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The impact of continuous multicolumn chromatography on biomanufacturing efficiency

Over the past few years, the capacity bottleneck in biomanufacturing has shifted from the upstream process to the purification process. In particular, capture chromatography steps have become a limiting factor in facility throughput. This is a direct result of the discrepancy between expression levels, which have increased by a factor of ten, and the capacity of biochromatography media, which has at best doubled. Continuous countercurrent multicolumn chromatography is a promising approach to deal with the capacity bottleneck, particularly if designed in a single-use format. BioSMB™ technology is a disposable bioprocess technology that allows standard capture chromatography steps, such as Protein A capture, to be executed with much less installed chromatographic media using a multicolumn chromatography process. This allows an economically favorable, single-use option where none has previously been available. Various case studies have demonstrated the advantages of this technology for various separations. In this paper, we provide theoretical background on designing continuous multicolumn chromatography processes. With these models, the advantages of continuous processing will be illustrated.

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Over the past decade, the science and technology of biopharmaceutical manufacturing has progressed rapidly [1]. Two developments have had enormous impacts on the field of bioproduction; the significant increase in expression levels and the large doses of biopharmaceutical therapeutics required for some chronic indications. The expression levels in animal cell cultures over the course of the past two decades in fed batch bioreactors have increased from well below 1 titer to 5 gm/l titer, with occasional titers of 10 gm/l and higher being reported. This advance was achieved through a combination of a better understanding of the cell biology, resulting in higher specific productivities, and improvements in cell culture technology and media, resulting in higher cell densities. The other trend that has changed the landscape is the success of monoclonal antibodies for vari-

ous indications. This class of molecules also has significant similarities, which allows the development of platform processes. A platform process is a template that suits a range of molecules with relatively small variations in process conditions. These two developments have triggered the biopharmaceutical industry to mature rapidly and to implement technological improvements in their manufacturing platforms. One of the best known examples of this is the adoption of single-use technologies. Single-use bioreactors have been adopted to avoid cross contamination and to increase operational efficiency. At this time, even moderately sized bioreactors can produce commercial amounts of products.

One of the consequences of the trends in the industry is that the bottleneck in biomanufacturing has shifted from the upstream to the downstream processing portion. In par-


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Key Term**Multicolumn chromatography:**

Continuous countercurrent chromatography system in which the chromatography media is held in multiple smaller columns and in which the transport of the chromatography media is established by periodically switching in- and outlet ports of these smaller columns.

particular, those steps whose throughput is related to the mass of product to be purified – rather than to the liquid volume to be processed – have become a limiting factor in many cases. The capture chromatography step is the most notable example of this. The binding capacities of chromatography media have increased significantly over the past few years, but it has not been able to keep pace with the developments in expression levels. As a result, capture chromatography columns have become larger whereas the bioreactor sizes have become smaller. The mitigation strategy for many companies has been to cycle the capture column multiple times during a batch, thereby allowing the same amount of product to be processed with a smaller volume of chromatographic media. This strategy, however, obviously leads to longer processing times and has a negative impact on the overall facility throughput. Furthermore, this strategy requires extra hold time, which may have an impact on product quality.

In many other process industries, similar problems have been addressed by adopting continuous **multicolumn chromatography** and in particular, simulated moving bed (SMB) technology. This has resulted in various designs for multicolumn chromatography equipment [2]. SMB technology, for instance, has been widely used for separating fructose from glucose in the production of high fructose syrups. More recently, this approach has also been adopted for chiral separations of synthetic active pharmaceutical ingredients. Other examples of large-scale commercial applications of continuous multicolumn processes can be found in

the large-scale purification of Lysine and antibiotics from fermentation broth [3], and the production of vitamin C [4]. These applications are not characterized by a binary fractionation as performed in a traditional SMB approach but are commonly referred to as continuous ion exchange. The principles, however, remain the same.

BioSMB™ technology

In a biopharmaceutical production environment, specific requirements relating to hygienic or sanitary processing must be strictly followed. In addition, both the hardware and the control software need to comply with high regulatory standards in order to be accepted by the industry. In earlier studies, the potentials of multicolumn chromatography and SMB technology for the capture of monoclonal antibodies on Protein A chromatography was demonstrated [5]. This work was performed on a carousel type SMB system that involved a sliding valve to connect 20 columns to the pumps and collection containers. The design of the sliding valve in these systems was not suitable for hygienic processing and hence alternative systems needed to be developed in order to materialize the promises of this technology. This has led to the development of suitable multicolumn chromatography systems for biopharmaceutical use, such as the Prochrom® BioSC from NovaSep (Pompey, France), the 3C-PCC and 4C-PCC system from GE Healthcare (Uppsala, Sweden), the BioSMB™ system from Tarpon Biosystems (MA, USA), the Contichrom® process from ChromaCon (Zürich, Switzerland) and the Octave™ system from Semba Bioscience (WI, USA).

Although these systems target the same, they differ in the way this is achieved. Particularly the valve design is different for the various systems. This has implications for the number of columns that can be used in the different processes and the number of pumps required for each system. Most systems are equipped with multiple pumps, each serving only one buffer throughout the entire process. This eliminates the need, while switching between different buffers, for flushing, and provides the best flexibility for scheduling the entire process over multiple columns. Only with relatively few columns, such as implemented in the 3C-PCC and 4C-PCC systems, fewer pumps can be used without running into scheduling limitations. On the other hand, the ability to connect more columns provides more flexibility in implementing and optimizing various chromatography processes. For this reason, the BioSC system and Octave system have been designed to accommodate six and eight columns, respectively and the BioSMB system can support up to 16 columns.

The BioSMB technology, designed by Tarpon Biosystems, is the first fully disposable multicolumn

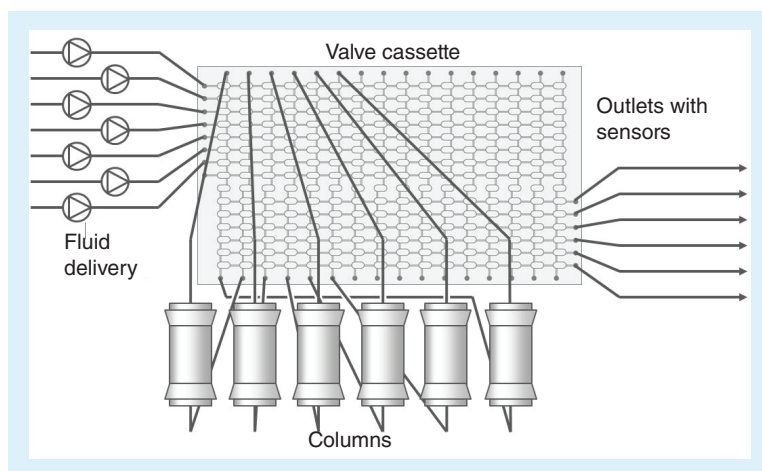


Figure 1. Global lay-out of the BioSMB™ system with multiple chromatography columns. The valves (shown in the center) are held in a single disposable cassette.

chromatography system. The entire fluid path, from pumps to most of the sensors, including the entire valve system, is designed in disposable format. It is designed to offer the versatility needed to perform any chromatography process application that one would normally expect for the purification of biotherapeutics [6,101]. The global architecture of the system is shown in Figure 1.

The valve system in the BioSMB system accommodates 240 individually addressable diaphragm valves. This provides the flexibility that is required to connect and disconnect the columns in virtually any configuration. This flexibility is key to using the system for both capture steps, such as Protein A affinity chromatography, and polishing steps, such as size exclusion chromatography. The first mode resembles continuous ion exchange operations as practiced with various continuous ion exchange technologies in other industries, such as the purification of Lysine [3] and vitamin C [4]. These applications require the process to be configured in isolated steps in which the outlet of each column is not always connected to the next. Size exclusion chromatography, on the other hand, can very effectively be used for binary fractionations. These applications required the columns to be configured according to the traditional SMB scheme in the outlet of each column is connected to the inlet of the next column.

The default configuration comprises four conductivity probes, two pH probes and four UV flow cells. These sensors can be configured in any position of the process, but most commonly they are configured to monitor a single effluent. This allows the use of a UV flow cell with a longer path length in effluents where only trace amount of proteins are expected and shorter path lengths where higher protein concentrations should be monitored.

The BioSMB system utilizes dual-head positive displacement pumps to deliver fluids to the BioSMB valve cassette and hence to the columns. Larger scale BioSMB systems are equipped with quaternary diaphragm pumps to ensure a well-controlled and consistent flow rate. Preliminary studies indicate that capacities up to 250 l/h can be easily achieved in a disposable valve cassette without significant modifications to the conceptual design of the valve system.

Principles of multicolumn chromatography

Countercurrent contact has greatly improved the efficiency of two phase separation processes such as absorption, distillation and extraction processes. The benefits of countercurrent processing reside in an optimization of the driving force for mass transfer throughout the overall trajectory of the two phases. This allows the mass transfer process to exceed the thermodynamic limitations of a (single stage) batch or concurrent process.

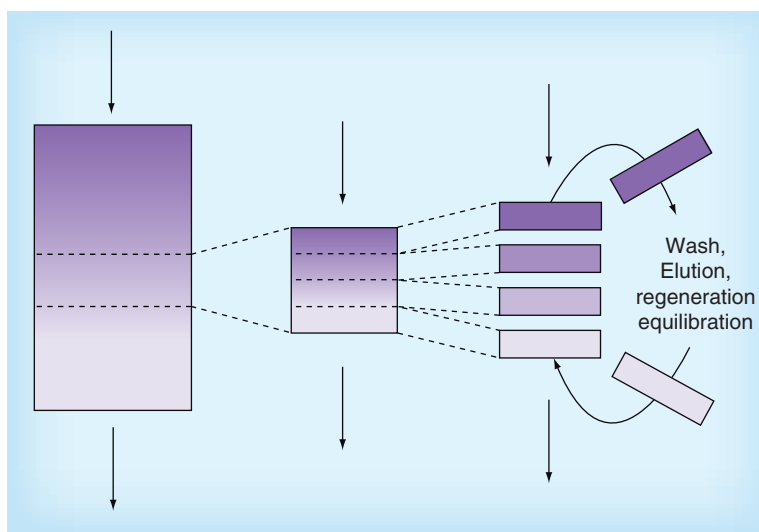


Figure 2. The principles of batch chromatography (left) and continuous countercurrent multicolumn chromatography (right).

During the industrialization of ion exchange processes in the 1950s, various attempts were made to establish a countercurrent process for ion exchange separations. Even with relatively rigid ion exchange beads common to many traditional ion exchange applications, transporting them through a piece of equipment resulted in very high attrition rates and hence very short resin life times. The solution to this was the implementation of SMB technology, which allowed the resin particles to remain in a packed bed and established transport of the resin by means of switching column in- and out-lets. In this way, the process travels along the columns rather than the other way around.

The concept of continuous countercurrent multicolumn chromatography can be visualized as shown in Figure 2. This figure shows a visualization of a batch column operated with the liquid flowing in downwards direction (left side of figure). As the feed solution flows through the column, a mass transfer zone will develop. Above the mass transfer zone, the chromatography media is saturated, which means it is in equilibrium with the feed solution. As a result, there will be no mass transfer occurring in that part of the column. Below the mass transfer zone, all target molecules from the feed solution have been removed. As a result, the mass transfer process only takes place in the mass transfer zone, which normally corresponds to a relatively small portion of the entire column.

As the chromatography process continues in the batch mode, the mass transfer zone will travel downwards to finally reach the outlet of the column. Before the first part of the mass transfer zone reaches the outlet of the column, the loading is stopped in order not to lose material. This leaves a significant portion of the static binding capacity of the column unused and in process

Key Terms

Separation factor: Dimensionless number describing the capacity ratio of the bed transport rate and the incoming material flux.

Number of transfer units: Dimensionless number describing the ratio between mass transfer flux and the convective flow in the load zone. This can also be considered as the ratio between the contact time in the load zone and the characteristic diffusion time in the load zone.

environments, large batch chromatography columns are often only loaded to 65% of their capacity.

In a countercurrent process, the mass transfer takes place throughout the entire course of the contact zone between the two phases. Over the entire course, the driving force for mass transfer is optimized, allowing a more efficient mass transfer process.

The second and third diagram in Figure 2 present a simplified explanation of the concept of continuous countercurrent multicolumn chroma-

tography. It does, however, illustrate how the top segment of the countercurrent capture zone is loaded beyond the dynamic binding capacity. This is possible since all material escaping from this column will be captured on the second column in the load zone. Once a saturated column has left the load zone, it is washed, eluted, regenerated and equilibrated before it becomes part of the load zone again.

Design of a countercurrent chromatography process

For binary fractionations, various design models have been published and discussed earlier [7]. These models, however, do not apply for continuous affinity chromatography and/or continuous ion exchange. For the 3C-PCC and 4C-PCC systems, a process design strategy was presented by Pollock *et al.* [8]. This approach is based on an empirical approach to the chromatography process. In this paper, a process design based on engineering principles is presented. The process design approach presented below is not limited to BioSMB systems only, provided that it features a continuous flow of the feed solution applied to the system.

A successful design of a countercurrent process relies on the following steps:

- » Set the flow rates of the two phases based on the thermodynamic equilibrium;
- » Determine the size of the countercurrent contact zone based on the required residence time for mass transfer;
- » Determine the dimensions of the columns in the contact zone based on the properties of the chromatography media.

Bed transport rate

In the design of countercurrent processes, the ratio between the flow rates of the two phases is commonly expressed in terms of a **separation factor** (S).

The separation factor expresses the ratio between the transport capacity of the receiving phase (or auxiliary phase) and the incoming flux of the target molecule(s) through the feed solution (primary phase). For chromatography processes, this can be written as:

$$S = \frac{\phi_{\text{Bed}} Q_{\text{st}}}{\phi_{\text{F}} C_{\text{F}}}$$

Equation 1

In this expression the transport capacity of the bed is evaluated as the simulated bed transport rate (ϕ_{Bed}) and the static binding capacity (Q_{st}). The incoming flux of product is expressed as the feed rate (ϕ_{F}) multiplied by the concentration of the target molecule(s) in the feed solution (c_{F}). If the process is designed with an S below unity ($S < 1$) the transport capacity of the chromatography media is lower than the incoming amount of the target molecule(s). As a result, not all of the target molecule(s) can be bound and the separation will be incomplete. An S that equals one ($S = 1$) corresponds to the situation where the transport capacity exactly matches the incoming flux of material. As a result, a complete separation could theoretically be achieved with infinite contact time and in the absence of axial dispersion. These are unrealistic conditions and hence any countercurrent separation process is designed with an S slightly above unity. In many cases, the S is chosen in the range of $S = 1.1$ – 1.4 [9]. Depending on the contact time in the system, this provides sufficient safety margin to address the mass transfer kinetics and to mitigate non-idealities in the flow patterns of both phases.

Sizing of the load zone

The required volume for the amount of chromatography media in the process is related to the residence time that is needed to allow the mass transfer to complete. This can be expressed in terms of the ratio between the mass transfer flux and the convective flow in the system. Following the parallel to other longitudinal two-phase countercurrent processes, this can be expressed in terms of the **number of transfer units** (NTUs):

$$\text{NTU} = \frac{k_{\text{oL}} \alpha V}{\phi_{\text{F}}}$$

Equation 2

The convective flow in this expression is represented by the liquid flow rate (ϕ_{F}). The mass transfer flux equals the total surface area between the two phases in the system, multiplied with the mass transfer coefficient (k_{oL}). The total surface area between the two phases equals the specific surface area ($a = 6/d_{\text{p}} [1 - \epsilon_{\text{L}}]$) and the resin volume in the load zone (V).

The NTUs can be considered as the ratio between the mass transfer between the two phases and the convective flow, but it can also be visualized as the ratio between the residence time of the liquid in the zone and the characteristic time associated with the mass transfer kinetics.

The mass transfer in a two-phase system includes diffusional resistance on both sides of the phase boundaries. For this reason, the mass transfer coefficient in the above expression should include both effects. This results in an overall or lumped mass transfer coefficient:

$$k_{oL} = \left(\frac{1}{k_L} + \frac{1}{Kk_s} \right)^{-1}$$

Equation 3

In this relation, k_L and k_s represent the partial mass transfer coefficients in the liquid and resin phase respectively and K represents the partitioning or distribution coefficient. This approach for evaluating the impact of the mass transfer kinetics relies on the linear driving force model or two-film diffusion model [10]. For design purposes, this approach has proven to provide sufficient accuracy.

Various models for the mass transfer coefficients in adsorption and ion exchange processes have been published. For design purposes, the Snowden–Turner relationship has proven to be sufficiently accurate [11]. These estimates provide a good starting point for the process design, but in many cases it is recommended to evaluate the mass transfer coefficients from analyzing the breakthrough behavior at different flow rates in laboratory scale batch chromatography processes.

Capture efficiency

In chromatographic columns, the degree of longitudinal backmixing (or axial dispersion) is relatively small compared with the impact of the mass transfer kinetics. This is caused by the fact that the packed bed of particles generally stabilizes the plug flow of the liquid. Particularly in countercurrent processes, where the required NTUs is much less than in batch chromatography, the impact of axial dispersion is very low compared with the impact of mass transfer kinetics.

From the mass balance equations, the overall performance of the countercurrent process can be described based on the S and the NTUs. Analytical solutions for this have been provided by Miyauchi and Vermeulen in 1963 [12]. These analytical solutions can be simplified to express the breakthrough from the load zone as a function of the S and NTU only:

$$\frac{c_{out}}{c_F} = \frac{(S-1)\exp(-NTU[1-S^{-1}])}{S - \exp(-NTU[1-S^{-1}])}$$

Equation 4

This analytical solution assumes that the columns were completely regenerated and equilibrated upon entering the load zone. Various case studies have confirmed that this predictive model provides an accurate estimate for the process performance [13,14]. As a result, this model has been adopted for design purposes. An example of such dataset is shown in Figure 3, showing model curves and experimental data under similar process conditions. In this graph, the horizontal axis represents the amount of antibody applied to the system per unit volume of chromatography media and the vertical axis represents the capture efficiency ($100\% - c_{out}/c_F$). This is the operating binding capacity of the process, which results from the static binding capacity divided by the S .

The experimental data were generated in a commercially available laboratory scale BioSMB Process development system, using MabSelect SuRe (GE Healthcare) to capture monoclonal antibodies from a clarified cell supernatant at concentrations around 2 mg/ml [13].

The required process parameters for a BioSMB design are therefore limited to the mass transfer coefficients and the equilibrium behavior. Since the BioSMB process relies on the same fundamental phenomena as a batch chromatography process, these parameters can be derived from batch chromatography experiments, using standard laboratory scale columns. The most accurate estimates for the parameters that describe the capture process can be obtained by evaluating breakthrough curves at different linear velocities. At lower linear velocities, the breakthrough curve is dominated by equilibrium behavior whereas the impact of mass

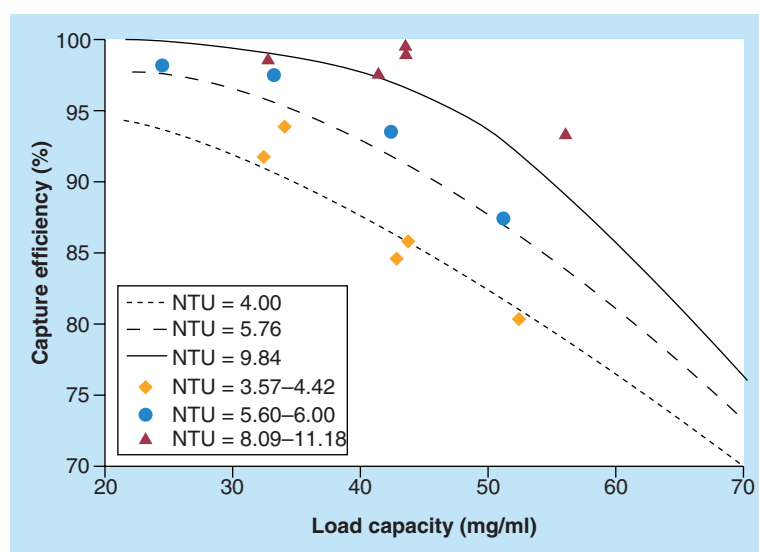


Figure 3. Performance of a BioSMB™ process for Protein A affinity chromatography. Curves represent the model prediction and dots represent experimental data [13].

NTU: Number of transfer units.

transfer kinetics becomes more pronounced at higher linear velocities.

A more sophisticated model for designing BioSMB processes is available. This model relies on numerical simulations to describe the process and hence takes more computing time. In addition to this, the numerical simulation model requires more detailed process information in order to be used. This model takes into account that chromatography processes generally are non-linear multicomponent separations.

Once the load parameters have been defined, the rest of the process can be assembled by adding the other steps in the process cycle. A convenient way of visualizing the assembled process is the Circular Chronogram™, shown in Figure 4. In this diagram, each of the columns are represented by a spoke in the wheel. The outer ring of the circular chronogram displays for each of the columns whose step of the process it currently is in. The inner ring shows which outlet each of the columns is connected to. In the example shown in Figure 4, the chronogram for a four column process is shown with two columns, in series, in the load zone. The effluent of the first wash step (W1) is added to the second pass of the load zone in order to recover any unbound product. The time for each of the steps is chosen based on the linear velocity and the required buffer volume in that step. In addition to this, the total time of the steps from the W1 to the equilibration (EQ) should match an integer number of switch times.

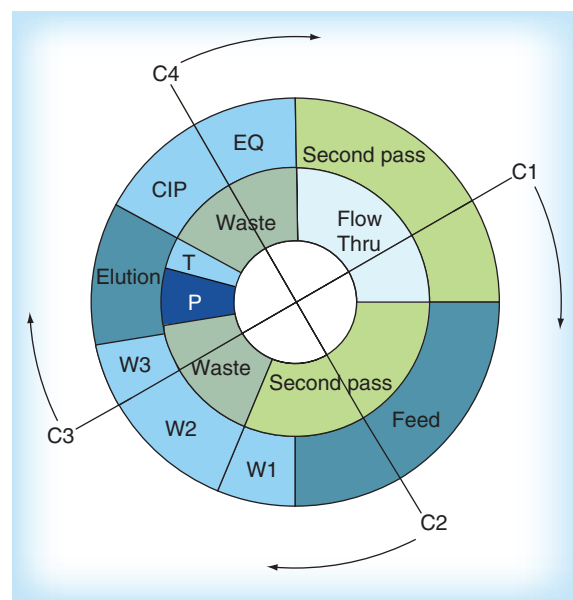


Figure 4. Example of a Circular Chronogram™ for typical Protein A affinity steps with four columns. The first two columns (C1 and C2) are in the load zone (Feed and Second Pass), the fourth column (C4) is being washed (W1, W2 and W3) and the third column is being EQ. CIP: Cleaning in place; EQ: Equilibrated.

Impact of titer

The process cycle time in this diagram corresponds to the rotation speed of the chronogram. The load time thus corresponds to the time between two columns, which equals the process cycle time divided by the number of columns. As the titer increases, the load time becomes shorter since the columns in the load zone are saturated earlier. The time for the other steps (W1 to E) is not affected by the titer and hence the time required for these steps remains the same. This needs to be compensated by adding columns to the system. This is visualized in the two chronograms in Figure 5. The circular chronogram on the left hand side represents the same process as in Figure 4. The circular chronogram on the right hand side represents a similar process, though at a higher titer. The time required to complete all steps from the W1 to EQ is the same, but the load time is shorter because of the lower load volume. As a result, the load time covers a shorter portion of the entire process cycle time. A high titer process therefore requires more columns. The column size, however, can remain the same provided that the process kinetics for the two different processes is not significantly different. The additional columns are not added in the load zone but serve to provide sufficient time to perform all other steps in the process cycle. As a result, adding such columns does not result in increased pressure drops.

From this approach, the total number of columns that is required to run a continuous process with uninterrupted feed solution can be calculated. For a typical Protein A affinity chromatography process this is shown in Figure 6. This graph is based on an operating binding capacity of 45 mg/ml and assuming that all other steps in the process cycle (W1–EQ) require a combined total of 24 CV of buffer. Furthermore, the scenarios presented in this graph assume that the linear velocity throughout the entire process cycle remains constant. A similar scheme, although based on more simplistic approach, has been presented earlier [6].

The solid curve represents the minimum number of columns, assuming two columns in the load zone. In some applications, it may be attractive to have four columns in the load zone, which can be connected as two in series and two in parallel. This offers more degrees of freedom in optimizing the process and allows running the same process with smaller column diameters. The number of columns required for such scenario may be larger but with smaller column diameters, these columns are easier to handle, cost less and are commercially available as pre-packed disposable-based products. Overall, the volume of chromatography media in the load zone remains the same and in the overall process, the total volume may actually be smaller. This is indicated by the

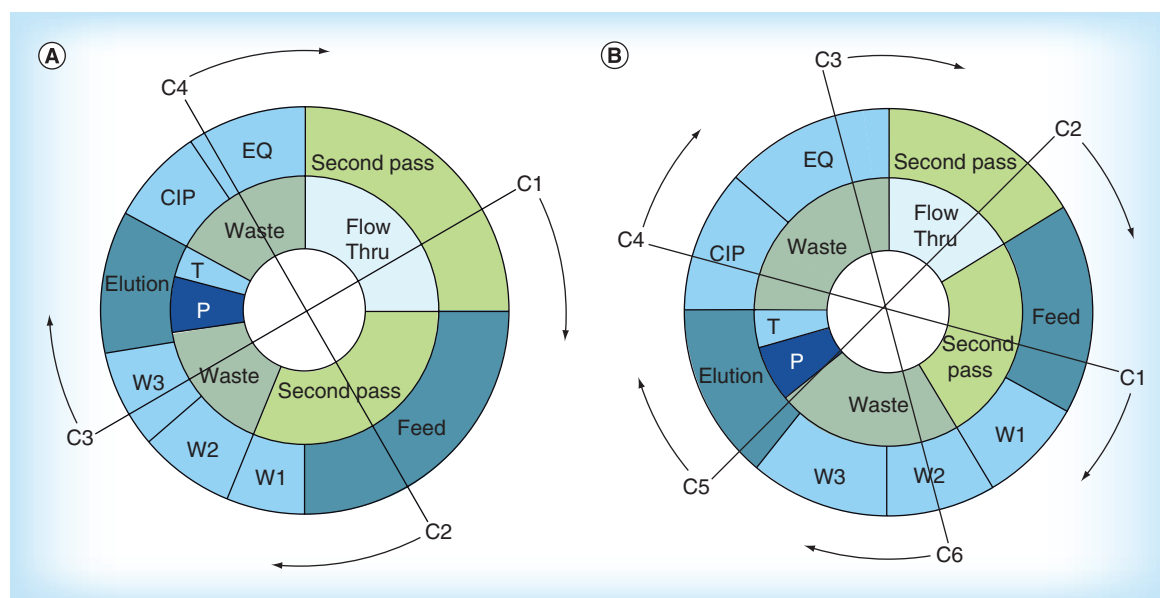


Figure 5. Circular chromatograms for a similar process with different feed concentrations. (A) Process with moderate titer and (B) process with higher titer.

C1–6: Column 1–6; CIP: Cleaning in place; EQ: Equilibrated; W1–3: Washed stages.

dashed curve in the graph, assuming a load zone with four columns in total, connected as two series of two columns each in parallel.

Scale-up

The S and NTU are dimensionless figures and hence these are scale-independent. The scale-up does not necessarily have to be limited to increasing the column diameters with flow rates. The approach allows varying the linear velocity through the scale-up, provided that the impact on the mass transfer characteristics is characterized.

This parametric scale-up approach based on the S and NTUs has been successfully applied in the scale-up of continuous ion exchange processes for small molecules [4]. This work describes how a laboratory process was translated into a commercial manufacturing scale that was more than four orders of magnitude larger than the laboratory process, while changing the dimensions of the columns and the total number of columns in the system.

Manufacturing scenarios

The design model described above relates the capture efficiency of the load zone to the required resin volume in the load zone (expressed as the NTUs) and the flow rates in the load zone (expressed in terms of the S). This allows optimizing the process design to meet certain business drivers, such as an optimized specific productivity or utilization of the binding capacity.

For clinical manufacturing, the largest economic impact of implementing a continuous chromatography

process is obtained by reducing the volume of chromatographic media. The volume of media needed for a clinical campaign is not often reused for different products and hence the entire volume of media is usually depreciated in the clinical campaign. A higher specific productivity can result in a significant reduction of this volume and hence in tremendous cost savings. A low inventory of chromatography media corresponds to a low NTUs. To achieve viable process efficiencies at low

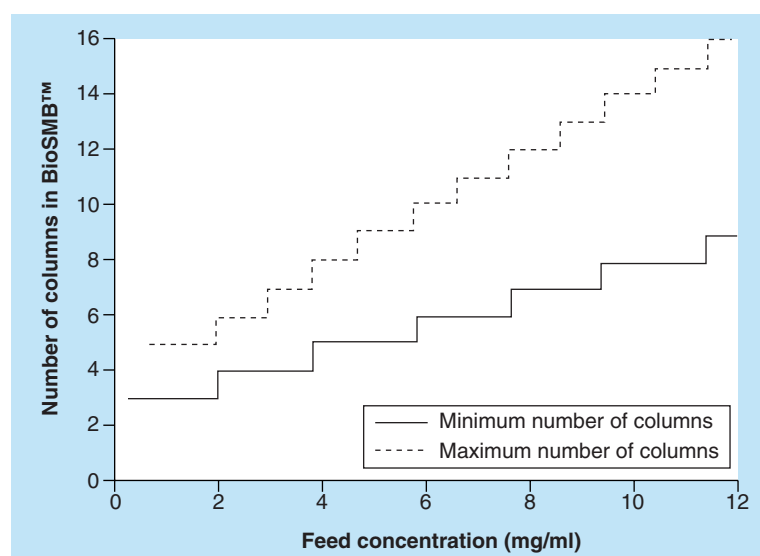


Figure 6. Typical number of columns in a multicolumn chromatography process that is required for continuous processing of a feed solution with a given titer. The dashed line (Maximum number of columns) represents the number of columns needed in case the load zone is operated with four columns instead of two.

Key Terms

Continuous countercurrent chromatography:

Chromatography process in which the feed solution is continuously applied to the system and in which the chromatography media is transported in the opposite direction.

Disposable bioprocessing: Use of technologies in which all parts of the equipment that have been in contact with the (intermediate) product and/or any other process solution are disposed of after a certain time. This could be a single batch or a (short) manufacturing campaign.

Hybrid processes: Processes in which some (consecutive) unit operations are seamlessly linked to each other to allow continuous flow of the product, whereas the other unit operations are conducted in batch mode.

resin volumes (or low NTU values), the S needs to be relatively high. An optimized specific productivity therefore comes at a lower utilization of the static binding capacity, which leads to a higher buffer consumption. For clinical manufacturing, however, the buffer consumption is often not a limiting factor.

In routine manufacturing, the chromatographic media will be exploited to its economic life time, which is generally expressed in terms of number of cycles. As a result, the cost contribution of chromatographic media is governed by the amount of protein purified per liter of media in each cycle. This is proportional to the capacity utilization and hence the BioSMB process should be operated at an S that is slightly above unity. This will require longer residence

times than in the clinical manufacturing scenario and hence the volume of chromatography media will be larger, yet still much lower than in the equivalent batch process. The cost contribution of the chromatography media, however, is mainly governed by the total amount of protein purified per liter of media throughout its lifetime.

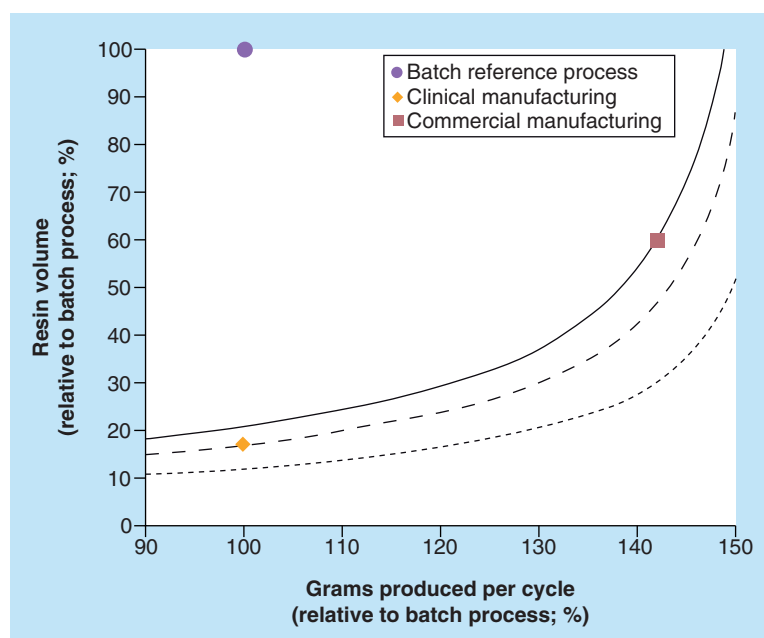


Figure 7. Typical scenarios for BioSMB™ operating conditions compared with a batch reference process. The curves represent operating conditions that correspond to 99% capture efficiency (solid line), 98% capture efficiency (dashed line) and 95% capture efficiency (dotted line).

Typical operating conditions for clinical and routine manufacturing scenarios are graphically shown in Figure 7. This graph illustrates how the relationship between the total volume of chromatography media, proportional to the NTUs ($V \mu NTU$), and the amount of product produced per liter of media in each cycle are related. The latter is proportional to the operating binding capacity, which is governed by the S (grams produced per cycle $\mu 1/S$). In this graph, the reference batch process is operated at 60% of the static binding capacity. This corresponds to 80% of the dynamic binding capacity at operating conditions that compare to $NTU = 50$. The process conditions for commercial manufacturing result in approximately 42% more product per cycle per liter of chromatography media, resulting in 30% direct savings in chromatography media. The impact for clinical manufacturing in the example below is even more significant. In this scenario, the same throughput can be achieved with approximately 83% lower costs for chromatography media.

Continuous processes lend themselves very well to adaptive control strategies. For (bio-) pharmaceutical applications, this would follow the concepts of the Process Analytical Technology initiative launched by the regulatory authorities. There are various ways to implement such control strategies that would allow adjustment of the process conditions to, for instance, variations in the titer. It is beyond the scope of this paper to provide a full discussion of the various options.

In the example shown above, the processing time for the batch process and the BioSMB process has been kept the same. This is not a necessity, since continuous processes can be operated for any length of time. Depending on the manufacturing schedule in the facility, the BioSMB process can be operated for 8 h, 24 h or even longer during each batch. The processing time is therefore an extra degree of freedom that can be used to optimize the process performance within the boundaries of product stability and manufacturing schedules.

Continuous & disposable bioprocessing

Biopharmaceutical manufacturing is dominated by batch processing. All steps in the process are conducted as separate unit operations that are not interconnected. Each unit operation is only started as the previous unit operation has finished and between such process steps the product is collected in intermediate product containers. This discontinuous mode of operation is inherently inefficient.

Continuous manufacturing has been adopted by almost all matured manufacturing industries. In most cases, this has been driven by improved process efficiency and superior process control that come naturally with continuous manufacturing. Continuous manu-

facturing is characterized by an uninterrupted flow of product through the cascade of unit operations. This requires that all unit operations should be designed to support such uninterrupted flow. The compatibility of typical downstream processing operations with continuous flow is summarized in Table 1.

Continuous countercurrent chromatography is one of the key technologies that enable a fully continuous manufacturing. Recent work in this field has resulted in preliminary proof-of concept for this approach [13,15]. In both studies, multicolumn chromatography was used to conduct the capture chromatography steps. The subsequent processes are either carried out fully continuously or with smaller sub lots. For the virus inactivation, for instance, the approach of holding small representative elution pools from the Protein A capture process has been presented [13,15].

Continuous manufacturing not only minimizes or even eliminates intermediate product containers; it also allows the size of most process systems to shrink. This facilitates the viable use of disposable or single-use bioprocessing technologies throughout the entire cascade of unit operations. In return, disposable technologies offer the flexibility that is needed to enable the use of continuous processing in a multiproduct environment as it reduces the validation requirements. This aspect is particularly relevant since continuous processing systems generally involve slightly more complex process equipment. Reducing validation requirements, for example, by eliminating the cleaning validation, will help the implementation of continuous process equipment. As such, continuous processing and disposable bioprocessing technologies are mutually supportive.

In traditional biopharmaceutical production processes, the entire batch is collected after each step and only then the next step is started. This results in relatively long batch processing times and often only one unit operation is active

When all unit operations in the downstream process are seamlessly linked, the overall processing time can be significantly shortened. This results from the fact that each step can start as soon as the first intermediate product has left the previous unit operation. The result is that the cascade of linked unit operations, which are linked as a continuous train can be operated in parallel, thereby saving time. This is illustrated in Figure 8. It should be noted, however, that all unit operations need not be seamlessly linked in order to take benefit from continuous processing. In other process industries one can also find hybrid processes, where some unit operations are conducted as a batch process step whereas other process steps are configured as a continuous cascade.

Table 1. The compatibility of typical downstream processing operations with continuous flow.

Step	Compatible with continuous processing	Examples and/or comments
Clarification	Yes	Continuous centrifugation Depth filtration
Capture chromatography	No	Protein A chromatography
Viral inactivation	Yes/No	Requires a cascade of mixers or a tubular contactor
Ultrafiltration	Yes	Single-pass Tangential flow filtration
Trace impurity removal	Yes	Q membrane adsorbers
Aggregate removal	No	Cation exchange chromatography Hydrophobic interaction chromatography
Virus filtration	Yes	–

In the framework of a fully disposable process, the use of other prepacked chromatography formats in combination with BioSMB technology can be very attractive. This has – for instance – been demonstrated for monoliths [16] and membrane adsorbers [17]. BioSMB

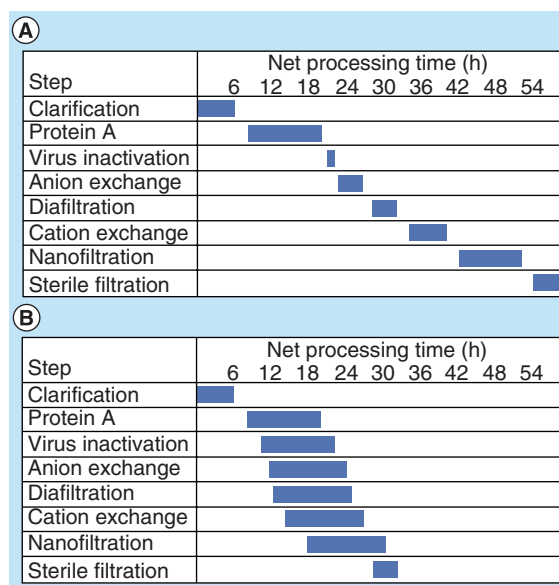


Figure 8. Typical manufacturing schedules for downstream processing operations, illustrating the potential time savings that result from continuous processing. (A) Traditional cascade of consecutive batch processes. (B) The same unit operations seamlessly linked as continuous process steps.

technology can provide a means to make membrane adsorbers suitable for capture processes in situations where this is not a viable option if they were used in a batch mode. Membrane adsorbers are known for their very fast binding kinetics and high volumetric throughputs. In a continuous countercurrent process, this will lead to very short contact times and hence very large throughputs can be processed with small membrane adsorbers. The downside of membrane adsorbers for capture processes is the relatively low binding capacity compared with packed beds. In a continuous countercurrent system, this is mitigated by automatic rapid cycling. The combination of these features will lead to very short process cycle times which results in a high number of cycles per batch. This favors the viability of disposable chromatography within a manufacturing campaign or even a single batch.

Conclusion

Continuous countercurrent multicolumn chromatography can resolve capacity bottlenecks in the downstream process and thereby contribute to improving the overall efficiency of biopharmaceutical manufacturing. The BioSMB technology has the unique feature of a fully disposable fluid path, thereby addressing most hurdles commonly associated with more complex separation equipment. The cyclic nature of the BioSMB process leads to a significant reduction in chromatography media, thereby supporting the economics of a single-use chromatography process both at clinical manufacturing and in commercial manufacturing. As a result of the high specific productivity and improved capacity utilization, this can translate into savings in the range of 30–80% on chromatography media.

Continuous countercurrent multicolumn chromatography is a technology that lends itself for a systematic design approach. The abbreviated design model presented in this paper already provides a good basis for designing and modeling the performance of the chromatography process. More sophisticated numerical process models are available to provide more in-depth modeling studies and to support, for instance, QbD studies.

Continuous countercurrent multicolumn chromatography allows transforming the downstream process into a fully continuous cascade, in which all unit operations are seamlessly linked together. This enhances overall facility throughput significantly, due to the overall shorter batch processing times. In addition to this, a fully continuous process will result in more compact process equipment for all steps, thereby promoting a viable disposable format throughout the entire downstream process.

Future perspective

With multicolumn chromatography processes emerging, continuous biomanufacturing can become a reality in a few years' time. Many of the complexities that one can expect with the implementation of continuous process equipment can be mitigated by implementing equipment in a disposable or single-use fashion. Implementing continuous bioprocess equipment in disposable formats will therefore definitely accelerate the acceptance in the industry.

The validation of continuous chromatography processes in a cGMP environment has not yet been done. Most challenges seem to be related to the hardware, the control software and the control strategies. The protein itself undergoes exactly the same steps as in the equivalent batch process and hence there is little reason to assume that the critical quality attributes will be affected. This has been demonstrated during various case studies in which the removal of impurities in a continuous system has been compared with the equivalent batch chromatography process. In most studies presented on multicolumn chromatography, the eluted product is collected for each process cycle. As a result, each sample contains one elution peak coming from each individual column in the system. This sample has been proven to be the most representative sample for the entire process.

Continuous process equipment inherently involves more valve, pumps and sensors than batch chromatography. This will translate into a more extensive equipment qualification or validation package. The types of instruments (pumps, valves and sensors) are no different than in batch chromatography and hence the generic validation approach will be very much the same.

An important aspect in the process validation will be to demonstrate process consistency. In this respect, continuous chromatography offers an advantage over batch chromatography in the sense that it involves multiple repetitive elution peaks during the processing campaign. In the control software of the BioSMB system, these peaks are automatically overlaid per process cycle, in order to visualize process consistency during the run. This approach would allow immediate detection of any failure in the hardware (pumps or valves) or in one of the columns.

Continuous purification does not necessarily have to cover the entire cascade of unit operations. In some situations, there will be good reasons to adopt a hybrid approach where not all unit operations are seamlessly interconnected. This could for instance be beneficial in case of in process controls. Such strategies could allow the design of the entire biomanufacturing process to fit the facility's production schedule.

The US FDA has stated repeatedly that they expect that continuous manufacturing will enhance product quality control through superior process control. Continuous biomanufacturing also aligns very well with the Process Analytical Technology initiative launched by the FDA. For small molecule active ingredients, this is already a reality. There are various approved drugs that have been purified using (partly) continuous production processes. For biomanufacturing, the promise of continuous manufacturing includes improved process economics, smaller more flexible facilities and eventually, real time process control. These are all being worked on today by companies wishing to

push into continuous bioprocessing. With continued technology development and FDA support, the future of a fully integrated, continuous bioprocess will soon be within reach.

Financial & competing interests disclosure

M Bisschops is an employee of Tarpon Biosystems Europe B.V., a fully owned subsidiary of Tarpon Biosystems Inc., MA, USA. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Executive summary

Principles of multicolumn chromatography

- » Multicolumn chromatography utilizes the concepts of simulated moving bed technology to establish a fully continuous countercurrent chromatography process.
- » Multicolumn chromatography processes are well established in other process industries to enhance the efficiency of ion exchange and/or chromatography processes.
- » Recently, various approaches to implement multicolumn chromatography processes in biopharmaceutical processes have been presented.
- » Continuous multicolumn chromatography involves the same chemistry and principles as batch chromatography and hence the required process design information can be derived from batch chromatography tests.

Design of a countercurrent chromatography process

- » The performance of a capture process can be described with two dimensionless numbers, the Separation Factor and the Number of Transfer Units.
- » The design methodology provides an accurate description of the process performance: the model predictions matched very well with experimental data obtained for the capture of monoclonal antibodies using Protein A chromatography.
- » These dimensionless numbers provide a basis for parametric scale-up of the chromatography process.

Continuous & disposable bioprocessing

- » Continuous chromatography enables a fully integrated continuous biomanufacturing process. All other unit operations are already available in a format that is compatible with fully continuous bioprocessing.
- » Continuous processes in general lead to more compact process equipment.
- » Single-use or disposable formats can reduce or even eliminate some of the validation issues associated with more complex equipment.

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