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A straightforward methodology for designing continuous monoclonal antibody capture multi-column chromatography processes

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ABSTRACT

A simple process development strategy for continuous capture multi-column chromatography (MCC) is described. The approach involves a few single column breakthrough experiments, based on several simplifying observations that enable users to rapidly convert batch processes into well-designed multi-column processes. The method was validated using a BioSMB® (Pall Life Sciences) lab scale multi-column system and a mAb capture process employing Protein A resin. The approach enables users to optimize MCC processes based on their internal preferences and constraints without requiring any mathematical modeling expertise.

1. Introduction

As of 2015, forty-seven monoclonal antibodies (mAbs) have been approved for the treatment of diseases in either the U.S. or Europe. Seven mAb biotherapeutics were granted marketing approval in 2014 alone, the most ever in a single year [1]. An additional 39 mAb biotherapeutics are currently in phase 3 studies [2]. If the number of mAb therapeutics continues to grow at the current rate it is predicted the global mAb market will reach almost $125 billion by 2020 [3]. The success of biotherapeutics along with advances to the upstream processes, either via higher titers or perfusion processes, has placed increased demand on the downstream purification process.

In other industries, the challenge of process intensification has been addressed by the move from batch to continuous manufacturing [4]. One of the earliest examples of continuous processing may be the Fourdrinier paper-making machine which was patented at the turn of the 19th Century [5]. However, bioprocessing has been slow to adopt continuous manufacturing. This can be attributed to a number of factors including the product being defined by its manufacturing process, surfeit of legacy facilities, resistance to change because of regulatory constraints and the barrier to adoption as the result of increased complexity.

The biopharmaceutical industry appears to be at a tipping point [6–8] where these barriers to adoption are counterbalanced by the need for improved downstream processing. The main driver for process change is cost, but the reduction in overall processing time is a key factor for adoption of continuous purification. Reducing processing time not only reduces cost, it may be especially important for enzymes which may not be as stable as mAbs. Additionally, one of the outcomes of process intensification is increased cycling of the capture columns. This leads to smaller volume columns which in turn facilitate higher relative flow rates, as smaller columns can be packed with shorter bed heights. Higher flow rates lead to increased productivity and can be leveraged in smaller unit operations to enable single-use manufacturing and factory of the future concepts.

To realize these advantages it is necessary to tip the balance in favor of continuous processing. On the regulatory side, the FDA appears to be actively encouraging the move to continuous processing: “Continuous pharmaceutical manufacturing is consistent with FDA Quality Initiatives – More modern manufacturing approach – Potential to improve assurance of quality and consistency of drugs – Enables quality to be directly built into process design” [9].

On the process side, multi-column chromatography (MCC) has been established as a facilitative technology for continuous bioprocessing. However, the process development for MCC is much more complicated than typical single column chromatography. This conflicts with the desire of the biopharmaceutical industry to drive toward shorter development timelines while maintaining

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product quality [10]. Thus, there is a necessity to simplify process development for MCC. To understand this challenge it is important to consider how MCC is best operated. The strategy applied for bind and elute processes is to increase operating binding capacity by loading columns beyond product breakthrough and by capturing the target molecule in the breakthrough on additional column(s).

Currently there are multiple approaches to optimize and operate MCC. One of the simplest process development (PD) approaches relies on performing a series of multi-column processes and incrementally increasing the loading amount in order to find the point that quantity of product eluted no longer correlates linearly with the amount of protein loaded [11]. Another approach is to measure the difference in reading of UV detectors positioned before the first column and after either the first or second column in the load zone to monitor the column breakthrough. This approach has been employed to control the load step either with an arbitrarily chosen breakthrough percent [12] or based on column switching when the UV placed between two columns reaches a pre-determined value [6]. Both of these approaches require multiple development MCC processes to be performed, even for operation at a single residence time. For a more complete optimization of productivity, based on an understanding of the effects of residence time on capacity and cycle time, these approaches become unfeasible.

An alternative approach has been to understand MCC via process modeling. For traditional simulated bed, which relies on isocratic operation, process modeling is a well understood and trusted form of process development [13]. For MCC a plethora of modeling approaches have been applied to bind and elute mode operations [14–18]. However, these approaches tend to be complicated and mathematically intensive and are thus not easily employed.

We set out with the goal of simplifying PD for MCC, thereby lowering the barrier to its adoption. Here we make observations from single column experiments that enable us to model a MCC process with just a few batch breakthrough curves. The process development approach described here allows for a more comprehensive optimization, yet it can be performed with minimal time and material and is easy to implement.

1.1. MCC process description

Before describing the MCC process operation it is necessary to introduce a few definitions:

- Breakthrough (%) – ratio of the outlet mAb concentration to the inlet mAb concentration when a mAb is injected on a solute free column.
- Thermodynamic binding capacity (mg/ml) – amount of mAb that can be loaded on an initially solute free column just before breakthrough, assuming no hydrodynamic dispersion or mass transfer limitations. This is a property of the mAb/chromatographic media couple; it depends on the feed concentration and temperature.
- Dynamic binding capacity (mg/ml) – amount of mAb that can be loaded on an initially solute free column to get a given breakthrough (typically 10%), under the hydrodynamic dispersion and mass transfer limitations prevailing in the investigated column. This depends on the mAb/chromatographic media couple, on the feed concentration, temperature, column length, and fluid velocity.
- Operating binding capacity (mg/ml) – user specified number of grams of product that is loaded on an initially solute free column. Typically, an amount that ensures no product will break through the column is selected.
- Residence time – calculated by dividing column volume with flow rate (column volume/flow rate). Ideally, this is the time it takes a non-interacting particle to travel the length of the column.
- Load time – time required to complete the load step/s within each chromatography cycle.
- Rest time – time required to complete the non-loading steps such as wash, elution, regeneration, and equilibration steps.
- Idle time – time before a column is about to enter the load step of a chromatography cycle. The column remains stagnant in equilibration buffer as while in queue.
- Cycle time – time required to perform the complete chromatography cycle including load time, rest time, and idle time.
- Productivity – grams of product purified per liter of sorbent per hour.

In the embodiment of MCC that we perform, the load step is performed with two or more columns in series. The feed sample is pumped through the inlet of a chromatography column and the outlet of this column is connected to the inlet of the succeeding column. This way the first column can be over-loaded and target product that would normally be lost to the waste is captured by the other columns connected in series. Here we will consider the columns that are connected in series and that receive feedstock to be in "the load zone". Thus, the basic concept of MCC is to have more than one column in the load zone to improve the binding capacity of the sorbent.

To understand MCC further it is important to consider the fate of the columns as they complete an entire cycle. A completed cycle requires that each column progresses through all of the chromatography steps. Fig. 1A–F depicts three columns as they progress through a MCC cycle. Columns A–C are each at the start of a phase in the cycle shown in Fig. 1A and D. Column A begins in the second pass, i.e. it would be the second column in series receiving the flow through from column B which is receiving feed sample directly from the pump. Column A enters the second pass without any bound product, so it is ideally suited to capture any product flowing through column B. The outlet of column A (second pass column) is directed to waste. Column B receives load directly, the flow out of column B is directed to the top of column A. Column C is performing the "rest" of the chromatography steps. One third of the way through the cycle, the fluid flow path must change in order for each column to begin the next phase in the process (Fig. 1B and E) column A, which was in the second pass, now receives load directly and column B which was previously in the load and has now entered into the "rest" phase. Column C completed the rest phase and now receives the flow-through of column A in the second pass. Two thirds of the way through the cycle, the fluid flow path changes once again (Fig. 1C and F) column A, which was previously in the load, now enters the rest. Column B, which was in the rest, now acts as the second pass and column C, which was in the second pass, now receives load directly from the pump. At the end of the cycle, the fluid flow path returns to its initial configuration (Fig. 1A and D) and the process steps are repeated until all of the required feed has been processed.

The challenge exists in determining the maximum optimum binding capacity of the process while ensuring no product is lost at the outlet of the second pass. Additionally, it is clear that the residence time of the load step should be addressed in process development. Increasing residence time tends to increase dynamic binding capacity, but requires more time to load the product, thereby increasing the cycle time. Both capacity and residence time can impact productivity, which can be calculated by dividing operating binding capacity by the cycle time. Therefore, it is critical to determine the optimum load residence time where these two opposing parameters deliver maximum productivity in order to realize the full advantages of continuous chromatography.