)
Organic solvents	No
Delay volume (UV to dispenser head)	1807 µL

Ordering information

ÄKTA pcc 75 is offered through direct sales as custom systems. For quotes or more information, please contact your local sales representative.

Related literature	Product code
Application note: Continuous chromatography in downstream processing of a monoclonal antibody	29170800
Application note: The use of dynamic control in periodic counter-current chromatography	29169950
Poster: Integration of continuous upstream and downstream operations in mAb production	29208615
Data file: UNICORN 7	29135786
Data file: MabSelect SuRe pcc	29177558

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