Integrated Continuous and Single-use (ICS) bio-production platform

Linking continuous perfusion bioreactor with Continuous Countercurrent Tangential Chromatography (CCTC)

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Continuous processing has been adopted in many industries leading to significant improvements in process efficiency and cost savings [1]. The well-known benefits of continuous processing include reduced capital equipment costs and facility size, increased productivity, greater flexibility, improved process reliability, and enhanced product quality. The pharmaceutical industry has successfully adopted continuous processing for a number of small molecules [2], and there is currently a significant effort in the biotechnology industry to develop and adopt continuous processing for biologics [3]. Perfusion bioreactors have been successfully adopted for several approved manufacturing processes for biologics. However, downstream processing is still dominated by batch column chromatography, resulting in significant production bottlenecks [3]. Thus, there are wide-ranging efforts across the biopharmaceutical industry to develop continuous downstream processes. This transition has been strongly encouraged by the FDA whose Director, Dr. Janet Woodcock, predicted at the 2011 AAPS meeting that current manufacturing practices will be abandoned "in favor of cleaner, flexible, more efficient continuous manufacturing". In a recent article published in MIT Technology Review [4], Dr. Konstantin Konstantinov, who previously led a team pursuing continuous bioprocessing at Genzyme-Sanofi, says that he "can get rid of about half the usual equipment and has squeezed a production line of steel vessels and piping down from something approaching the size of a football field into a space the size of a squash court." Currently, a number of development efforts are on-going for adoption of multi-column chromatography systems in continuous and semi-continuous modes [3, 5]. However wide scale adoption of these approaches has been limited due to multiple drawbacks of these systems including high complexity, expensive hardware, high pressure operation, and cyclic product output.

Continuous Countercurrent Tangential Chromatography (CCTC) [6-10], developed by Chromatan Corporation, has overcome many of the limitations of batch columns without the drawbacks of multi-column systems. The CCTC platform is a true moving bed technology that runs at steady-state and doesn't utilize columns. The novel design of the system enables short residence times, consistent product concentration and quality, as well as easier implementation of advanced Process Analytical Technology (PAT) and process control. In addition CCTC is uniquely suited for processing sensitive molecules due its ability to independently control buffer and micro-mixing conditions for all chromatographic steps, thereby eliminating product-related concentration and buffer gradients that always accompany column-based separations. As a result of these features, The National Institutes of Health (NIH) has decided to fund a $1.75 Million Fast-Track Phase II SBIR program to develop and commercialize a fully integrated continuous and single-use (ICS) bio-production platform that combines the advantages of perfusion bioreactors with CCTC in order to deliver a compact and steady-state solution for manufacturing and purification of biologics (Figure 1A).

In this paper we present data from the Phase I portion of this project. The results of the testing in this phase enabled the larger Phase II investment from the NIH which will be used for ICS platform commercialization. The testing was supported bya commercial partner, which provided a mAb producing CHO cell line, as well as Applikon Biotechnology Inc., which provided their EZ control bioreactor system, bioreactor vessel and probes, and cell retention device (Biosep). The schematic of the Biosep system is shown in Figure 1B. The Phase I data include perfusion platform evaluation, long-term CCTC hydrodynamic testing, as well as CCTC purification testing of a model mAb provided by a commercial partner.

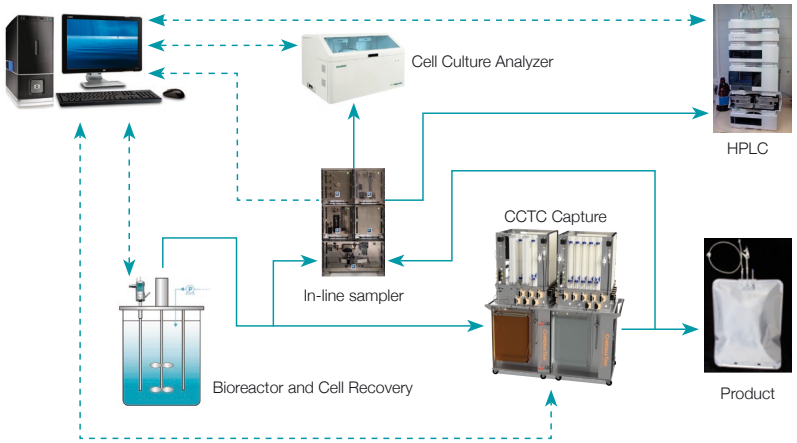

Figure 1A | Schematic of the integrated system. The integrated system contains a combined bioreactor – cell retention device (BioSep).

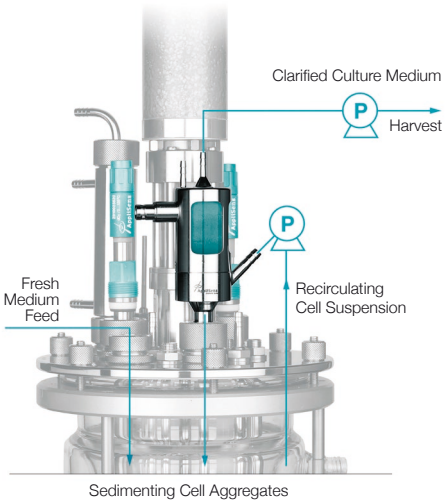

Figure 1B | The schematic of the BioSep cell retention device.

CCTC system for continuous downstream bio-processing

The patented CCTC system includes all of the traditional chromatography steps such as bind, wash, elution, strip, and regeneration, but these operations are conducted on a moving slurry instead of a packed column as shown in Figure 2A. Each step is made of multiple stages arranged in a countercurrent configuration, each of which is composed of a static mixer and a hollow fiber membrane module. The schematic in Figure 2B shows a 2-stage chromatographic step. In a CCTC process, the resin slurry flows through these modular stages, while all of the chromatographic operations are performed simultaneously and at steady state. The static mixers are sized to provide appropriate residence times for all operations while the microporous hollow fiber membranes (0.5-1 µm pore size) retain the large resin particles (25-90 µm) while allowing all dissolved species like proteins and salts to pass through into the permeate.

Countercurrent staging allows better yield and impurity removal. Increasing the number of stages increases the yield and impurity removal while reducing buffer consumption. The number of stages in each step can thus be optimized based on yield, purity, and buffer consumption requirements. More details are provided in Dutta et al. [9].

The CCTC system has been shown to operate continuously with 5-10x greater productivity vs. batch columns, enabling a significant reduction of resin volume compared to batch column requirements. In a recent paper [8], the versatility of the CCTC system was demonstrated by successfully purifying mAbs from both high and low titer clarified cell culture fluid (CCF) using a commercially available Protein A resin. CCTC was also featured in a polishing mixed-mode mAb purification step post protein A, achieving >10x productivity improvement and comparable purity to batch columns [10].

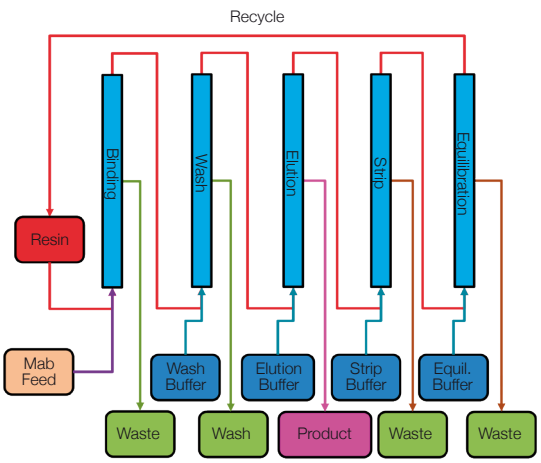

Figure 2A | Schematic of the CCTC system operating in continuous resin recycle mode. The system consists of five steps: 1) binding, 2) wash, 3) elution, 4) strip, and 5) equilibration.

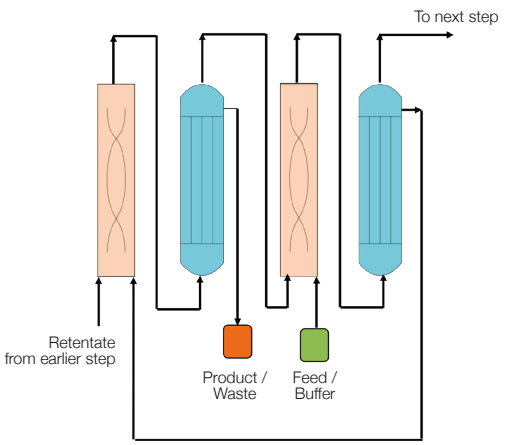

Figure 2B | Schematic of a step showing 2 countercurrent stages, each consisting of a static mixer (red) and hollow fiber membrane module (blue).

Perfusion bioreactor for continuous upstream bio-production

Perfusion bioreactors are now well-established for continuous upstream production of mAbs and other recombinant products, but these bioreactors have yet to be used in a truly continuous process due to the lack of available technology for continuous product capture and purification. Remicade, one of the blockbuster mAb products currently on the market, employs perfusion culture in manufacturing, as does Factor VIII, manufactured by Bayer. Perfusion bioreactors can achieve high cell density and high productivity in a relatively small size when compared with batch/fed-batch systems, enabling a 200 L perfusion bioreactor to produce the same amount of product as a 2,000 L fed-batch reactor within a comparable timeframe. Another major advantage of perfusion systems is that the short residence time allows for production of unstable proteins that would be denatured in a batch bioreactor after long process times.

Integration of perfusion bioreactor and CCTC system

The continuous nature of both perfusion bioreactors and CCTC, and the associated advantages when compared to batch bio-production, provide excellent opportunities for integration. Three unit operations will be combined into a single system. A perfusion bioreactor (Applikon) will be linked with a cell recovery system which will also be linked with a Protein A CCTC operation (Figure 1A). Two cell recovery technologies will be evaluated. The use of the Biosep acoustic separation device from Applikon Biotechnology (Figure 1B) is well suited for perfusion bioreactors targeted for continuous cultures. Acoustic waves inside the Biosep chamber retain cells without physical filtration so there is no risk of clogging during long-term use, however additional filtration maybe required to the product stream to remove trace levels of cells that pass through the acoustic field. The membrane based Spectrum KrosFlo system will also be evaluated. Its single-use design is well suited for bio-manufacturing, however long-term use of this system can be prone to membrane fouling and decreased protein transmission.

The CCTC system's modular design creates flexibility, while the accessible flow path makes the platform relatively easy to customize. This includes integration with automated sampling for improved automation. The in-line HPLC and cell culture analyzer will be used for bioreactor monitoring and feedback control. For example, in-line measurement of product titer by HPLC will be used to fine-tune the feed flow rate to the CCTC system to provide optimal resin loading in the CCTC system in response to any changes in perfusion reactor performance. The integrated control system will collect data from the bioreactor, the cell recovery system, CCTC capture, and analytical instruments (pH, conductivity, cell count, CCTC pressure profiles, titer, media composition, etc.). These data will be used to control key process variables such as perfusion rate, CCTC feed and buffer flow rates, sampling frequency, process duration, and startup / shutdown.

Phase I results

The Phase I efforts were focused on generating a convincing data set that enabled the larger NIH investment for Phase II. The data include perfusion platform evaluation, long-term CCTC hydrodynamic testing, and CCTC purification testing with the commercial partner mAb.

An Applikon 3L bioreactor with EZcontrol and Biosep perfusion device were used to perform two bioreactor runs; one with a higher steady state viable cell density (50-56 x 106 cells/mL) operated over a short time period (13 days) and one with a lower steady state cell density (10-15 x 106 cells/mL) operated over a longer time period (30 days). The Biosep acoustic-based cell separation device maintained high cell viability (> 94%) for both runs. Biosep efficiency was calculated from the formula described in equation (1) and is plotted in Figure 3.

$$\text{Efficiency}(\%) = \left(1 - \frac{\text{Number of viable cells in harvest}}{\text{Number of viable cells in bioreactor}}\right) \times 100 \quad [1]$$

Figure 3 | Biosep performance over a 30 day run showing >90% efficiency. Efficiency was not affected by the perfusion rate from 0.4 to 1.2 volume/reactor volume/day (VVD).

The efficiency was reproducible and greater than 90% for both runs. Furthermore, the effect of varying perfusion flowrates and harvest flowrates were examined as part of a single run. Tuning the recirculation flowrate according to the harvest collection flowrate resulted in stable operation with high efficiency during 30 days of operation (Figure 3).

After testing the performance of the perfusion bioreactor, the long term hydrodynamic stability of the CCTC system was tested by flowing protein A resin slurry through a representative system consisting of binding, strip, and equilibration steps. This system demonstrated stable pressure profiles as shown in Figure 4.

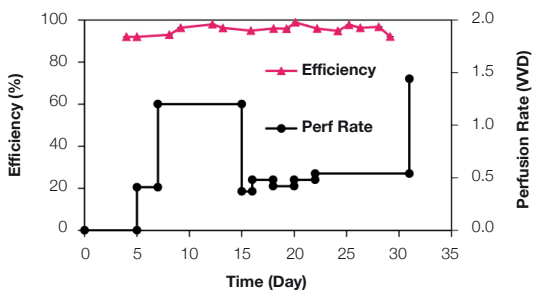

Figure 4 | Pressure profile in different stages of the CCTC system showing long term hydrodynamic stability. The gaps in the pressure profile were due to resin exchange every 2 days which corresponds to about 600 cycles.

The CCTC system was also used to purify mAb from clarified cell culture fluid obtained from a perfusion bioreactor. The CCTC system consisted of six process steps (binding, wash I, wash II, elution, strip, and equilibration), and was continuously monitored via pressure sensors. A run with 21-hr steady state operation was carried out to demonstrate the potential of the CCTC system for long term operation. Samples were collected hourly for yield and purity analysis. The pressure profile, yield, HCP and DNA content in the product are shown in Figure 5. The pressures in all steps were <10 psi, which is about 5-10x lower than the pressure in a typical chromatography column. The average mAb yield was 92% while steady state removal of HCP and DNA were 4.9 and 4.7 logs, respectively.

Future work

With the successful completion of Phase I, Chromatan Corporation is currently carrying out Phase II research which focuses on the development and commercialization of the ICS platform for production and purification of monoclonal antibodies. This program will be supported by Applikon Biotechnology, which will provide and co-develop "process control and management software" and "in-line sampling" system, to enable integration of the ICS platform with powerful PAT solutions and advanced process control. Our objective is to achieve significant benefits for the bioprocessing industry by reducing cost of goods and floor space requirements by at least 50%, as well as to enable a new bio-manufacturing platform that can be rapidly deployed in flexible single-use facilities while providing scalable, reliable, and robust operation.

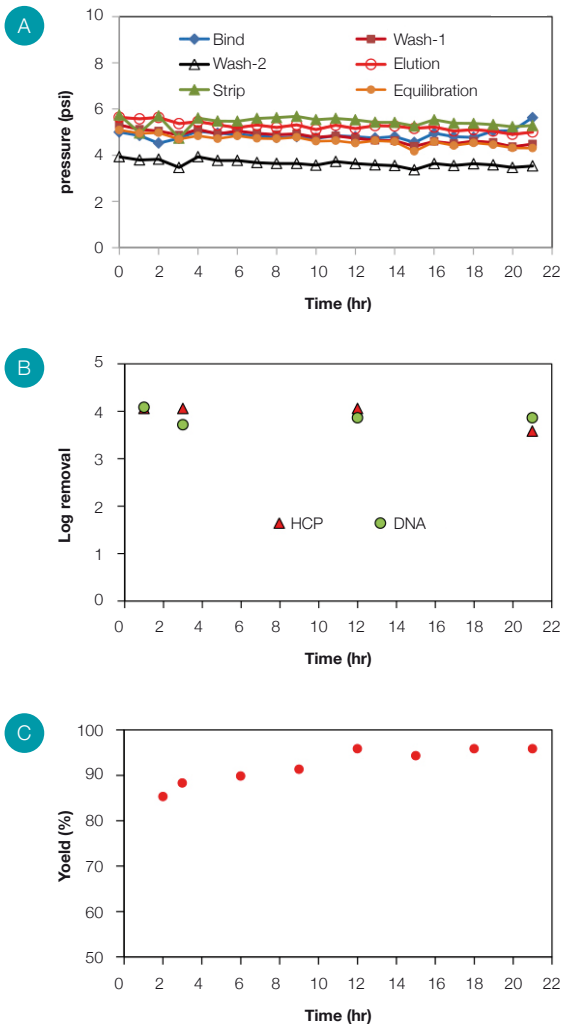

Figure 5. Performance of the CCTC system: (A) pressure profile, (B) yield, (C) HCP and DNA content in the product.

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