Continuous Chromatography Is Now Possible for Clinical Manufacturing

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Intensified and integrated bioprocess technologies are creating a paradigm shift toward more efficient, higher flexibility facilities for biopharmaceutical manufacturing. Continuous technologies that are designed as single-use systems help to greatly facilitate process intensification, delivering further efficiencies with reduced set-up times and elimination of the need for cleaning and cleaning validation.

Chromatography is often considered to be a challenging bioprocess step, which has caused great interest in a simplified, safer solution. Continuous multicolumn chromatography using a single-use flow path is an economically viable, disposable chromatographic solution for even the most demanding and costly chromatographic steps in a manufacturing process.

The Cadence BioSMB PD platform from Pall Life Sciences is the first disposable flow path, continuous multicolumn chromatography solution that is scalable from a process development (PD) laboratory to GMP manufacturing and is designed for easy integration with other unit operations to allow integrated continuous manufacturing.

**Simulated Moving Bed Technology**

Continuous chromatography is known to reduce costs and increase facility throughput using inherent unit operation efficiency rather than dramatic process change. Unlike batch chromatographic processing in which only 60–70% of the total binding capacity is used, with simulated moving bed (SMB) technology, a series of multiple, much smaller columns work together to improve process efficiency and make use of chromatographic resin more effectively.

During multicolumn countercurrent chromatography, all of the chromatographic steps normally run in a batch mode are conducted simultaneously in different zones (Figure 1). Each small column sees the same exact process steps that a batch column would go through multiple times. Because the chromatographic media reaches its full lifetime within a campaign through this rapid cycling, disposable use of the chromatographic media is possible, even with the most expensive sorbents.

The first column in the load zone is allowed to reach breakthrough, and the product breakthrough is captured on a second column often referred to as the second-pass column. The binding capacity of the first column can thus be exploited well beyond its batch dynamic binding capacity. In many cases, such processes can be operated close to equilibrium or to the static binding capacity of the chromatographic media, thus significantly reducing chromatographic media use.
In addition, because continuous countercurrent loading is used, the load zone must accommodate only the mass transfer zone, which generally represents a small portion of an overall chromatographic batch column. As a result, the entire process can be conducted in a much smaller system carrying only a fraction of the chromatography media used in a standard batch process.

**Advantages of the Single-Use Cadence BioSMB PD System**

The Cadence BioSMB system was designed to integrate single-use components with the already proven and widely accepted manufacturing efficiencies and economic benefits of continuous chromatography. It consists of 240 diaphragm valves in an integrated, acrylic block and a specially designed and patented single use valve cassette, which does not require cleaning and enables use of one valve cassette per campaign, including for early phase clinical manufacturing runs. The entire product-contact flow path of this multicolumn system is single use, and a user can choose to operate from one column to 16 columns in a process development environment or from one column to eight columns in a manufacturing environment. The single-use valve cassette allows column positions to be added easily because all of the necessary interconnections are already completed in the valve cassette.

In addition, the valve cassette contains all of the valves necessary to fully automate a wide variety of chromatographic processes, including bind/elute chromatography (Protein A affinity, ion-exchange, mixed-mode, and hydrophobic-interaction), as well as size-exclusion chromatography and membrane chromatography. By using more column positions to distribute the process efficiently into the various zones, a user can ensure that the system is always receiving load or feed and always eluting. This feature supports the integration of adjacent unit operations around the Cadence BioSMB PD unit — currently in development for late 2016 availability — which enables process development scientists to easily explore the added value of process integration. It also supports integrated continuous processes design in manufacturing, given that matching the flow rates between process unit operations can be a challenge.

The Cadence BioSMB PD system enables easy conversion from an existing batch process into a continuous chromatography step, with no change to the chromatographic sorbent, buffer system, or product quality assays. The BioSMB PD approach (Figure 2) is a standard, simple technique based on a process model that enables users to rapidly convert single-column breakthrough data for a batch process to the parameters appropriate for a BioSMB continuous process. Typically, a conversion can be completed in one week.

**Reducing the Consumable Cost Burden**

Chromatographic media gets expensive, so optimization of media is often a critical priority for manufacturers. The Cadence BioSMB system allows a significant reduction in chromatographic media use.
Table 2: Comparison of batch and continuous protein A capture steps for 2,000-L mAb culture

<table>
<thead>
<tr>
<th>BioSMB</th>
<th>Batch Reference1</th>
<th>Clinical Manufacturing2</th>
<th>Commercial Manufacturing3</th>
<th>Commercial Manufacturing High Titer (7 g/L)4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column Size</strong></td>
<td>ID 80 cm x H 20 cm</td>
<td>ID 20 cm x H 7 cm</td>
<td>ID 30 cm x H 13 cm</td>
<td>ID 30 cm x H 15 cm</td>
</tr>
<tr>
<td><strong>Number of Columns</strong></td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total Sorbent Volume</strong></td>
<td>100 L</td>
<td>17 L</td>
<td>39 L</td>
<td>50 L</td>
</tr>
<tr>
<td><strong>Capacity Use</strong></td>
<td>35 g/L</td>
<td>41 g/L</td>
<td>52 g/L</td>
<td>52 g/L</td>
</tr>
<tr>
<td><strong>Specific Productivity</strong></td>
<td>8.17 g/L/h</td>
<td>50 g/L/h</td>
<td>25 g/L/h</td>
<td>39 g/L/h</td>
</tr>
</tbody>
</table>

1 Batch process in which the total amount of antibody in each batch is captured in two cycles on a single column within eight hours
2 A BioSMB process targeted for clinical manufacturing: The process is designed for optimum specific productivity to reduce sorbent volume used. This comes at a short contact time in the load zone (approximately one to two minutes per column). At such short contact times, the sorbent capacity can be used beyond normal dynamic binding capacity, although not too close to static binding capacity.
3 A BioSMB process targeted for routine manufacturing: The process is designed for optimum capacity use of the media. For process economics, this corresponds to the maximum amount of protein purified throughout the life time of the media. This involved longer contact times and, hence, a somewhat lower specific productivity (2.5-minute contact time).

Overall, the use of fewer consumables results in supply chain, warehousing, and risk management advantages, along with elimination of the need for internal column-packing resources.

**PROCESS INTEGRATION AROUND THE CADENCE BIOSMB PD SYSTEM**

The Cadence BioSMB PD valve cassette is designed for developing integrated unit operations. Numerous combinations are possible. For example, a capture chromatography step can be combined with pH adjustment and flow through polishing. Multiple chromatography steps can also be run continuously.

As one example, a single-pass tangential flow filtration (SPTFF) step using the Cadence Inline Concentrator (ILC) integrated with the Cadence BioSMB PD has been operated over an extended period of time. In-line concentration before Protein A capture was demonstrated for a monoclonal antibody (mAb) feed with a low titer. In this case, the preconcentration occurs directly prior to the BioSMB step as the load is concentrated as it continuously feeds to the BioSMB. Preconcentration shortens the loading time and thus reduces the process time and increases the productivity and volumetric processing rate (L/h) (Table 1). This integrated continuous process is particularly valuable for relatively low-titer feeds and bioprocess fluids from perfusion cell culture processes.

**PROOF OF CONCEPT**

The Cadence BioSMB PD system was not only designed for use in the process development laboratory, but it also has the capability of processing protein quantities on a scale appropriate for the manufacture of clinical trial material.

For instance, a continuous mAb chromatography process was operated for four hours using the feed from a 10-L CHO fed-batch culture process. The overall process throughput for this system was 8.0 g/h for an initial mAb titer of 3.6 g/L. Note that a Cadence ILC was used one day earlier to raise the titer of the initial 40 L of cell culture from 0.9 mg/mL to 3.6 mg/mL using a 4x VCF.

These results clearly demonstrate the type of throughput that may be achieved with small footprint continuous chromatography equipment and indicate that continuous, intensive manufacturing is on the horizon. It is also important to note that the BioSMB PD valve cassette and flow path can be cleaned with 1M NaOH for reuse studies as required.

**DIRECT SCALE-UP FROM PD TO PROCESS**

Pall is developing a process-scale version of the Cadence BioSMB PD system that will be introduced in Q1 of 2017. The Cadence BioSMB Process system will support up to eight columns and have flow rates up to 350 L/h. As a result, it will be capable of purifying the output from a 2,000-L single-use bioreactor in less than eight hours.

To illustrate the flexibility of the BioSMB technology, process designs were generated for capturing a monoclonal antibody from a 2,000-L bioreactor with a titer of 3.5 g/L. The results indicate that the BioSMB scales predictably from the lab bench to the 2,000-L scale (Table 2). The process development system is equipped with the same flow-path characteristics found in the GAMP-5-compliant production system, providing ease of transition from the process development lab to full GMP production.
Because the PD and Process systems are both designed with the exact same flow path, processes developed using the Cadence BioSMB PD system are directly transferable to the Cadence BioSMB Process system for running in a GMP environment.

In addition, to ensure simplicity of operation, user interfaces and visualization software for the PD and Process systems were designed from the ground up with operator needs in mind. Furthermore, the human machine interface developed for the Cadence BioSMB PD system has been directly translated for the BioSMB Process system with incorporation of all of the necessary security features required for GMP operation.

The Cadence BioSMB Process system will provide many benefits for continuous GMP chromatography; no cleaning or cleaning validation is required for complex valving/piping systems at clinical scale. Integrated unit operations can be developed, improving process performance, efficiency, and throughput.

Two–four columns can be used for low concentration feedstocks (mg/L), and four–eight columns are available for >1 g/L bioprocess fluids. The Cadence Inline Concentrator also can be used to boost volumetric throughput for feed streams with concentrations that are less than desired.

For most other applications (e.g., size-exclusion chromatography), four–eight columns are recommended to maintain productivity.

**Leveraging the Benefits**

Pall Life Sciences’ scientific team has dedicated a great number of resources to provide a complete continuous chromatography solution to drug manufacturers at any scale. The Cadence BioSMB Process system enables manufacturers to leverage the benefits of continuous simulated moving bed chromatography with single-use technology for more efficient and flexible downstream processing of biopharmaceuticals with a drastically reduced footprint and cost.

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Also available in this portfolio, the Cadence BioSMB Process system for GMP manufacturing is designed with the same single-use flow path for direct scale up and the same HMI for a similar visualization and operation experience.

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**Featured Products**

**KANEKA KanCapA™**

*Improving mAb Bioprocesses with Higher Purification Productivity*

As part of its commitment to continuously improving bioprocesses from process development to commercial scale, Pall has actively developed advanced technologies for batch and continuous processing. Following its acquisition of the BioSMB multicolumn continuous chromatography platform from Tarpon Biosystems and licensing of FloDesign Sonics’ Acoustic Separator technology*, Pall is now offering state-of-the-art KANEKA KanCapA™ protein A chromatography sorbent from Kaneka Corporation in Japan for primary capture of monoclonal antibodies (mAbs) from clarified cell culture fluids.

With the ability to provide leading technologies for all steps in both batch and continuous biomanufacturing processes — from bioreactors and supporting systems to clarification/filtration, capture, and final purification equipment — Pall provides a one-stop shop for mAb bioprocessing and is the supplier of choice for KANEKA KanCapA protein A sorbent.

**Designed for High Performance**

The ideal protein A sorbent is readily scalable, easy to pack, has a high binding capacity to match current expression levels, and is highly selective under mild conditions — and it maintains this performance over long service periods. Composed of a proprietary recombinant protein A (rProtein A) ligand covalently bound to a rigid cellulose matrix, KANEKA KanCapA chromatography sorbent has been designed to meet these specific needs.

The rProtein A ligand is a pentamer of the mutated C domain of the native protein A molecule. It exhibits good sodium hydroxide (NaOH) resistance even after multiple cleaning cycles (effective for at least 200 runs) with no Fab–protein A interactions. That allows for milder elution pH conditions with the potential for less unwanted mAb aggregation and denaturation.

The dynamic binding capacity of KANEKA KanCapA sorbent is >50 g/L at a six-minute residence time and meets the need for high flow performance at low pressure for commercial-scale capture of mAbs from cell culture processes with titers >5 g/L.

KANEKA KanCapA is easy to pack and demonstrates reliable packing performance that is scalable from process development to commercial scale (60-cm ID) columns. The sorbent is available in a range of prepacked columns and high-throughput screening tools, as well as in bulk for use in Pall’s industry leading Resolute® AutoPak columns. All components of the sorbent are free of animal content.

**Ideal for Batch and Continuous**

With these properties, KANEKA KanCapA is the rProtein A sorbent of choice for mAb purification processes. Its high dynamic binding capacity, good flow performance, and improved alkali stability for reuse make KANEKA KanCapA sorbent ideal for mAb capture in both single-column batch operations and continuous processes based on multicolumn chromatography systems. KANEKA KanCapA also enhances Pall’s comprehensive line of ion exchange, mixed-mode, hydroxyapatite, affinity and solvent-detergent removal chromatography sorbents by improving downstream purification performance, speed, safety and reliability to reduce purification costs.

* On June 15, 2015, Pall announced the exclusive licensing agreement with FloDesign Sonics for the Cadence Acoustic Separator, a disruptive technology for cell culture clarification for both fed-batch and perfusion applications.

** KANEKA KanCapA is a trademark of Kaneka Corporation.**