# Optimizing Productivity of Multi-Column Continuous Chromatography for Processing High-titer Feed Streams

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## Introduction

The benefits of simulated moving bed (SMB) or multicolumn continuous chromatography (MCC) for increasing the productivity of separations have been known for over 60 years. When compared with single column batch systems, MCC offers:

- Smaller columns: lower adsorbent volume
- Higher throughput: operation at “overload” conditions with high purity and recovery
- Process flexibility: adjustable column number, size and configuration to suit feed and adsorbent properties and run time requirements
- Continuous operation

Gottschlich and Kasche described an MCC process for mAb Protein A capture in 1996 (1). With the recent development of hundreds of therapeutic mAb candidates, steady increase in upstream titers, and current high cost of manufacture, the Protein A capture step has become a primary target for MCC process improvement.

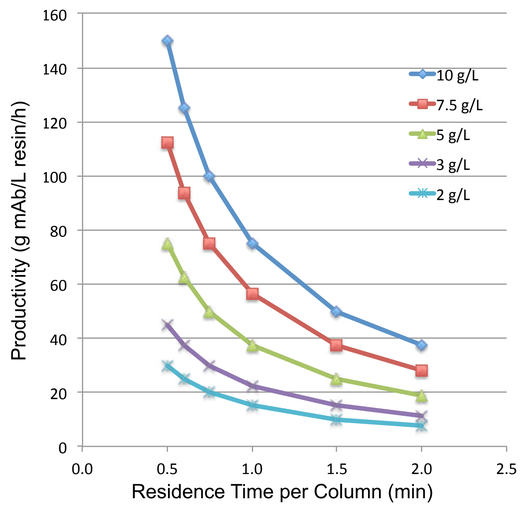
One major challenge for achieving maximum productivity is driving mAb adsorption to the full static binding capacity of the resin as fast as possible while maintaining high recovery. MCC makes this possible by including multiple columns in the capture zone, which allows recovery of the breakthrough fraction from the first column on one or more columns connected downstream. Several processes and detailed models have been described for optimizing MCC Protein A capture, with wide variation in predicted and realized productivities (2–5). Much of the variation can be attributed to differences in the assumptions used for the models (e.g. mAb titer, adsorbent and hydrodynamic properties of the resin, residence and regeneration times).

## Residence Time

The productivity of a Protein A capture process is driven by residence time. The chart shows the theoretical productivity achieved from an MCC process at different mAb feed titers, assuming 100% yield and no restraints on the process parameters.

Gains in productivity are observed as residence times decrease, and are most dramatic at titers above 3 g/L.

In practice, three interdependent factors restrain the ability to achieve low residence times with maximum productivity.



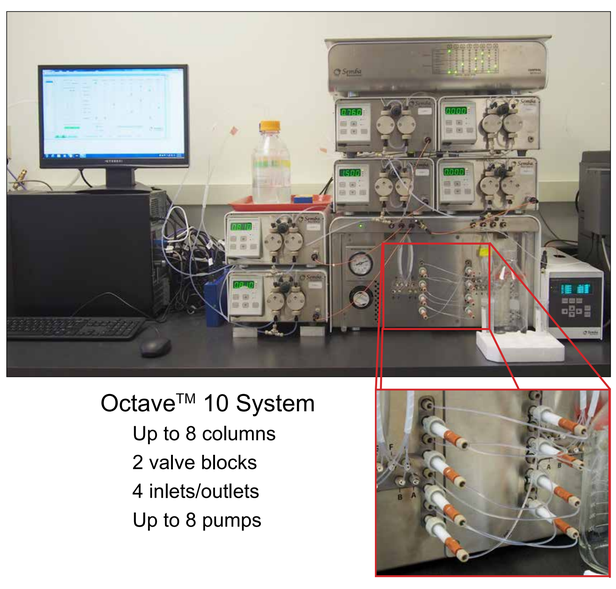

**Restraints** on residence time:

1. Limitation of **flow velocity** due to pressure drop through the columns.
2. Dynamic binding capacity limitations due to **mass transfer** characteristics of the adsorbent.
3. Process time constraints due to an insufficient **number of columns**.

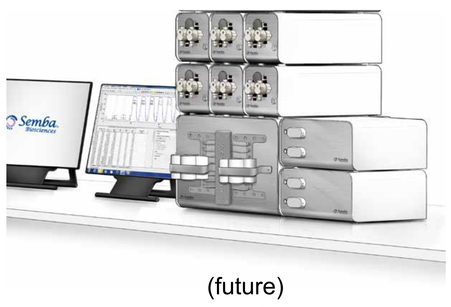
## Process Development

We have taken an experimental approach to optimize productivity using single column binding data, a simple Excel-based modeling tool, and small-scale MCC.

1. Determine dynamic and static binding capacities of the Protein A adsorbent; single column breakthrough analysis for DBC, saturation binding for SBC.
2. Model MCC process at various configurations (e.g. number of columns, zone flow pattern).
3. Test process models and optimize parameters using bench top MCC instrument (Octave 10 System).



Octave™ 10 System
Up to 8 columns
2 valve blocks
4 inlets/outlets
Up to 8 pumps



(future)
Semba ProPD™ System
Semba ProGMP™ System
Up to 8 columns
2 valve blocks
6 inlets/outlets
6 pumps

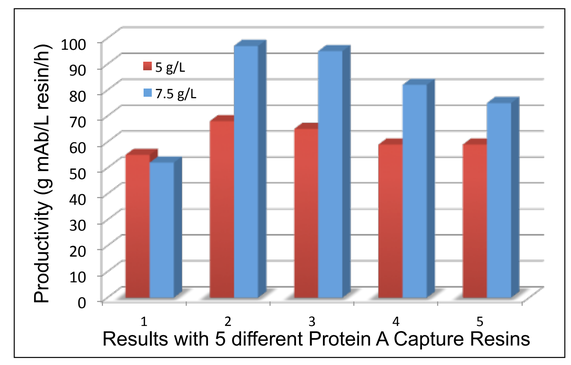
## Process Modeling

| Column Properties | | |
|---|---|---|
| Column diameter, cm | 0.68 | Blue entries = user input |
| Column length, cm | 2.80 | |
| Column volume, ml | 1.00 | ← $V_C$ |

| Adsorbent Properties | | |
|---|---|---|
| Maximum linear velocity allowed, cm/h | 1000 | ← Max flow of non-loading steps; based on hydrodynamic properties of the support media |
| Maximum flow rate, ml/min | 5.96 | |
| Static binding capacity (SBC), mg/ml | 72 | ← $K_S$ Measured SBC |

| Loading Properties | | |
|---|---|---|
| Residence time per column, min | 0.50 | ← $R_T$ Adjusted to desired flow of loading step based on breakthrough and pressure drop data |
| Loading flow rate, ml/min | 2.00 | |
| Linear velocity of loading step, cm/h | 336 | |
| Binding capacity per column, mg | 72.1 | |
| Loading ratio, % of SBC | 100% | ← Adjustment for resin capacity utilization |
| Target concentration in Feed, mg/ml | 7.50 | ← $C_F$ |
| Target loaded per column, mg | 72.1 | |
| Total loading time, sec | 288 | ← $T_{Load} = \frac{K_S R_T}{C_F}$ |
| Number of substeps during loading | 2 | |
| Step Time, sec | 144 | |

| Process Properties | |
|---|---|
| Total amount of Feed to be processed, ml | 300 |
| Cycle time, h | 0.64 |
| Amount of Target processed per cycle, mg | 577 |
| Volume Feed processed per cycle, ml | 77 |
| Number of cycles to complete process | 4.46 |
| Time to complete process, h | 2.86 |
| Target yield @ 100% recovery, g | 2.25 |
| Productivity @ 100% recovery, g/L resin/h | 98.3 |
| Steady-state productivity @ 100% recovery | 112.5 |
| [Purified Target] @ 100% recovery, mg/ml | 10.5 |
| Purified Target volume, ml | 215 |




| Process Step | Col/Zone | CV | Time, s | Flow Rate | Inlet | Pump | Vol, ml | Substep |
|---|---|---|---|---|---|---|---|---|
| Load | 3 | N/A | 288 | 2.00 | A | 1 | 300 | A&B |
| Wash | 2 | 10.0 | 288 | 2.09 | C | 3 | 358 | A&B |
| Elute | 1 | 6.0 | 144 | 2.50 | B | 2 | 215 | A&B |
| Clean | 1 | 4.0 | 144 | 1.67 | D | 5 | 143 | A |
| Equilibrate | 2 | 10.0 | 144 | 4.17 | D | 4 | 401 | B |

Flow rates of non-loading steps calculated based on $T_{Load}$, CV, and limited by maximum linear velocity and/or pressure drop tolerated by the support media.

Inlet assignments based on 4 inlets available on the Octave System.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1A | P1 A Col 1 | Col 2 | Col 3 | P5 D Col 4 | Col 5 | P2 B Col 6 | P3 C Col 7 | Col 8 |
| | Load | Load | Load | | CIP | Elute | Wash | Wash |
| 1B | P1 A Col 1 | Col 2 | Col 3 | P4 D Col 4 | Col 5 | P2 B Col 6 | P3 C Col 7 | Col 8 |
| | Load | Load | Load | Equil | Equil | Elute | Wash | Wash |

## Process Flexibility

Various titers and individual properties of target mAbs or other proteins, adsorbent media, column sizes, and feed volume may require the use of different numbers of columns and flow configurations to achieve optimal productivity, purity, and recovery. For example, the use of Protein A support media having better flow characteristics while retaining high binding capacity would enable flow rates over 1000 cm/h and corresponding productivities over 200 g/L/h (5, 6). Pressure drop becomes more important as column size increases with scale up. Process equipment should be flexible enough to accommodate these needs without undue complexity or cost.

| Process Step | Col/Zone | CV | Time, s | Flow Rate | Inlet | Pump | Vol, ml | Outlet | Substep |
|---|---|---|---|---|---|---|---|---|---|
| Load | 3 | N/A | 292 | 314.16 | A | 1 | 100000 | H | A |
| Purge | 3 | 1.0 | 58 | 161.38 | B | 6 | 65885 | H | B |
| Wash | 2 | 8.0 | 350 | 107.59 | C | 2 | 43923 | I | A&B |
| Elute | 1 | 5.5 | 350 | 147.93 | D | 3 | 60395 | G | A&B |
| Clean | 1 | 4.0 | 350 | 107.59 | E | 4 | 43923 | K | A&B |
| Equilibrate | 1 | 6.0 | 350 | 161.38 | F | 5 | 74141 | L | A&B |

8-column configuration for processing 100 L feedstock at 7.5 g/L with 5 x 8 cm columns for 9 cycles in 6.8 hours. Process includes a brief purge substep to recover void volume of the first column in the load zone (increases yield 5-10%).

System has 6 inlets, 6 outlets and 6 pumps.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1A | P1 A Col 1 | Col 2 | Col 3 | P5 F Col 4 | P4 E Col 5 | P3 D Col 6 | P2 C Col 7 | Col 8 | |
| | Load | Load | Load | Equil | CIP | Elute | Wash | Wash | |
| 1B | P6 B Col 1 | Col 2 | Col 3 | P5 F Col 4 | P4 E Col 5 | P3 D Col 6 | P2 C Col 7 | Col 8 | |
| | Purge | Purge | Purge | Equil | CIP | Elute | Wash | Wash | |

| | | | | | |
|---|---|---|---|---|---|
| 1A | P1 A Col 1 | Col 2 | P4 E Col 3 | P2 C Col 4 | 4-column configuration for the same process protocol; expect longer $R_T$ and run time, lower productivity, but lower pressure drop and column cost. |
| | Load | Load | CIP | Wash | |
| 1B | P1 A Col 1 | Col 2 | P5 F Col 3 | P2 C Col 4 | |
| | Load | Load | Equil | Wash | |
| 1C | P6 B Col 1 | Col 2 | P5 F Col 3 | P3 D Col 4 | |
| | Purge | Purge | Equil | Elute | |

## Conclusions

1. Optimal productivities with high-titer mAb feedstocks were obtained using a lab-scale 8-column MCC Protein A capture process at 0.5 min residence time with 3 columns in the capture zone. For example, 97 g/L resin/h was achieved with high purity and recovery using Toyopearl AF rProtein A HC resin. This value represents 86% of the modeled process (112.5 g/L/h), which assumes 100% yield.
2. Optimal performance at low residence times requires adsorbents having high DBC and low pressure drop and MCC equipment that accommodates multiple process configurations. Both of these considerations will be important to maximize process economy at production scale at any feed titer.

### References

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